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Edina histološko usmerjena citostatična terapija

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Zdravljenje prvega reda pri bolnikih z nedrobnoceličnim pljučnim karcinomom, ki nimajo pretežno luskaste histologije

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12,6 meseca pri bolnikih z adenokarcinomom pljuč[1]

*vs. Gemcitabine/Cisplatin
1. Scagliotti GV et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 2008;26(21):3543-51.

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Ime zdravila ALIMTA 100 mg prašek za koncentrat za raztopino za infundiranje in ALIMTA 500 mg prašek za koncentrat za raztopino za infundiranje **Kakovostna in količinska sestava** ALIMTA 100 mg: vsaka viala vsebuje 100 mg pemetrekseda (v obliki dinatrijevega pemetrekseda). Po pripravi vsebuje vsaka viala 25 mg/ml pemetrekseda. Pomožne snovi: Vsaka viala vsebuje približno 11 mg natrija. Manitol, klorovodikova kislina, natrijev hidroksid. ALIMTA 500 mg: vsaka viala vsebuje 500 mg pemetrekseda (v obliki dinatrijevega pemetrekseda). Po pripravi vsebuje vsaka viala 25 mg/ml pemetrekseda. Pomožne snovi: Vsaka viala vsebuje približno 54 mg natrija. Manitol, klorovodikova kislina, natrijev hidroksid. **Terapevtske indikacije:** ALIMTA je v kombinaciji s cisplatinom indicirana za zdravljenje bolnikov z neresektabilnim malignim plevralnim mezoteliomom, ki jih še nismo zdravili s kemoterapijo. ALIMTA je v kombinaciji s cisplatinom indicirana kot zdravljenje prvega izbora za bolnike z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim karcinomom, ki nima pretežno luskaste celične histologije. ALIMTA je indicirana kot monoterapija za zdravljenje lokalno napredovalega ali metastatskega nedrobnoceličnega pljučnega karcinoma, ki nima pretežno luskaste celične histologije pri bolnikih, pri katerih bolezen ni napredovala neposredno po kemoterapiji na osnovi platine. Zdravljenje prvega izbora naj bo platinasta dubleta z gemcitabinom, paklitakselom ali docetakselom. ALIMTA je indicirana kot monoterapija za zdravljenje drugega izbora bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim karcinomom, ki nima pretežno luskaste celične histologije. **Odmerjanje in način uporabe:** ALIMTO smemo dajati le pod nadzorom zdravnika, usposobljenega za uporabo kemoterapije za zdravljenje raka. ALIMTA v kombinaciji s cisplatinom Priporočeni odmerek ALIMTE je 500 mg/m2 telesne površine (TP), dan kot intravenska infuzija v 10 minutah prvi dan vsakega 21-dnevnega ciklusa. Priporočeni odmerek cisplatina je 75 mg/m2 TP, infundiran v dveh urah približno 30 minut po zaključku infuzije pemetrekseda prvi dan vsakega 21 dnevnega ciklusa. Priporočeni odmerek cisplatina je 75 mg/m2 TP, infundiran v dveh urah približno 30 minut po zaključku infuzije pemetrekseda prvi dan vsakega 21 dnevnega ciklusa. Bolniki morajo prejeti zadostno antiemetično zdravljenje, pred in/ali po prejemanju cisplatina jih moramo tudi ustrezno hidrirati. ALIMTA kot samostojno zdravilo Priporočeni odmerek ALIMTE je 500 mg/m2 TP, dan kot intravenska infuzija v 10 minutah prvi dan vsakega 21 dnevnega ciklusa. Režim premedikacije Da zmanjšamo incidenco in resnost kožnih reakcij, dajemo kortikosteroid dan pred dajanjem pemetrekseda, na dan dajanja pemetrekseda in naslednji dan. Kortikosteroid naj ustreza 4 mg deksametazona, danega peroralno dvakrat dnevno. Za zmanjšanje toksičnosti morajo bolniki dnevno jemati tudi peroralno folno kislino ali multivitaminski pripravek, ki jo vsebuje (350 do 1000 mikrogramov). V sedmih dneh pred prvim odmerkom pemetrekseda morajo vzeti vsaj pet odmerkov folne kisline, odmerjanje pa morajo nadaljevati ves čas zdravljenja in še 21 dni po zadnjem odmerku pemetrekseda. Bolniki morajo prejeti tudi intramuskularno injekcijo vitamina B12 (1000 mikrogramov) v tednu pred prvim odmerkom pemetrekseda in enkrat vsake tri cikluse zatem. Kasnejše injekcije vitamina B12 lahko dajemo isti dan kot pemetreksed. **Kontraindikacije:** Preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov. Dojenje. Sočasno cepljenje proti rumeni mrzlici. **Posebna opozorila in previdnostni ukrepi:** Pemetreksed lahko zavre delovanje kostnega mozga, kar se kaže kot nevtropenija, trombocitopenija in anemija (ali pancitopenija). Pri bolnikih, ki pred zdravljenjem niso prejemali kortikosteroidov, so poročali o kožnih reakcijah. Uporabe pemetrekseda pri bolnikih z očistkom kreatinina < 45 ml/min ne priporočamo. Bolniki z blagim do zmernim popuščanjem delovanja ledvic naj se izogibajo jemanju nesteroidnih protivnetnih zdravil (NSAID), denimo, ibuprofena in acetilsalicilne kisline 2 dni pred dajanjem pemetrekseda, na dan dajanja in še 2 dni po dajanju pemetrekseda. Vsi bolniki, ki jih lahko zdravimo s pemetreksedom, naj se izogibajo jemanju NSAID-ov z dolgimi razpolovnimi časi izločanja vsaj 5 dni pred dajanjem pemetrekseda, na dan dajanja in še vsaj 2 dni po dajanju pemetrekseda. Poročali so o resnih ledvičnih primerih, vključno z akutno ledvično odpovedjo, s pemetreksedom samim ali v povezavi z drugimi kemoterapevtiki. Pri bolnikih s klinično pomembno tekočino tretjega prostora moramo razmisliti o drenaži izliva pred dajanjem pemetrekseda. Kot posledico toksičnosti pemetrekseda v kombinaciji s cisplatinom za prebavila so opažali hudo dehidracijo, zato moramo bolnike pred prejemanjem terapije in/ali po njej ustrezno hidrirati, prejeti morajo zadostno antiemetično zdravljenje. Občasno so v kliničnih študijah pemetrekseda, običajno ob sočasnem dajanju z drugo citotoksično učinkovino, poročali o resnih srčnožilnih dogodkih, vključno z miokardnim infarktom in možganskožilnimi dogodki. Odsvetujemo uporabo živih oslabljenih cepiv. Spolno zrelim moškim odsvetujemo zaploditev otroka v času zdravljenja in še 6 mesecev zatem. Priporočamo ukrepe prosti zanositvi ali vzdržnost. Zaradi možnosti, da zdravljenje s pemetreksedom povzroči trajno neplodnost, naj se moški pred začetkom zdravljenja posvetujejo o shranjevanju semena. Ženske v rodni dobi morajo v času zdravljenja s pemetreksedom uporabljati učinkovito kontracepcijo. Poročali so o primerih radiacijske pljučnice pri bolnikih, ki so jih zdravili z radiacijo pred, med ali po zdravljenju s pemetreksedom. Poročali so o radiacijskem izpuščaju pri bolnikih, ki so se zdravili z radioterapijo pred tedni ali leti. Zdravilo Alimta 500 mg vsebuje približno 54 mg natrija na vialo. Pomembno za bolnike, ki so na dieti z nadzorovanim vnosom natrija. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Sočasno dajanje nefrotoksičnih zdravil (denimo, aminoglikozidov, diuretikov zanke, spojin platine, ciklosporina) lahko potencialno povzroči zakasnjeni očistek pemetrekseda. Sočasno dajanje snovi, ki se tudi izločajo s tubulno sekrecijo (denimo, probenecid, penicilin), lahko potencialno povzroči zakasnjeni očistek pemetrekseda. Pri bolnikih z normalnim delovanjem ledvic lahko visoki odmerki nesteroidnih protivnetnih zdravil (NSAID-i; denimo, ibuprofen) in acetilsalicilna kislina v visokih odmerkih zmanjšajo eliminacijo pemetrekseda in tako lahko povečajo pojavnost neželenih učinkov pemetrekseda. Pri bolnikih z blagim do zmernim popuščanjem delovanja ledvic se moramo izogibati sočasnemu dajanju pemetrekseda z NSAID-i (denimo, ibuprofenom) ali acetilsalicilne kisline v visokih odmerkih 2 dni pred dajanjem pemetrekseda, na dan dajanja in še 2 dni po dajanju pemetrekseda. Sočasnemu dajanju NSAID-ov z daljšimi razpolovnimi časi s pemetreksedom se moramo izogibati vsaj 5 dni pred dajanjem pemetrekseda, na dan dajanja in še vsaj 2 dni po dajanju pemetrekseda.Velika različnost med posamezniki v koagulacijskem statusu v času bolezni ter možnost medsebojnega delovanja med perioralnimi antikoagulacijskimi učinkovinami ter kemoterapijo proti raku zahtevata povečano pogostnost spremljanja INR. **Kontraindicirana sočasna uporaba:** Cepivo proti rumeni mrzlici: tveganje za smrtno generalizirano bolezen po cepljenju. **Odsvetovana sočasna uporaba:** Živa oslabljena cepiva (razen proti rumeni mrzlici): tveganje za sistemsko, potencialno smrtno bolezen. **Neželeni učinki** Klinične študije malignega plevralnega mezotelioma *Zelo pogosti:* znižani nevtrofili/granulociti, znižani levkociti, znižan hemoglobin, znižani trombociti, nevropatija-senzorična, diareja, bruhanje, stomatitis/faringitis, slabost, anoreksija, zaprtje, izpuščaj, alopecija, povišan kreatinin, znižan očistek kreatinina, utrujenost. *Pogosti:* dehidracija, motnje okusa, konjuktivitis, dispepsija. Klinične študije nedrobnoceličnega pljučnega karcinoma - ALIMTA monoterapija, zdravljenje 2. izbora. *Zelo pogosti:* znižan nevtrofili/granulociti, znižani levkociti, znižan hemoglobin, diareja, bruhanje, stomatitis/faringitis, slabost, anoreksija, izpuščaj/luščenje, utrujenost. *Pogosti:* znižani trombociti, zaprtje, povišanje SGPT (ALT), povišanje SGOT (AST), srbenje, alopecija, povišana telesna temperatura. Klinične študije nedrobnoceličnega pljučnega karcinoma - ALIMTA v kombinaciji s cisplatinom, zdravljenje 1. izbora. *Zelo pogosti:* znižan hemoglobin, znižani nevtrofili/granulociti, znižani levkociti, znižani trombociti, slabost, bruhanje, anoreksija, zaprtje, stomatitis/faringitis, diareja brez kolostomije, alopecija, izpuščaj/luščenje, povišan kreatinin. *Pogosti:* nevropatija-senzorična, motnje okusa, dispepsija/zgaga. Klinične študije nedrobnoceličnega pljučnega karcinoma - ALIMTA monoterapija, vzdrževalno zdravljenje: *Zelo pogosti:* znižan hemoglobin, slabost, anoreksija, utrujenost, izpuščaj/luščenje, utrujenost. *Pogosti:* infekcija, znižani levkociti, znižani nevtrofili, nevropatija-senzorična, bruhanje, mukozitis/stomatitis, diareja, povišanje ALT (SGPT), povišanje AST (SGOT). Občasno so v kliničnih študijah pemetrekseda poročali o primerih resnih srčnožilnih in možganskožilnih dogodkov, vključno z miokardnim infarktom, angino pektoris, cerebrovaskularnim insultom in prehodnimi ishemičnimi atakami; primerih kolitisa ter o primerih intersticijske pljučnice z respiratorno insuficience, primerih edema in o ezofagitisu/radiacijskem ezofagitisu. Redkeje pa o primerih potencialno resnega hepatitisa in pancitopenije. Po uvedbi zdravila na trg so poročali o primerih akutne odpovedi ledvic s pemetreksedom samim ali v povezavi z drugimi kemoterapevtiki, primerih radiacijske pljučnice pri bolnikih, ki so jih zdravili z radiacijo pred, med ali po njihovem zdravljenju s pemetreksedom, primerih radiacijskega izpuščaja pri bolnikih, ki so se v preteklosti zdravili z radioterapijo in o primerih periferne ishemije, ki je včasih vodila v nekrozo okončin. **Imetnik dovoljenja za promet** Eli Lilly Nederland B.V., Grootslag 1 5, NL 3991 RA, Houten, Nizozemska. Datum zadnje revizije besedila 21.09.2009. **Način izdaje zdravila:** H
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Progress of Radiology and Oncology

In front of you is a new issue of Radiology and Oncology, an international journal devoted to publications in the field of radiology, nuclear medicine and oncology. The journal's scope is to publish review papers, scientific papers and case reports on these and related topics, including experimental oncology, radiophysics and radiation protection.

It is my pleasure to inform you that Radiology and Oncology has grown over the past two years, thanks to inclusion into Web of Science and the Journal Citation Reports/Science Edition database from 2008. In other words, we will gain an Impact Factor (IF), probably in 2010 or in 2011. The inclusion into Web of Science is already reflected in a higher submission rate of papers, which enabled us to increase the number of published articles. It is additionally reflected in higher average citations per item and in a higher h-index.

Along with the printed version, Radiology and Oncology is also published electronically with open access on www.versita.com. This enables swift publication of the papers as E-ahead of print - early birds.

In light of making our journal more attractive, we have prepared a new format of the journal with a more attractive appearance. We now publish the journal in full color text, so that the papers in the printed and electronic edition of the journal will be more appealing.

At the end I would like to encourage you to submit papers to Radiology and Oncology and also encourage your colleagues and students to choose our journal for their publications.

We in the editorial office will strive to be swift in processing the papers and to publish papers with a higher impact and influence in the scientific community.

Best regards,

Prof. Gregor Serša, Ph.D.
Editor in Chief

Viljem Kovač, M.D.
Executive Editor

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review

Role of radiotherapy in melanoma management

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Background. In melanoma, radiotherapy has generally been considered as a palliative treatment option indicated only for advanced cases or disseminated disease. In the 70s of the previous century, the technological advances in radiotherapy, linked to rapid development of computer sciences, resulted in restored interest for radiotherapy in melanoma management. Although a fundamental lack of well designed prospective and/or randomized clinical trials critically influenced the integration of radiotherapy into treatment strategies in melanoma, radiotherapy was recently recognized as an indispensable part in the multidisciplinary management of patients with melanoma. Altogether, approximately 23% of melanoma patients should receive at least one course of radiotherapy during the course of the disease. In this review, radiobiological properties of melanoma that govern the decisions for the fractionation patterns used in the treatment of this disease are described. Moreover, the indications for irradiation and the results of pertinent clinical studies from the literature, creating a rationale for the use of radiotherapy in the management of this disease, are reviewed and a brief description of radiotherapy techniques is given.

Conclusions. Basic treatment modality in melanoma is surgery. However, whenever surgery is not radical or there are adverse prognostic factors identified on histopathological examination of resected tissue specimen, it needs to be supplemented. Also, in patients with unresectable disease or in those not being suitable for major surgery or who refuse proposed surgical intervention, other effective mode(s) of therapy need to be implemented. From this perspective, supported by clinical experiences and literature results, radiotherapy is a valuable option: it is effective and safe, in curative and palliative setting.

Key words: melanoma; radiobiology; radiotherapy; fractionation; indications; toxicity

Introduction

Changes in human behavior, particularly those related to sun exposure and global environmental alterations have contributed to an observed increase in the incidence of cutaneous melanoma in Europe since the 1950s.[1] In Slovenia with the population of two million, the melanoma incidence doubled during the last decade, being 10.2 per 100.000 inhabitants in 1997 and 19.6 in 2006.[2,3] As melanoma is a significant health burden, its management was continuously in focus of extensive laboratory and clinical research.

Surgery is basic and the most effective treatment modality for melanoma, whereas radiotherapy, one of the corn stones of anti-cancer management, has been evolving steadily and, during the time, taking over greater role in the management of this disease. It has long been negatively marked by the lack of well designed prospective and/or randomized clinical trials which finally gave more credit to lucid observations of clinicians dealing with the disease.[4]

First experiences of radiation oncologists with melanoma were marked with technologically inferior irradiation devices and the label of tumor as radioresistant, which originated from categorization of tumor radiosensitivity by histological type introduced in 1930s.[5] Consequently, radiotherapy was generally considered as a palliative treatment option indicated only for advanced cases or disseminated disease.

In the 70s of previous century, the interest for radiotherapy in melanoma management was restored. During the decades, new knowledge on radiobiological characteristics of melanoma cells

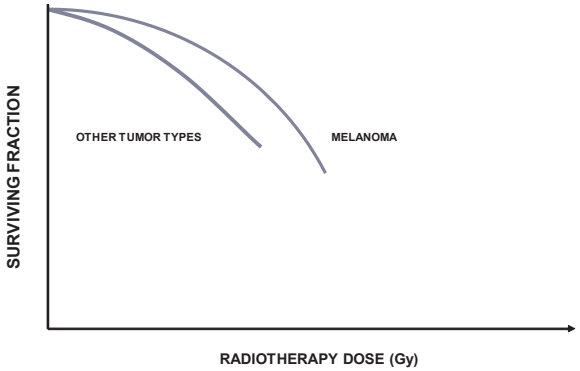


FIGURE 1. Dose-response curve for melanoma cells. High intrinsic capacity of melanoma cells for repair of sublethal DNA damages caused by irradiation is graphically presented by a distinctly broad shoulder in the low-dose portion of the logarithmic cell survival curve. Accordingly, the ability of melanoma cell to overcome sublethal DNA injuries suggests increased sensitivity to large doses per fraction (hypofractionation).

as well as more favorable clinical experiences were obtained. Furthermore, modern radiotherapy devices, including treatment planning systems, appeared on the market, allowing more sophisticated treatment planning and accurate targeting. These novelties contributed to a change in perception of clinicians confronted with this disease, which directly contradicted to long standing belief that melanoma is radioresistant tumor.

Nowadays, RT is recognized as the most effective non-surgical mode of locoregional therapy of melanoma and is an integral part of the multidisciplinary management, thus providing a valuable input to the best treatment of melanoma. According to Delaney *et al.*, the recommended proportion of all patients with melanoma who, according to the best available evidence, should receive at least one course of radiotherapy is 23%.[6]

Clinical radiobiology of melanoma and fractionation pattern

Response of melanoma to irradiation depends on tumor volume, radiotherapy dose and fraction size. From preclinical studies as well as clinical observations, an abundance of evidences emerge confirming a positive relationship between response to irradiation and radiotherapy dose corrected for tumor volume, *i.e.* the number of clonogenic cells that need to be sterilized. Within the timeframe of the schedules used, the overall treatment time showed no effect on response rate.[7,8]

From radiobiological perspective, the most intriguing is the observation that melanomas have a wide range of sensitivities to ionizing irradiation.[9-13] The results of *in vitro* studies on melanoma radiosensitivity suggest high intrinsic capacity of melanoma cells for repair of sublethal DNA damages caused by photon beam radiotherapy.[11-14] This particular characteristic of melanoma cells is graphically presented by a distinctly broad shoulder in the low-dose portion of the logarithmic cell survival curve (Figure 1).[13] However, variations in the cellular radiosensitivity recognized *in vitro* and in clinic imposed other factors to add to the observed heterogeneity among treated tumors.[10,12] There are several candidates, *i.e.* intra-tumor variability (clonogenic subpopulations with different radiosensitivity, variations in tendency to apoptosis); tumor physiological factors (the existence of hypoxic fraction and/or differences in reoxygenation capacity of tumor clonogens, the intracellular level of glutathione – scavenger of free radicals responsible for DNA damage); tumor cell kinetics (different propensity to cell cycle disruption); and host-related factors (immune competence of the patient).[9-12] According to the results of *in vitro* studies, sublethal irradiation doses increase the risk of metastases, possibly due to increased hypoxic fraction and hypoxia-induced up-regulation of urokinase-type plasminogen activator receptor in regrowing primary tumors.[15]

Theoretically, the ability of melanoma cell to overcome sublethal DNA injuries caused by irradiation suggests that, clinically, melanoma should be more sensitive to large doses per fraction (hypofractionation) than to lower fraction doses (hyperfractionation).[14] This concept is mirrored in a low value of the ratio of the parameters α and β in the linear-quadratic model, a determinant of the shape (or bendiness) of survival curve in the model and an indicator used to quantify the fractionation sensitivity of tissues. As derived from clinical data, the α/β ratio for cutaneous melanoma ranges from 0.6 Gy to 2.5 Gy and is characterized by wide confidence intervals, implying large variations in the sensitivity of individual tumors to radiotherapy.[7,8] Also, wide range of values of the α/β ratio resulted from calculations in preclinical studies.[16]

Despite seemingly firm theoretical arguments, clinical data on optimal fractionation pattern are not equivocal and no consensus was accepted on the best radiation regimen. This issue is further complicated by an increased probability of morbid-

ity from late reacting normal tissue injury when using hypofractionated regimens.[13,14] Whereas good arguments for the use of fraction doses of ≥4 Gy were provided by several retrospective studies (for review see Bello *and* Ang[17]), the results of the only prospective randomized trial addressing the issue of low versus high fraction doses neglected the expected advantage of hypofractionation. In RTOG 83-05, 137 patients with measurable lesions were randomized between 20 x 2.5 Gy in 26-28 days and 4 x 8 Gy in 21 days.[18] No differences in local control, either complete or partial, were reported between the two arms; unfortunately, no data on the duration of responses were provided from this trial.[18] On adjuvant setting, a retrospective comparison of conventional and hypofractionated regimens[19-22] and reported in-field relapse rates from rare retrospective[23] or prospective[24] series implementing more conventional fraction doses (*i.e.* 1.7–2.4 Gy) support this observation.

On the other hand, prospective randomized comparison of different hypofractionation schedules (3 x 9 Gy versus 5 x 8 Gy, 2 fractions per week) in recurrent or metastatic melanoma resulted in virtually identical durable complete response rates of 65% and 72%, respectively, in the two arms of the trial.[25] In another randomized study, after adding hyperthermia as an adjuvant to radiotherapy (3 x 8-9 Gy in 8 days) for macroscopic lymph node and skin disease, multivariate analysis with either complete response or 2-year local control rates as an endpoint showed that tumor dose, on the top of additional hyperthermia and tumor size, was an independent prognostic variable.[26]

Indications for radiotherapy

Considering the treatment intent and the time point at which radiotherapy is to be introduced into melanoma management, indications for irradiation can be divided into four groups: upfront radiotherapy (as the main treatment modality, replacing surgery); adjuvant radiotherapy (after surgery), elective and palliative radiotherapy.

Radiotherapy as primary therapy

Radiotherapy is rarely used as a primary treatment modality instead of surgery which is the curative treatment of choice for all types of primary melanoma lesions. Poor performance status of the patient with severe comorbidities or refusal of proposed surgery are potential but less plausible motives in clinic for replacing surgery with radiotherapy.

More frequent indication for upfront radiotherapy is lentigo maligna melanoma (LMM). Particularly when LMM is extensive and located on the face of elderly patient, radiotherapy is a good alternative to surgery. In three larger series with a total of 107 patients, 3 local recurrences were observed 13-44 months after radiotherapy (85 lesions) or combination of surgical excision of the nodular part followed by irradiation of the lentiginous part of the lesion (22 lesions).[27-29] Time to complete regression of the lesion after irradiation took up to 24 months. Regional node metastases developed in 3 patients 6, 8 and 18 months after therapy, respectively, whereas in one patient, pulmonary metastases occurred 44 months after treatment. All theses patients had their primaries controlled.[27-29] Thus, whenever surgery attempting to achieve clear margins would result in excessive mutilation, either cosmetic or functional, or in elderly patients, it should be replaced with radiotherapy, which is effective and has curative potential in LMM. Because the incidence of regional metastases is extremely low, no elective irradiation of regional lymphatics is required.

Primary curative radiotherapy should be attempted also in localized inoperable mucosal melanoma (MM) where it is considered the most effective treatment modality. After the extensive literature review, Krengli *et al.* summarized their analyses in a way that high local control rates, over 70%, can be achieved with radiotherapy alone in MM, which could be – taking into account some preliminary results – further improved by utilizing high-linear energy transfer (LET) radiation.[30] Primary tumor area only should be included into the irradiation field in clinically N0 disease as it is unlikely that elective nodal treatment affects the overall course of the disease.[30,31] The exception might be the oral cavity primaries with higher regional failure rates.[32]

Adjuvant radiotherapy - primary melanoma

After excision of primary lesion, the decision about the use of postoperative radiotherapy is dictated by the risk estimate for recurrence, treatment related side-effects and the possibility for successful salvage when recurrence occurred. Because of superficial nature of the target tissue(s), the risk for serious complications after local radiotherapy is low.

TABLE 1. Nodal field relapse rates (number of relapses/dissections) after therapeutic surgery according to adverse clinicopathological features negatively impacted disease control in dissected nodal basin

Parameter	Nodal basin recurrence (%)	References
No. of involved nodes		
1	9, 9, 25, 45	45,46,47,48
1-3	19, 14, 25[a], 15, 24	47,49,50[a],51,52
2-4	15, 10	45,46
≥4	17, 22, 20, 60, 53, 46[a], 8, 37	45,46,47,48,49,50[a],51,52
>10	33, 26, 63[a], 47	45,46,50[a],52
Diameter of largest node		
<3 cm vs. 3-6 cm vs. >6 cm	25 vs. 42 vs. 80[a]	50[a]
Extracapsular tumor spread		
No	15, 38, 23[a], 9	45,48,50[a],51
Yes	28, 54, 63[a], 24, 43	45,48,50[a],51,52
Matted nodes		
Yes	29, 44, 12	45,46,52
Nodal basin		
Parotid & neck	41,15, 19, 50, 43[a], 14, 43, 50, 50	22,45,47,48,50[a],51,52,53,54
Axilla	15, 60, 28[a], 30, 14, 10	45,48,50[a],52,53,55
Groin	17, 44, 18, 23, 19, 8, 34, 19, 34, 8	45,48,49,50[a],52,53,56,57,58,59
All nodal sites	16, 52, 18, 30[a], 28, 15, 34, 41	45,48,49,50[a],52,53,60,61

[a]Actuarial nodal basin control rates at 10 years are reported.

Factors that adversely influence local control after wide excision alone are close or positive margins, early and/or multiple recurrences, extensive satellitosis, desmoplasia or neurotropism, and MM primaries. The incidence of local recurrence when tumor satellites are noted histologically was reported to be 12-14%[33,34], and in desmoplastic tumors, as high as 11-48%.[35-38] In the latter case, it appears that local recurrence may be related to the presence of neutropism[38] and to inadequate surgical margins[36-39], which could be of importance for lesions arising in anatomically critical regions of the head and neck. In high-risk clinical situations, postoperative radiotherapy has a potential to reduce the risk of local recurrence significantly.[37,40-42] In MM, a number of retrospective studies suggest that postoperative radiotherapy yields better outcome, although it has no influence on survival. Combined approach is currently recommended after non-radical surgery, but seems to improve local control also after excision of large primary tumors, especially those in sinonasal localization, and those with perineural invasion.[30]

Adjuvant radiotherapy - regional lymphatic metastases

After dissection of regional lymph nodes, radiotherapy adds significantly to an improved control in the operative bed. Only two randomized controlled trials were conducted to clarify this issue. The first was carried out in the 1970s with small sample size (56 patients) using an unusual regimen (split course, 50 Gy total and 1.78 Gy daily midplane dose, one field was treated daily) and was found inconclusive.[43] Only recently, the results of the intergroup multicenter randomized trial (ANZMTG 01.02/TROG 02.01) were published (in an abstract form, Henderson *et al.*[44]). After lymphadenectomy for isolated regional recurrence of melanoma, 250 patients considered to be at high risk (>25%) of in-field recurrence were randomized into radiotherapy group (126 patients) and control group (127 patients); 227 patient were available for analysis. After a median follow up of 27 months, a statistically significant improvement in lymph node field control was observed with radiotherapy (hazard ratio 1.77, 95% confidence interval 1.02-3.08, P=0.041), but not also in median survival times (P=0.14).[44]

Thus far, identification of factors increasing the risk for regional recurrence after lymphadenectomy and recommendations for adjuvant irradiation were based on retrospective analyses or rare non-randomized prospective studies. In high-risk setting, the rates of relapse in nodal basin could reach 50% or even more after surgery alone. The factors contributing to an increased recurrence in surgical field are the presence of residual disease after sur-

TABLE 2. Therapeutic lymph node dissection in melanoma patients with or without adjuvant radiotherapy: comparison of nodal basin recurrence rates

Surgery			Surgery plus radiotherapy		
Author, year[Ref.]	No. of pts.	Nodal basin recurrence (%)	Author, year[Ref.]	No. of pts.	Nodal basin recurrence (%)
Parotid & neck			***Parotid & neck***		
Bayers, 1986[54]	28	50	Ang *et al.*, 1994[62]	95	8
Calabro *et al.*, 1989[45]	287	15	O'Brian *et al.*, 1997[47]	45	7
O'Brian *et al.*, 1997[47]	107	19	Shen *et al.*, 2000[51]	21	14
Shen *et al.*, 2000[51]	196	14	Ballo *et al.*, 2002[63]	160	8
Pidhorecky *et al.*, 2001[52]	44	43	Strojan *et al.*, 2010[22]	45	18
Strojan *et al.*, 2010[22]	42	40	*Total*	366	10
Total	704	20			
Axilla			***Axilla***		
Bowsher *et al.*, 1986[53]	22	14	Ballo *et al.*, 2002[64]	89	10
Calabro *et al.*, 1989[45]	438	15	Beadle *et al.*, 2009[65]	200	10
Pidhorecky *et al.*, 2001[52]	116	30	*Total*	289	10
Kretschmer, *et al.*, 2001[55]	63	10			
Total	639	17			
Groin			***Groin***		
Bowsher *et al.*, 1986[53]	36	8	Ballo *et al.*, 2004[66]	40	23
Kissin *et al.*, 1987[56]	44	34			
Calabro *et al.*, 1989[45]	276	17			
Hughes *et al.*, 2000[57]	132	19			
Pidhorecky *et al.*, 2001[52]	93	19			
Kretschmer *et al.*, 2001[58]	104	34			
Allan *et al.*, 2008[59]	72	8			
Total	757	20			
All sites			***All sites***		
Bowsher *et al.*, 1986[53]	66	15	Burmeister *et al.*, 1995[67]	26	12
Calabro *et al.*, 1989[45]	1001	16	Corry *et al.*, 1999[23]	42	21
Miller *et al.*, 1992[49]	55	18	Stevens *et al.*, 2000[68]	174[1]	11
Monsour *et al.*, 1993[48]	48	52	Cooper *et al.*, 2001[41]	40[1]	8
Pidhorecky *et al.*, 2001[52]	253	28	Fuhrmann *et al.*, 2001[69]	58	16
Mayer *et al.*, 2002[60]	140	34	Chang *et al.*, 2006[21]	54	12
Henderson *et al.*, 2009[44]	108	31	Burmeister *et al.*, 2006[24]	234	7
Agrawal *et al.*, 2009[61]	106	41	Ballo *et al.*, 2006[70]	466	9
Total	1777	23	Henderson *et al.*, 2009[44]	123	18
			Agrawal *et al.*, 2009[61]	509	10
			Total	1726	11

gery, extracapsular tumor extension, nodes measuring ≥3 cm in the largest diameter, multiple nodal involvement or recurrence after previous lymph node dissection (and RT was not used at that time) (Table 1).[22,45-61] The criteria for using adjuvant irradiation vary slightly among different nodal basins, reflecting various potential outcomes for distinctive anatomical body region. In recent ANZMTG/TROG randomized trial, the high-risk features (in addition to non-radical surgery and recurrent disease) were as follows: ≥1 parotid, ≥2 cervical or axillary or ≥3 groin nodes; extracapsular spread of tumor; maximum metastatic node diameter ≥3 cm in neck and axilla or ≥4 cm in the groin.[44]

Comparison of studies using surgery alone or surgery plus radiotherapy provides a strong argument for the effectiveness of adjuvant irradiation when adverse prognostic factors are found at histopathological examination of resected specimen. In-field tumor control is roughly 90% in adjuvantly irradiated patients using either conventional or hypofractionated schedules (Table 2).[21-24,41,44-49,51-70] However, in these studies, only the patients with less favorable disease characteristics were referred

TABLE 3. Nodal basin control after surgery with or without adjuvant radiotherapy

Author, year[Ref.]	No.	Nodal Basin	Risk factors (%)			FUP, median (mos.)	Nodal basin recurrence (%)		Survival, at 5 yrs. (%)	
			ECE	N_+ ≥2	N_+ >3		Absolute	Actuarial (at 5 yrs.)	Melanoma specific	Overall
O'Brian *et al.*, 1997[47]										
Surgery	107	Parotid & neck	20	43	9	56	19	40	35	n.r.
Surgery + XRT	45	Parotid & neck	49	67	24	38	7	17	40	n.r.
Shen *et al*,. 2000[51]										
Surgery	196	Parotid & neck	23	n.r.	27	32[a]	14	17	n.r.	32
Surgery + XRT	21	Parotid & neck	43	n.r.	48	n.r.	14	25	n.r.	n.r.
Fuhrmann *et al.*, 2001[69]										
Surgery	58	All sites	n.r.	74	n.r.	n.r.	21	26	n.r.	25
Surgery + XRT	58	All sites	n.r.	74	n.r.	n.r.	16	22	n.r.	23
Moncrieff *et al.*, 2008[71]										
Surgery	587	Parotid & neck	n.r.	n.r.	n.r.	35	n.r.	6[b]	n.r.	n.r.
Surgery + XRT	129	Parotid & neck	n.r.	n.r.	n.r.	35	n.r.	10[b]	n.r.	n.r.
Henderson *et al.*, 2009[44]										
Surgery	108	All sites	All patients at high risk for regional recurrence			27	31	n.r.	n.r.	n.r.
Surgery + XRT	109	All sites				27	18	n.r.	n.r.	n.r.
Agrawal *et al.*, 2009[61]										
Surgery	106	All sites	All patients at high risk for regional recurrence			60	41	48	30	n.r.
Surgery + XRT	509	All sites				60	10	13	51	n.r.
Strojan *et al.*, 2010[22]										
Surgery	42	Parotid & neck	21	38	n.r.	25	40	44[a]	n.r.	58[a]
Surgery + XRT	45	Parotid & neck	44	64	n.r.	25	18	22[a]	n.r.	51[a]

ECE – Extracapsular extension of tumor; N_+ - Number of positive nodes; FUP – Follow-up; XRT – Radiotherapy; n.r. – Not reported.
[a]At 2 years.
[b]At 6 years.

to radiotherapy; thus, the existing selection bias should be aware of when comparing the results between these two groups of studies. Finally, while matching the results of studies simultaneously reporting on the outcome in surgically and postoperatively irradiated patients, it seems that adjuvant radiotherapy compensates effectively for the negative impact of adverse histopathological features to the disease control in the dissected nodal basin (Table 3).[22,44,47,51,61,69,71] No effect of postoperative irradiation on survival was observed in these studies. To the contrary, Agrawal *et al.* recently reported that adjuvant radiotherapy also could have a positive impact on melanoma specific survival.[61]

Owing to the increased probability of serious treatment-related side effects after adding radiotherapy to surgery, particularly lymphedema, and due to high likelihood of distant metastases in the patients with extensive lymph node involvement and no survival advantage for the adjuvantly irradiated patients, the question has been raised on the meaning of adjuvant use of radiotherapy. In view of these obstacles, the primary goal of postoperative irradiation must be emphasized, *i.e.* to prevent the uncontrolled regional recurrences with local destruction and associated infection (with secretion and stench), hemorrhages, edema, disfigurement or pain, which produce considerable morbidity that significantly reduces the quality of patient's life.

The probability of systemic dissemination, which is the most powerful predictor of the risk of dying due to the disease, seems to be associated with the number of involved nodes.[22,70] Thus, it sounds reasonable to use as cut point a certain number of involved nodes at which the risk of distant failure is that high that regional radiotherapy should not be delivered, despite its proven effectiveness in controlling regional disease. A reasonable number might be between 10-15 nodes, at which point the risk of distant metastasis reaches 70%.[22,70]

When a comprehensive nodal resection is not done and only local excision of palpable node(s) is performed instead, either due to significant medical comorbidities or patient's refusal of more extensive surgical procedure, radiotherapy seems to have a potential to compensate for this deficiency. In a series of 36 patients with parotid or cervical node metastases from melanoma treated with local excision of palpable nodal disease and postoperative radiotherapy (to the primary site – if known, the site of nodal excision and the undissected ipsilateral neck), the disease, after the median follow up of 5.3 years, recurred within the regional basin in two patients only and at distant sites in 14 patients.[72] In this setting, it seems unlikely that a comprehensive surgical dissection would improve the regional control, but observation only would place the patient at unnecessary risk of regional recurrence.

Elective radiotherapy – regional lymphatic metastases

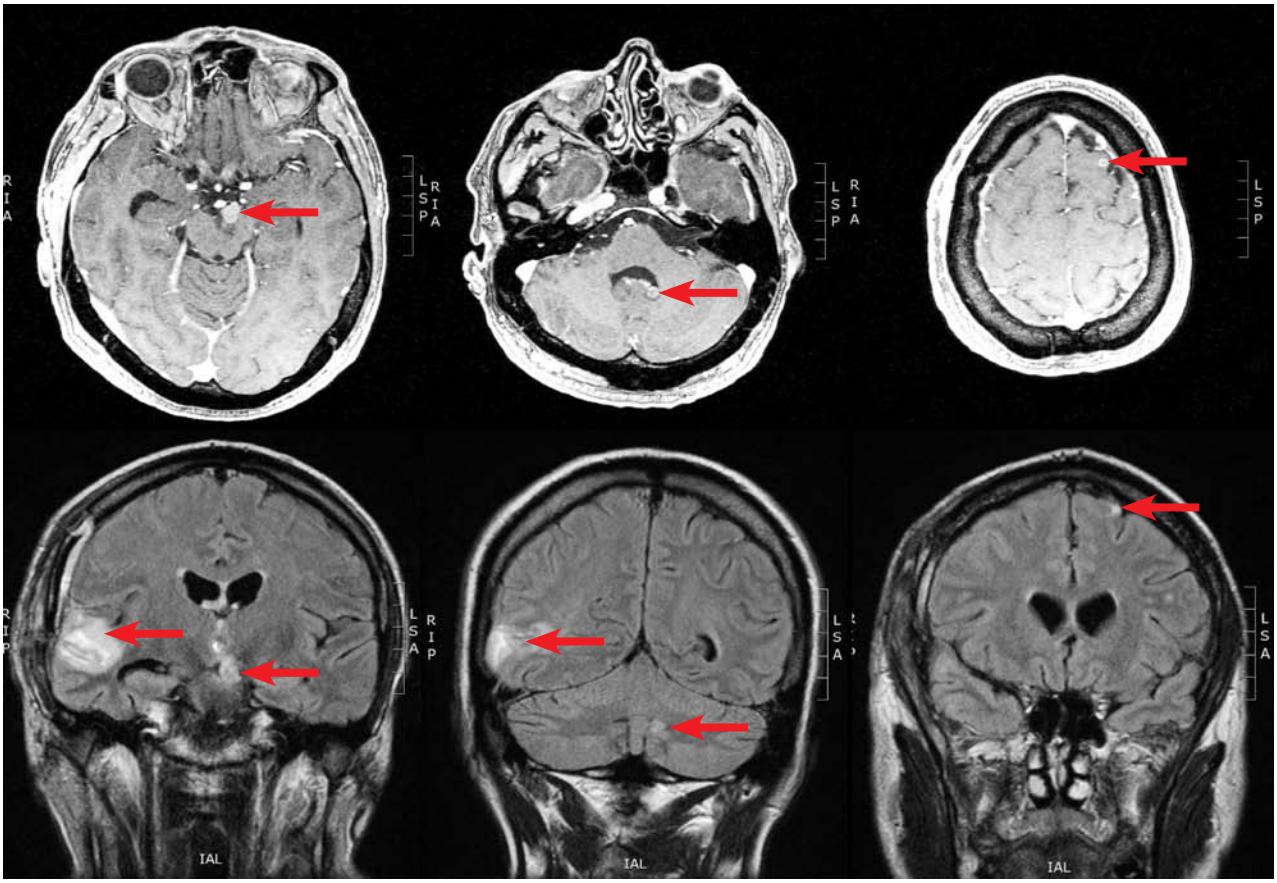
Elective neck irradiation is a viable treatment option for the patients at risk for nodal micrometastases who are not candidates for sentinel lymph node biopsy.[62,73] In a retrospective series of 157 patients with high risk cutaneous melanoma of the head and neck for lymph node involvement (stages I or II), elective regional radiotherapy was found effective and safe treatment option. After a median follow up of 68 months, the disease recurred in the neck lymph nodes in 15 patients and distantly in 57 patients.[73] However, in the sentinel lymph node dissection era, this particular indication is less relevant.

Palliative radiotherapy

The primary goal of palliative radiotherapy is to reduce signs and symptoms related to the disease and improve quality of patients' life; eventual prolongation of her/his life is in the second plane.

Palliative RT is to be introduced whenever surgery is not possible (*i.e.* technically unresectable tumors, poor general condition of the patient) or is deemed ineffective (*i.e.* multiple metastases, particularly when occurring in different organs). In general, all types of metastases or metastatic sites can be irradiated, including cutaneous, lymphatic, brain, bone, and visceral lesions. The effectiveness of radiotherapy in palliative setting is primarily dependent on tumor burden and site. According to the results of *in vitro* studies, cells from metastatic lesions are more radioresistant than those from primary tumors.[74]

Whereas more than 85% complete response rate could be expected after irradiation of small-size (*i.e.* ≤1 cm in diameter) cutaneous lesions, the frequency of complete response is less than 30% in tumors of 5 cm in diameter or larger.[7] Single-shot or fractionated radiotherapy of bone metastases results in complete or partial pain relief at one month after the completed therapy in more than 65% of cases.[75] In the case of impending or known patho-



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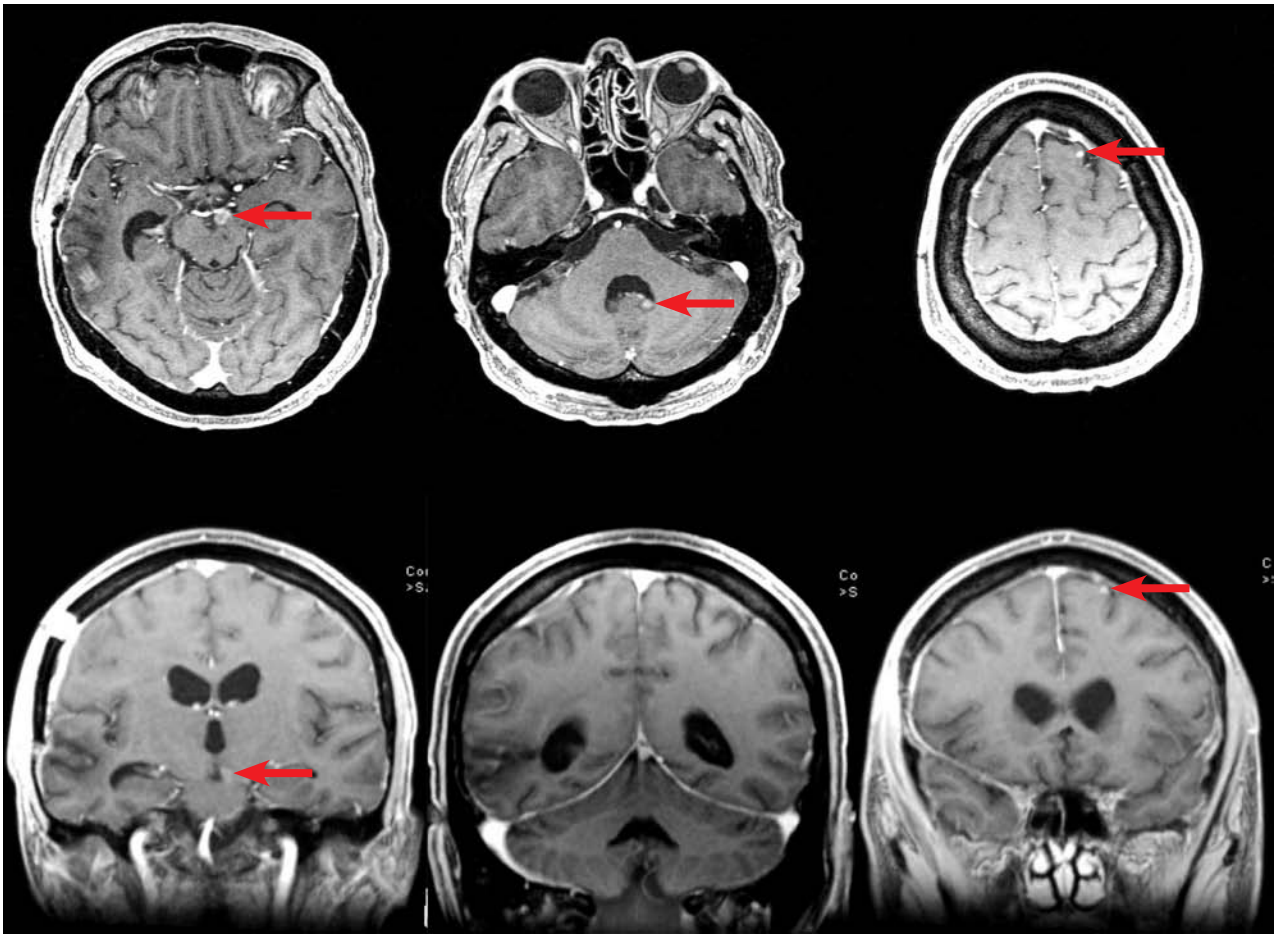

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logic fracture, a combination of adjuvant irradiation that follows upfront surgical intervention resulted in immediate pain relief and prolongation of disease-free interval. Surgical treatment of existing bone fracture is indicated when the expected survival exceeds 6 weeks and the patient's condition permits operation, when no greater benefit from nonoperative treatment is expected, when internal stability can be obtained and when early mobilization is possible. The criterion for choosing between radiotherapy and combined treatment approach when impending fracture is diagnosed is metastasis in weight-bearing bones of a diameter >2-3 cm or with cortical destruction of >50%.[75]

Patients with brain metastases are usually referred to whole brain radiotherapy (WBRT), whenever their number or location excludes surgical intervention or stereotactic radiotherapy. In combination with corticosteroids, WBRT resulted in life prolongation for uninspiring 1-2 months[76], whereas an improvement of performance status, at least temporary, could be expected in 60-70% of patients.[17] In a recent report on 686 melanoma patients with brain involvement, supportive carealone resulted in a median survival of only 2.1 months, WBRT 3.4 months, neurosurgery 9.7 months and combination of surgical resection and WBRT 8.9 months.[77] For patients with lower number of metastatic lesions (usually ≤3) and of maximal diameter between 2.5-3 cm, stereotactic radiosurgery, either with linear accelerator or gamma-knife-based, represents a comparative alternative to surgery (Figure 2).[78] In this clinical scenario, local control in the range of 90% with sporadic long-term survivorships can be expected, whereas in the majority of patients treated with stereotactic irradiation the prevailing cause of death is progression of extracranial disease.[77,78] Recently, as no difference in local control or survival was found when WBRT and stereotactic radiosurgery versus surgery plus WBRT and a boost were compared, the less invasive of the two combinations, WBRT and stereotactic irradiation, was recommended as a treatment of choice for the patients with one or two brain metastases.[79]

In metastases causing spinal cord compression, radiotherapy can be used as a single modality (in conjunction with high dose corticosteroids) or in combination with surgery to reverse neurological impairment or to prevent further loss of motor functions.[80] The decision on the use of upfront surgery versus radiotherapy alone depends on the assessment of neurological deficit, mechanical instability, radioresponsiveness and extent of malignant disease, patient's performance status and comorbidities. Combined treatment offers good chance for pain relief and restoration of affected neurological functions as well as delay in tumor regrowth and prolongation of symptoms-free period.[80,81]

Radiotherapy regimens and techniques

As the best radiotherapy regimen for melanoma remains undetermined, fractionation pattern should be in line with treatment intent and adapted to treated patient: *i.e.*, anatomical localization and extent of radiation volume/target, life expectancy and convenience for the patient, taking into account her/his performance status and preferences. All existing radiotherapy armamentarium can be used when irradiating melanoma, from simple kilovoltage machines or telecobalts to sophisticated linear accelerators, tomotherapy units or cyber-knife.

FIGURE 2. Stereotactic radiosurgery. This radiotherapy technique is characterized with maximal accuracy and is used for focal irradiation of small brain lesions (usually up to 3 tumors of 3.5 cm maximal diameter). After rigid fixation of the head with specific frame, several small beams coming from various directions are focused on one spot inside of the target, creating a steep dose gradient on periphery of the target. Tumor doses in the range of 16-25 Gy are prescribed on 80% isodose encompassing the lesion, whereas 1-2 mm from the edge of the target, the dose drops to 20-30% of its prescribed value. Local control is in the range of 90% and the prevailing cause of death is progression of extracranial disease.

In January 2009, a 59-year-old male with melanoma, diagnosed 4 years earlier, presented with 4 metastatic lesions in the brain. On PET-CT, two additional metastases were identified elsewhere in the body, occupying the third lumbar vertebra and the musculature of the posterior abdominal wall. T1-weighted post-contrast MRIs revealed a lesion of 30x20 mm (long arrow) in the right temporoparietal region, a smaller one in the left half of the pons (short arrow), a 6 mm lesion in the left frontal lobe (thick arrow) and a 7 mm lesion in the left cerebellar hemisphere (arrowhead) (Figure 2A). The patient was treated with surgical resection of the large temporoparietal metastasis, whole brain irradiation (10 x 3 Gy), temozolamide and stereotactic radiosurgery of other three (smaller) brain metastases with the irradiation doses to 80% isodose of 20 Gy (the lesion in the frontal lobe) and 18 Gy (the lesions in the pons and cerebellum). Four months after the procedure, the size of all three irradiated tumors was reduced and no new lesion was identified in the brain (Figure 2B). In September 2009, disease progression was recorded after detecting a metastasis in the spinal cord which was treated with surgery, postoperative irradiation and chemotherapy. No progression of treated brain metastases occurred so far (January 2010, 11 months after stereotactic radiosurgery).

At the Institute of Oncology Ljubljana, in melanoma patients irradiated with curative intent, the choice of fractionation pattern and total dose is governed mainly by the region to be irradiated. If there is no particular risk for lymphedema, *e.g.* targets on the trunk or neck region, higher fraction doses are used (4-6/fx Gy), although, owing to the risk of subcutaneous fibrosis particularly on the neck, lower fraction doses are sometimes preferred. In other clinical scenarios (axilla, groin), more conventionally fractionated radiotherapy regimens are implemented (1.8-2.5 Gy/fx). In palliative radiotherapy, smaller number of higher daily doses is usually employed (4-8 Gy/fx). The complexity of treatment plans, including the number of beams implemented, beam shaping and irradiation techniques (simple 2D, 3D-conformal, intensity modulated, image-guided) is also adjusted to the treated region and treatment intent.

For macroscopic disease, curative dose should be in a range of 66-70 Gy (equivalent dose, *i.e.* when conventionally fractionated with 2 Gy/day and 5 fractions/week, α/β = 2 Gy; Jones *et al.*[14]). Radiotherapy dose prescribed postoperatively to the operated side of the neck should be in the range of ≥60 Gy[22,41,47,61-63,68,70,71], although a favorable outcome was also reported with lower doses.[21-24] For irradiation of axillary and inguinal nodal basins, a total dose of 50-55 Gy, causing a tolerable profile of irradiation induced side-effects, is used as recommended.[24,44] In palliative setting, the radiotherapy doses are usually lower (equivalent dose 24-50 Gy).

The stereotactic technique is a valuable option for clearly defined subset of patients with brain metastases. It based on rigid fixation of the head with specific frame, allowing more accurate positioning of the head (and tumor – target) in 3-dimensional space compared to non-stereotactic conditions. Several small beams coming from various directions are focused on one spot inside of the target, creating a steep dose gradient on periphery of the target. Tumor doses in the range of 16-25 Gy are prescribed on 80% isodose encompassing the lesion, whereas 1-2 mm from the edge of the target the dose drops to 20-30% of its prescribed value (Figure 2).

Conclusions

Basic treatment modality in melanoma is surgery. However, whenever surgery is not radical or there are adverse prognostic factors identified on histopathological examination of resected tissue specimen, it needs to be supplemented. Also, in patients with unresectable disease or in those not being suitable for major surgery or who refuse proposed surgical intervention, other effective mode(s) of therapy need to be implemented. From this perspective, supported by clinical experiences and literature results, radiotherapy is a valuable option: it is effective and safe, in curative and palliative setting. However, the highest benefit in terms of best achievable disease control rates and, simultaneously, minimal treatment-related toxicity is obtainable when modern radiotherapy equipment and techniques are used and indications for irradiation are followed consistently, on patient-to patient basis.

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review

Genetic markers in oligodendroglial tumours

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Background. Oliogodendrogliomas are brain tumours composed of the cells resembling oligodendrocytes. They represent the third most common glial tumour, comprising 2.5% of all primary brain tumours and 5-20% of all gliomas. **Conclusions.** Oligodendroglial tumours with 1p and 19q loss demonstrate a better overall prognosis due to more indolent clinical behaviour and higher sensitivity to treatment. Additionally, 1p and 19q loss is a marker of clinical utility, helping to assess tumour sensitivity to chemotherapy and harbouring the potential for improving the diagnosis and survival of oligodendroglioma patients as well as future clinical practice.

Key words: oligodendroglioma; genetic markers; chemotherapy; prognosis

Introduction

Over the past decade, a remarkable progress in the aspects of cancer treatment was made.[1] Advances in imaging methods, molecular biology, surgical and chemotherapeutic techniques and radiation delivery have all improved the prognosis of cancer patients more than ever before.[1-6] Despite optimal diagnostics and treatment, cancer burden in the society is still ranking too high.

After cardiovascular disease, cancer is the second leading cause of morbidity and mortality worldwide.[7] The most frequent brain tumours are gliomas. According to cell morfology they may be divided into astrocytomas, oligodendrogliomas, ependymomas and oligoastrocytomas.[4,8] Due to a favourable response to chemotherapy, oligodendrogliomas have gained much interest in the past decade. They may be additionally subdivided into prognostic subgroups according to the histopathology, molecular and biological characteristics. The genetic alterations in tumour cells, together with clinical and histolpathological properties, may all define the most appropriate therapy and predict the outcome of the treatment.[4,9]

Epidemiology and aetiology

Oliogodendroglial tumours arise from oligodendroglial cells or immature glial precursors and represent the third most frequently encountered type of glial tumours, ranking after glioblastoma and astrocytomas. They constitute 5-20% of all gliomas and 2.5% of all primary brain tumours.[4,9-11] About 1500 new cases are diagnosed in Europe, and in Slovenia there are about 20 cases each year.[12,13] The annual incidence rate of oligodendrogliomas is two to four per 1,000,000 people and the incidence is increasing every year.[4,9] It has significantly increased over the past years, but this may primarily be due to the use of additional diagnostic criteria in recent years.[4,9,11,12]

Although oligodendrogliomas may occur at any age, there is a peak incidence between 40 and 45 years.[4,11] In spite of, the brain tumours in children are very frequent[14], oligodendrogliomas are rare and representing 2% of all brain tumours in patient younger than 14 years.[11] Male-to-female ratio is 1.5 to 1. In males, peak incidence was described between 45 to 49 years and in females between 55 to 59 years.[4,9] In younger patients, low grade oligodendrogliomas predominate. Familial clustering of tumours was found, with neither genetic factors nor special pattern of inheritance.[15-17] Moreover,

causes for oligodendroglioma evolvement are unknown, no lifestyle or environmental factors are discovered, even though rare individual cases of oligodendroglioma in patients previously irradiated for other reasons have been documented.[9,11,15,18] More than 90% of oligodendrogliomas arise supratentorially in the cerebral white matter, predominantly in the frontal lobes, but patients have been reported with oligodendroglima in basal ganglia, posterior fossa, or spinal cord. Supratentorial locations according to tumour frequency are as follows: 55% in frontal lobes, 47% in temporal, 20% in parietal and 4% in occipital lobes.[4,9,17]

Pathology and neurooncology

Oligodendrogliomas exhibit an infiltrative growth pattern, although not to such an extent as astrocytomas.[4,19,20] According to the growth pattern and histological characteristics, two grades of malignancy are distinguished by the WHO: well differentiated oligodendroglioma of grade II and anaplastic oligodendroglioma of grade III.[17,19,21] The latter may evolve from a low-grade oligodendroglioma, becoming gradually more anaplastic over time, or present *de novo*, without a preceding low-grade tumour. There are 77% of low-grade and 23% of anaplastic oligodendrogliomas.[4,9,21] According to the tumour cell morphology, two types have been described: pure oligodendrogliomas and mixed gliomas or oligoastrocytomas, containing neoplastic cells of oligodendroglial and astroglial phenotype.[4,13,21-23] Oligodendrogliomas may sometimes invade meninges.[4] Very rarely, the tumours may metastasize to other locations, such as lung, liver, bone and cervical lymph nodes.[24,25] Although extremely rare, metastatic disease is encountered more frequently due to the improved survival of oligodendroglioma patients.[4,9]

Because no specific immunohistological markers for oligodendrogliomas exist, the histological diagnosis may be challenging for a pathologist.[9] However, a chromosomal alteration has been reported, which is the most common lesion found in oligodendroglial tumours and involves a deletion at chromosomal loci 1p and 19q. A combined loss of 1p and 19q identifies a group of good prognosis tumours and has been reported, depending on the literature, in the range of 50% to 90% or 60% to 70% of oligodendrogliomas of any grade. On the other hand, the incidence of either 1p or 19q deletions alone is 75%.[4,9,17,26,27]

Tumour signs and symptoms

The symptoms of oligodendroglial tumours are similar to other primary and solitary secondary brain neoplasms[28,29], with epileptic seizures being the most common symptom, presenting in 35% to 85% of patients.[4] Seizures may be generalised, simple or complex partial, or a combination of these. They may be experienced for a number of years before the diagnosis.[30] Other symptoms include headaches, sensory and motor disturbances in terms of localised limb weakness, sudden or insidious change in personality and mood, visual complaints, nausea and dizziness. Symptoms usually precede the definitive diagnosis for 2.9 months to 5 years.[4,31,32]

Oligodendroglioma treatment

There are three therapeutic modalities for the treatment of oligodendrogliomas that are connected and combinable: surgery, radiation therapy and chemotherapy.[4,9] All three are often used successively. Surgery remains a most frequently employed method both in order to perform a tumour reduction or a gross resection where possible and to obtain tissue samples for the definite diagnosis.[9,33] The resection decreases the tumour mass effect on the brain with concomitant neurological consequences and reduces the tumour load during radiotherapy, which is the next and often the following form of the treatment in grade 3 tumours.[24,35] Radiotherapy is used due to an invasive nature of tumour growth where a deep infiltration of tumour cells cannot be determined during surgery and, therefore, prevents a complete removal. As a consequence, the disease relapses slowly but inevitably.[35,36] A tumour relapse after a removal, which was not possible to be detected by clinical means, is termed a recurrence. It takes place at the operative site in the form of a high grade tumour, an anaplastic oligodendroglioma or even glioblastoma. While low-grade tumours may recur after many years, anaplastic ones tend to do so sooner.[4]

The third option of the treatment is chemotherapy, which is being widely used, again for grade 3 tumours. The most frequently employed agents are procarbazine, vincristine, and lomustine (CCNU) (PCV scheme).[37-39] It has been reported that 60% to 75% of patients respond to PCV chemotherapy with 10-32 months of median response duration.[9] Chemotherapy is used as a treatment option and with or without radiotherapy, the latter option in

children, where radiation is usually withheld due to adverse effects on the developing nervous system. Chemotherapy application before radiotherapy is becoming a standard practice also in adults in order to spare the side effects of radiation and to have a second line of the treatment option in case of tumour progression with comparable time to progression and overall survival.[4,37-40]

Besides standard PCV chemotherapy, temozolomide is being widely used as an alternative or supplement treatment both in primary and in metastatic disease.[10,41-43] In comparison to PCV chemotherapy, there are fewer side effects reported and the therapy regimen is more convenient.[38,40,44-46] On the contrary to low-grade oligodendrogliomas, where radiation is delayed until tumour progression, patents with anaplastic oligodendrogliomas receive both radiation and chemotherapy and this combination is superior to either treatment alone.[4,9,34,35] Other chemotherapeutic agents used are carboplatin, cisplatin, etoposide, melphalan, thiotepa and other nitrosourea drugs, as well as interferon-β and recently bevacizumab.[47] A reason for an increase of chemotherapy comes from the observations that low-grade and anaplastic oligodendrogliomas are chemosensitive tumours.

Many genetic abnormalities are encountered in brain neoplasm and many of these identified may emphasize potential diagnostic, therapeutic and prognostic implications.[9,40]

Genetic markers in oligodendrogliomas

Various genetic markers have been described in connection to oligdendroglial tumours and are briefly discussed below.

Chromosomes 1 and 19

As already stated, abnormalities in chromosomes 1 and 19 are the most significant. 1p and 19q losses are encountered in 80% to 90% of grade 2 and in 50% to 70% of grade 3 oligodendrogliomas.[17] On the contrary, childhood oligodendrogliomas only rarely exhibit chromosomal abnormalities. In adults, in the majority of cases chromosome losses involve the entire long arm of chromosome 19 and are present in connection with losses from chromosome 1p. They were observed to be more common in frontal, occipital and parietal lobes than in temporal lobe tumours.[48,49]

Methylation of MGMT genes

Another common finding in oligodendrogliomas is methylation of DNA regions that code for MGMT genes, present in approximately 93% of cases. The end result is transcriptional silencing of genes responsible for DNA repair enzyme, which may contribute to higher chemosensitivity.[17,49]

Mutations in p53 gene

Mutations in p53 gene are described in 10% to 15% of tumours without 1p and 19q loss.[17] Such tumours arise most commonly in the temporal lobes; histologically they are anaplastic or mixed oligoastrocytomas and express poor chemosensitivity.[50,51] Response rate or efficacy of the chemotherapy treatment was observed only in 33% of patients with p53 mutation and intact 1p and 19q chromosomes, as opposed to tumours with intact p53 gene and 1p and 19q or only 1p mutation, where the response rate was 100%.[17,50]

Growth factors and other genetic abnormalities

Growth factors overexpression includes epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF).[17,52] EGFR overexpression was observed in 50% of oligodendrogliomas; the percentage of other two overexpressed factors is somewhat lower. Other chromosomal abnormalities consist of genetical abnormalities or losses from chromosomes 10q and 9p. They are preferentially encountered in anaplastic oligiodendrogliomas without of 1p and 19q loss.[17,48] Additionally, oligodendrocyte transcription factors, such as Olig 1 and Olig 2 that may be used as markers of oligodendrogliomas, are highly expressed in oligodendrogliomas, as well as in astrocytomas.[17,53,54]

Prognostic and diagnostic value of 1p and 19q abnormalities

Pure oligodendrogliomas show a better prognosis than astrocytomas of the same grade and oligoastrocytomas are prognostically in between the former two.[9] For the management of tumours and for prognostic and therapeutic decisions, it is important to

identify the tumour type correctly.[23] Microscopical appearance, which forms the basis for the distinction of gliomas, is not always as clear as to set the diagnosis directly. It is sometimes particularly difficult to distinguish oligodendrogliomas and oligoastrocytomas. In literature studies, the diagnostic concordance observed in these tumours may range from 52% to 86% among pathologists.[48] This fact necessitated a search for an additional diagnostic tool for the oligodendrogliomas. Two factors influence the difficulties in histopatological diagnostics: (1) lack of a specific immunohistochemical cell marker for oligodendroglial tumours and (2) a variation in tumour microscopic morphology. Genetically, a combined loss of 1p and 19q is typical for oligodendrogliomas and rare in gliomas of other type, while isolated 19q loss occurs in mixed oligoastrocytomas and in astrocytomas.[48,49,55] Chromosome 1p and 19q status may be assessed by a variety of techniques, such as microsatellite analysis, fluorescence in situ hybridization (FISH), genomic hybridization and quantitative polymerase chain reactions.[17,48,55] Besides being a valuable diagnostic marker due to its specificity, it was discovered that 1p and 19q loss also acts as a powerful marker in the prognosis of the disease and as a predictor of chemotherapeutic response and survival.[42,48,56] The follow-up period described in the studies varies from two to five years.[23,57] This is somehow short, when one takes into account the survival period in oligodendroglioma, which varies between four to seven years, depending on the grade. Oliogodendrogliomas harbouring 1p and 19q deletion behave more indolently and respond favourably to PCV chemotherapy and temozolomide as well as to radiotherapy.[42,55] For example, the reported correlation between 1p and 19q loss and PCV regimen in the treatment response ranged from 93% to 100%. Temozolomide as a replacement for PCV therapy, due to a better toxicity profile, showed 46% to 55% response rate to the treatment. Also, time to progression of the disease correlated with 1p and 19q loss.[9,58,59] On the other hand, the therapeutic sensitivity of 1p and 19q-intact tumours is less favourable and the survival is therefore shorter.[58,60,61]

Another factor reported to bear the prognostic significance is o6-methylguanine-DNAmethyltransferase (MGMT), an enzyme involved in DNA repair.[62,63] In many tumours, including gliomas, alterations in DNA may be found, such as methylation of the promoter region and their genes. Methylated DNA is less readily accessible to transcription factors and results in the loss of gene function. As MGMT is one of the key factors in resistance to chemotherapy, hypermethylation inhibits the repair mechanism due to a lower level of the active enzyme.[6,17,58,63] MGMT methylation rates in oligodendrogliomas range from 25% to 85% and were reported to be strongly associated with 1p and 19q loss.[58,59] However, the response rate to chemotherapy and time to progression of oligodendrogliomas were not observed to be in correlation with the degree of MGMT methylation, as is the case with glioblastoma, where promoter methylation correlated with response to the alkylating agent treatment and survival. The cause probably lies in different genes and pattern of promoter methylation, which is present in astrocytic cells.[17,58,62-64]

1p and 19q deletion is a predictive factor of tumour response principally to chemotherapy, and radiotherapy as well.[48,59-61] A number of centres employ evaluation of 1p and 19q status as a laboratory test, which is used in conjunction to clinical status, imaging and patohistological diagnosis for predicting the patient response to the treatment. This enables to tailor the most effective and appropriate therapy for the individual patient.[23,55,58] However, there are still unexplained issues in connection to 1p and 19q loss. To begin with, the genes and their exact functions in the pathogenesis of oligodendrogliomas, located on the long arms of chromosomes 1 and 19, need to be identified for some patients with 1p and 19q intact tumours which respond well to the therapy and vice versa.[9,58] Despite the fact that 1p and 19q status helps in selecting patients with respect to therapeutic regimen, there were no revolutionary improvements in the treatment outcomes.[55] A further investigation is required in order to elucidate the unsolved questions in oligodendroglioma biology.

Conclusions

Oligodendroglial tumours with 1p and 19q loss demonstrate a better overall prognosis due to a more indolent clinical behaviour and higher sensitivity to treatment. The 1p and 19q status acts as a prognostic marker, since its loss is associated with an improved outcome compared to non-1p and 19q deleted oligodendrogliomas and astrocytomas of a same grade. 1p and 19q testing proved to be particularly useful for determining the tumour type in morphologically ambiguous cases, as it acts as a valid marker of classical oligodendroglial tumours, when present. Additionally, 1p and 19q

loss is a marker of clinical utility, helping to assess tumour sensitivity to chemotherapy and harbouring the potential for improving the diagnosis and survival of oligodendroglioma patients as well as future clinical practice.

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research article

CT colonography in detection of colorectal carcinoma

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Background. Diagnostic methods used in screening and detecting colorectal carcinoma are digitorectal examination, faecal occult blood testing, sigmoidoscopy, DNA stool analysis, barium enema, colonoscopy, and as of recently CT colonography. The aim of this study was to establish diagnostic accuracy and comfort of CT colonography compared to colonoscopy and barium enema.

Patients and methods. We included 231 patients in the prospective study. For all patients CT colonography and barium enema followed by colonoscopy were performed. After the procedures a comfort assessment was done in all patients. Diagnostic positive results were verified by the pato-histological examination. Sensitivity, specificity, positive predicative value (PPV) and negative predicative value (NPV) were calculated for each procedure.

Results. With CT colonography, barium enema and colonoscopy 95 lesions were found, 56 (59%) of them were tumours and 39 (41%) were polyps. Among polyps pato-histology revealed 34 adenomas, 3 tubulovillous adenomas and 2 lipomas, among tumours there were 55 adenocarcinomas and 1 lymphoma. Results showed CT colonography sensitivity to polyps to be 89.7%, barium enema 48.7%, and colonoscopy 94.9%. Sensitivity to tumours of CT colonography and colonoscopy was 100% and of barium enema 94.6%. Specificities and PPV were 100% in all procedures. The comfort assessment showed CT colonography as the far most comfortable out of three procedures.

Key words: CT colonography; barium enema; colonoscopy; colorectal polyp; colorectal carcinoma

Introduction

Colorectal carcinoma (CRC) is the second leading cause of illness and the third leading cause of death in Western countries.[1] Pato-histologically (PH) CRC is most commonly adenocarcinoma in 98% of cases. CRC starts as a polyp, representing precursor of CRC. Consumption of meat and animal fats, physical inactivity, smoking and consumption of alcohol increase the risk for CRC.

Prevention and screening of CRC are very complex and depend on financial and organizational capacities of health institutions where they are performed. There are several basic tests applied in the screening of CRC: digitorectal examination, faecal occult blood testing (FBOT), sigmoidoscopy, colonoscopy, barium enema, DNA stool analysis and recently CT colonography (CTC).[2,3]

We conducted this comparative study to establish the diagnostic accuracy and comfort of CTC comparing with C and barium enema.

Patients and methods

Of 231 patients included in the study 106 (47%) were males and 125 (53%) were females. The average age of patients was 57.9 years (SD ± 11.3y, range 23-83y). Only patients with suspected symptoms of CRC were included with the history of blood in the stool, anaemia, constipation, and changes in the stool or positive FBOT test.

In all patients CT colonography, barium enema and colonoscopy were performed. Positive diagnostic findings were correlated with PH results of biopsies taken during colonoscopy. Two hundred

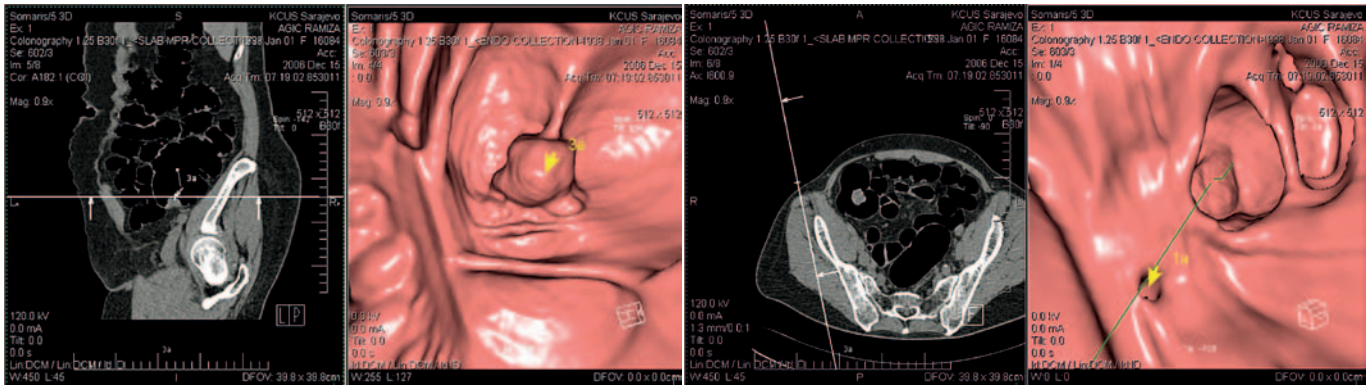

FIGURE 1. Polypous adenoma of cecal region in a 55 years old female patient, obtained by our CTC evaluation.

TABLE 1. Age distribution of patients with positive hystologic findings

Age	Polyps		Tumours	
	n	%	n	%
20-30	4	10.26%	0	0.00%
31-40	5	12.82%	1	1.79%
41-50	10	25.64%	4	7.14%
51-60	10	25.64%	19	33.93%
61-70	9	23.08%	11	19.64%
71-...	1	2.56%	21	37.50%
Total	**39**	**100.00%**	**56**	**100.00%**

and twenty-seven patients were included in the statistical analysis; four patients were excluded due to undetermined PH results.

An identical protocol for cleansing the bowels (Dulcolax® tablets and suppositories, as well as Coloclens® syrup) was performed before the commencement of each of three procedures. The CTC procedure was performed after the air had been insufflated in the cleansed colorectal region until an optimal extension, with an intravenous application of spasmolytics. Patients with intraluminal residual content or suboptimal distension of the bowels were excluded from the study so that reliable images could be achieved. CT scanning was performed on 4 slice MDCT (Volume zoom Siemens, Erlangen, Germany) equipment in the prone and supine position of the patient. 2D and 3D reconstructions were performed on the «Syngo» software work station.

Double contrast barium enema was performed on an X-ray diascopic equipment (Practix 100, Philips, Aidhoven The Netherlands). The colonoscopy procedure was performed by a gastroenterologist on Videocolonoscopic device (CF Q-165 L Olympus» Tokyo Japan). PH examination was done on the tissue obtained by polypectomy or tumour sample that was taken either during an endoscopic examination or a surgical procedure.

In relation to PH findings sensitivity and specificity as well as PPV and NPV, using Kappa statistical method for all three procedures were calculated. All hypotheses were tested for the statistical significance of $p < 0.05$ value. Confidence intervals (CI) were also presented. Patients self evaluated comfort of all three procedures as being comfort, less comfort or discomfort.

Results

The histological examination was conclusive in 227 patients. There were 39 benign lesions in 31 patients and 56 malignant lesions in 56 patients. Benign lesions were present among females in 22 cases (56%), and in males in 17 cases (44%). In male patients tumours were found in 30 cases (54%), and in the females in 26 (46%). Age distribution of the patients regarding benign lesions and tumours is presented in Table 1 (Table 1). The most common symptoms in the case of polyps were: bowel disturbances in 14 cases, constipation in 14 cases, blood in the stool in 7 cases, followed by anaemia and abdominal pain each in 1 case. In case of tumours, most commonly reported symptoms were: blood in the stool in 35 cases, anaemia in 11 cases and constipation in 10 cases.

Most polyps were detected in colon descedens, followed by rectum, colon transversum, caecum and colon ascendens. The most frequent localization of carcinoma was rectum in 27 cases followed by sigmoid part of colon in 13 cases while the descedent part of colon in 5 cases. In the remaining nine cases, carcinoma was found in colon ascedens, transversum and caecum, three cases in each of

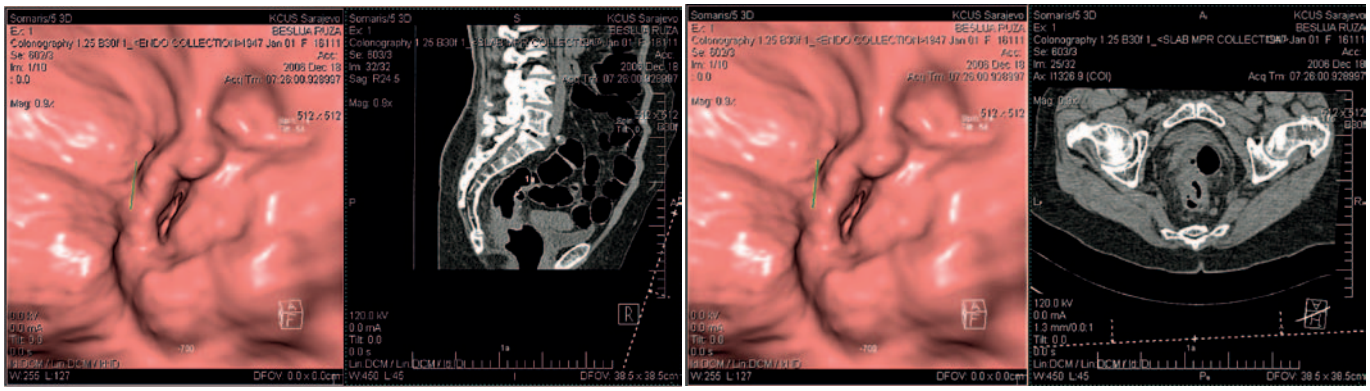

FIGURE 2. Adenocarcinoma in the middle third part of the rectum with infiltration of mesorectal fat tissue and reactive lymph nodes, obtained by our CTC evaluation.

TABLE 2. Comparison of results regarding all three methods

	Polyps			Tumours			All		
	CTC	BE	CC	CTC	BE	CC	CTC	BE	CC
Sensitivity	89.7%	48.7%	94.9%	100.0%	94.6%	100.0%	95.8%	75.8%	97.9%
Specificitiy and PPV	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

these localisations. In one case carcinoma was located on hepatic flexure whilst a single case of lymphoma was located on Valvula Bauchini.

With the CTC procedure the size of benign lesions (polyps) detected was: less than 6 mm in 2 cases, 6 to 10 mm in 15 cases and larger than 10 mm in 18 cases. The size of carcinomas detected by CTC was more than 10 mm in all 56 cases (Figure 1).

Barium enema detected benign lesions between 6 and 10 mm in 2 cases and in 17 cases larger than 10 mm. This procedure did not detect any polyps smaller than 6 mm. With BE 53 carcinomas were found, all of them were larger than 10 mm.

Colonoscopy detected benign lesions smaller than 6 mm in 6 cases, 6 to 10 mm in 9 cases and larger than 10 mm in 22 cases. The size of carcinomas detected by colonoscopy was larger than 10 mm in all 56 cases.

Amongst polyps, there were 34 adenomas, followed by tubuloviluous adenomas in three cases and lipomas in three cases. According to PH analysis adenocarcinoma was far most common (in 98% of cases, n=55), since there was only a single case of lymphoma. (2%). (Figure 2). In all 231 patients CTC side findings were found in 25 cases and extracolic extension in 36 cases.

Sensitivity, specificity and PPV for all three procedures are presented in Table 2. We obtained statistically significant results validating CTC procedure regarding sensitivity and specificity on polyps and tumours, which are approximately identical in comparison with colonoscopy, and significantly above the method of barium enema. It is important to point out that the CTC method missed to locate 4 polyps which were found by colonoscopy, but did not miss any tumours. Out of 22 cases which were missed by barium enema, all 22 were located by colonoscopy, and 19 by CTC. 3 of those cases were carcinoma and all were diagnosed by both colonoscopy and CTC. Colonoscopy did not miss any carcinoma; however it missed two polyps located by CTC. Both were later confirmed by colonoscopy and PH; however, it did not miss any tumours. To evaluate staging of carcinoma, we used Dukes method of clinical staging and achieved conformity in 96.4% of cases in comparison with the post-surgical oncology staging.

In a survey of all examinees in our research, the CTC procedure was assessed as the most comfortable in comparison with the barium enema and colonoscopy; all 231 patients assessed the CTC procedure as comfort. Barium enema was assessed as a less comfort procedure by 224 of patients, and as discomfort by 7 patients. CC was assessed as the least comfort procedure by 224 patients, assessed it as discomfort, whilst 7 patients assessed it as less comfort.

Discussion

In recent years there has been an extremely rapid development of CT due to the development of CT multislice technology. Its more frequent use in detecting CRC is also due to the fact that it has not yet been established an optimal procedure regarding comfort and high reliability in detection of colorectal lesions.[1]

The CTC could become an important method in CRC and polyps screening due to its efficacy, cost-effectiveness and because it is an ultra-low dose radiation technique.[4,5] The more recent method is MR imaging. However, it is usually used in the diagnostic of colorectal lesions and not in the screening proceeding.[6] The most significant advantage of CTC is that it can detect the extraluminal tumour extension, which is not possible by other procedures.[7] It is extremely important for discovering the extent of the disease and enabling the proper choice of the treatment. On that way we can influence on better surviving and quality of life of our patients.[8,9] Regarding comfort, the CTC procedure is undoubtedly in advance compared to other two procedures. It is also much safer, although the colorectal injuries during barium enema are very rare.[10] In our research the comfort of the procedure was assessed as being 100%. Gluecker published that 72% and Svenson stated that 82% of patients would rather have a CTC than any of the other two procedures.[11,12]

There are many reports regarding detecting benign and malignant colorectal lesions in the literature. Winawer published the lowest result regarding sensitivity of barium enema in detecting polyps to be 48%.[13] Smith reported sensitivity of barium enema in detecting tumours as 83% and of colonoscopy as 97.5%.[14] Hara stated that sensitivity of CTC for polyps larger than 1 cm was 75%, or 85% in a follow up study.[15] Fletcher reported that sensitivity of CTC for polyps larger than 1 cm was 85%.[16] Gennen published that sensitivity of barium enema regarding carcinoma is in the range of 85-95%, and that sensitivity in detecting polyps smaller than 1 cm is between 50-80%.[17] Johnson published that sensitivity of CTC to polyps larger than 1 cm was 81%, and of barium enema of 45%. For those smaller than 1 cm, sensitivity of CTC was 72%, compared to barium enema which was 44%. Specificities of CTC were 96-99%, compared to 99-100% of barium enema.[18] Cotton's multicentric study included 600 participants and showed that sensitivity of CTC to lesions smaller than 6 mm was 39%, to those larger than 1 cm was 96%.[19] Macari reported 100% sensitivity of CTC regarding polyps larger than 1 cm, and 52.9% regarding those between 6-9 mm.[20] Iacanconne found 100% sensitivity of CTC regarding polyps of 1 cm and larger, and 86% for those smaller than 6 mm, which is slightly more compared to colonoscopy, which was 84%.[21] Mulhal reported 48% sensitivity of CTC regarding polyps smaller than 6 mm; 70% for those between 6-9 mm; and 85% for those larger than 9 mm.[22] Ramjii reported sensitivity of 71-93% for polyps larger than 1 cm, 55-71% for those between 5 and 9 mm, and 39% for those smaller than 6 mm.[23] In 2008, Johnson acquired 90%sensitivity of CTC for polyps larger than 9 mm.[24]

In our research CTC was equally sensitive (100%) in detection of CRC lesions as colonoscopy and much better than barium enema (94.6%). The CTC demonstrated similar sensitivity in detecting polyps larger than 1 cm (89.7%) compared to the colonoscopy (94.9%), and better sensitivity compared to barium enema (sensitivity of 48.7%). The CTC is very efficient in pain-intolerant patients and in cases of tumours causing obstruction, dolichocolons, spasms, and other reasons preventing the colonoscope to reach the caecum. The CTC is suitable for screening and staging of tumours, as well as for obtaining unexpected findings on other abdominal or pelvic organs. In the detection of lesions smaller than 5 mm, colonoscopy showed to be better in regard to other two methods.

Having considered all results of our study and having compared all three procedures, we have obtained statistically significant differences regarding sensitivity and specificity of CTC regarding polyps and tumours. These results are quite similar to those compared to colonoscopy, but much more advanced compared to the barium enema. We could state here that our results regarding sensitivity of CT colonography are much better compared with the results of initial studies published in the early nineties in the world, and are quite close to the results of the latest studies published at the beginning of this century.

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research article

Diffusion weighted MR imaging in the differential diagnosis of haemangiomas and metastases of the liver

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Background. The purpose of the study was to evaluate the value of diffusion-weighted imaging in the differential diagnosis of haemangiomas from metastases of the liver.

Patients and methods. We analyzed 69 lesions in 38 patients (33 haemangiomas; 36 metastases) in the retrospective study. Diffusion-weighted imaging was performed using a breath-hold single-shot echo-planar spin echo sequence with three b factors (0, 500 and 1000 sec/mm^2), and apparent diffusion coefficients (ADCs) were calculated. For the quantitative evaluation, signal intensity of the lesions, lesion-to-liver signal intensity ratios, ADC of the lesions, and lesion-to-liver ADC ratios were compared between the groups. The statistical significance was determined by student's-t test.

Results. With the b factor 500 sec/mm^2, no statistical significance was achieved ($p>0.05$). With the b factor of 1000 sec/mm^2, both the signal intensity and lesion-to-liver signal intensity ratio of the metastases were significantly higher than those for haemangiomas ($p<0.001$). The cut-off value at 2.6 yielded a sensitivity of 86% and a specificity of 82% for the lesion-to-liver signal intensity ratio. The ADC, and lesion-to-liver ADC ratio of the metastases were significantly lower than those of haemangiomas ($p<0.001$). With cut-off value of 1.7, ADC ratio had a sensitivity of 88% and a specificity of 72% for ADC lesion/liver.

Conclusions. Diffusion-weighted imaging with high b value may help in the differential diagnosis of metastases from haemangiomas of the liver.

Key words: liver; haemangioma; metastasis; magnetic resonance imaging; diffusion-weighted imaging, apparent diffusion coefficient.

Introduction

A liver lesion detected in a patient with the known malignant disease requires a further assessment, as the liver is common site for metastatic spread and haemangiomas are encountered in about 7-20% of the population.[1] The radiologic imaging plays a critical role in the differential diagnosis of these lesions. On postcontrast computed tomography (CT) and magnetic resonance (MR) images, most haemangiomas have a typical enhancement. However, atypical haemangiomas may imitate metastases. The differential diagnosis of these lesions is essential to determine the therapy.[2] A variety of radiologic imaging is currently available for the clinical use in these cases.[3] CT arterioportography has been widely used in the differential diagnosis, however, this technique is invasive and the results are not always reliable.[4] Over the years, the success rates have increased with the development of new MR contrast agents such as superparamagnetic iron oxide (SPIO).[3] However, we may encounter some problems in interpreting SPIO-enhanced MR images because of the difficulty in differentiating thin vessels, small cysts, haemangiomas, and metastases.[5] Therefore, a non-invasive method is required in the diagnosis of such lesions.

In this study, we evaluated the contribution of diffusion-weighted (DW) imaging in the differentiation of metastases from haemangiomas, particularly in a patient with the known malignant disease, which poses a challenge in the differential diagnosis.

Patients and methods

Patients

Our retrospective data were obtained in a 14-month period (September 2007 to October 2008). During this period 63 patients were referred for MR imaging of the liver for the following indications: suspected haemangioma or metastatic liver mass based on the findings of other imaging modalities and the evaluation for metastases in patients with known primary cancer. However, 25 patients were excluded from the study because of size (< 1cm) (n = 11), low image quality of DW images (n = 8), and incomplete characterization of lesions on the follow-up imaging or the histopathologic examination (n = 6). As a consequence, a total of 69 solid lesions with a diameter of at least 1 cm in 38 patients (20 women, 18 men) were included in this study. Of these lesions, 36 (in 15 patients) were metastases and 33 (in 23 patients) were haemangiomas. Fifteen patients had multiple lesions (two metastases in 1 patient, three metastases in 3 patients, five metastases in 1 patient, six metastases in 2 patients, two haemangiomas in 6 patients, three haemangiomas in 2 patients). For subjects with more than six lesions only six largest lesions and one region of hepatic parenchyma were analyzed. Imaging was performed prior to the administration of the neoadjuvant treatment or biopsy.

The diagnosis of all metastases was confirmed histopathologically after MR imaging. For subjects with multiple metastases only one lesion was analyzed histopathologically. Remaining similar radiologic appearing lesions were accepted as metastases because all of them increased in size during the radiologic follow-up (4-10 months). The primary cancer sites in each patient were as follows: 3 colorectal cancers, 2 pancreatic cancers, 1 common bile duct cancer, 3 lung cancers, 2 breast cancers, 1 non-Hodgkin's lymphoma, 1 neuroblastoma, 1 endometrial cancer, 1 adenoid cystic carcinoma of the appendix. All patients with a tentative radiological diagnosis of haemangiomas showed no change during the clinical and radiological follow up (US every 3 months for 9-14 months). Five patients with haemangiomas had a primary cancer as following sites: 3 colorectal cancers, 2 breast cancers.

MR imaging

The study was approved by the institutional review board and the protocol review committee.

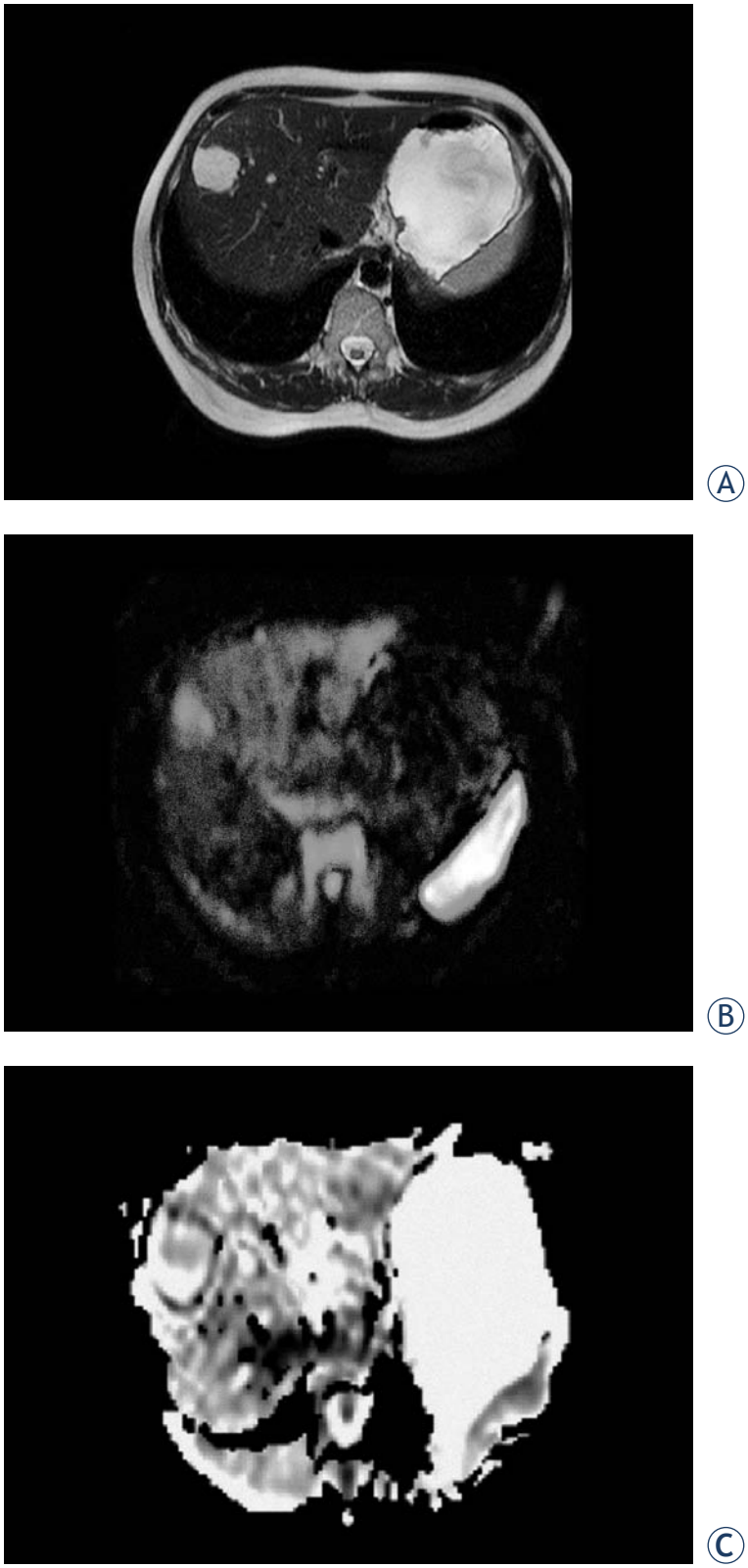

FIGURE 1. 44-year-old woman with a haemangioma of the liver. Axial T2-weighted TSE (A) MR image shows a haemangioma in the right lobe of the liver. This haemangioma appears isointense relative to the liver on the diffusion-weighted image with b factor 1000 sec/mm^2 (B). ADC map. Lesion-to-liver ADC ratio=1.9 (C).

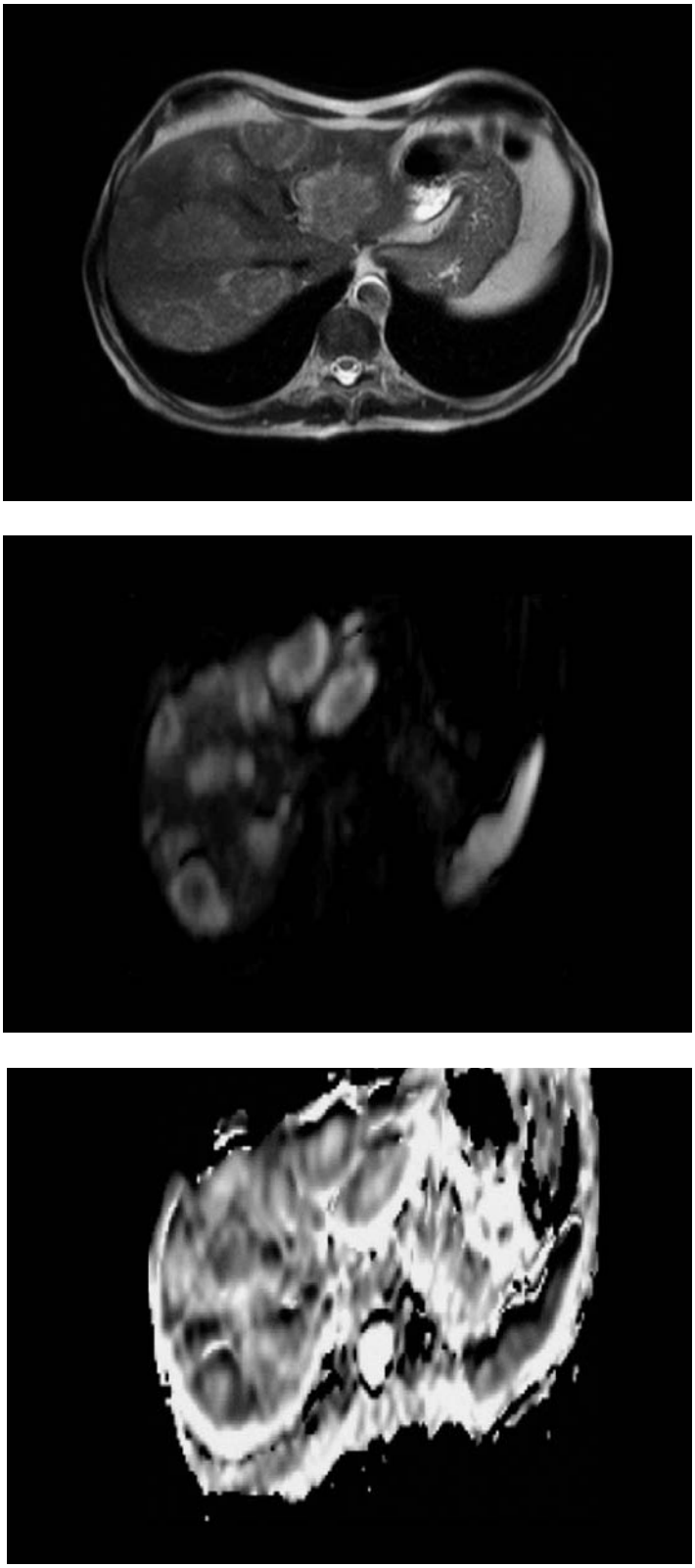

FIGURE 2. 69-year-old woman with multiple metastases of the liver. Axial T2-weighted TSE (A) MR images show multiple metastases in the right and left lobe of the liver. These metastases appear hyperintense compared to the liver on the diffusion-weighted image with b factor 1000 sec/mm^2 (B). ADC map. Lesion-to-liver ADC ratio=1.5 (C).

Since the tests employed were a part of the routine clinical work-up of these patients, the informed consent was not required by the review board.

All patients were examined with a 1.5 Tesla MR scanner (Gyroscan Intera; Philips Medical Systems, Eindhoven, The Netherlands) using a four element phased-array body coil. This system has a maximal gradient strength of 30 mT/m and a slew rate of 150 mT/m/msec. All patients were examined initially with the routine MR imaging protocol for the upper abdomen that included: precontrast axial T1-weighted breath-hold spoiled gradient echo (fast field echo: FFE) with and without fat suppression (TR/TE/FA/NEX:169/4.6/80/1), coronal and axial T2-weighted single shot turbo spin echo (SS-TSE) (TR/TE/NEX/TSE factor: 700/80/1/72), and axial T2-weighted SS-TSE with fat suppression (TR/TE/NEX/TSE factor: 700/80/1/72). Subsequently, 3 series of axial single-shot spin-echo echo-planar (SS-SE-EP) DW images (TR/TE/echo-planar imaging factor: 1000/81/77; sensitizing gradients in x, y, z directions) were acquired using the following b values: 0, 500 and 1000 sec/mm^2. ADC maps were reconstructed from these images. The fat suppression was performed by using a spectral saturation inversion recovery (SPIR) technique. Subsequently, 0.1 mmol/kg Gd-DTPA (Magnevist, Schering, Germany) was administered as a hand-injected bolus in 5 seconds followed by a rapid flush with 10-20 ml of saline. Five dynamic series and an additional late phase (5th min) image were acquired with a T1-weighted breath-hold FFE (TR/TE/FA:169/4.6/80) sequence. MR imaging, including the DWI, consisted of a multisection acquisition with a slice thickness of 6 mm, an intersection gap of 1mm, and an acquisition matrix of 128x256. The field of view varied between 455 and 500 mm. All sequences were acquired using a partially-parallel imaging acquisition and SENSE reconstruction with a reduction factor (R) of 2. The scan time of the acquisition of each DW imaging series during a single breath-hold was 26 seconds.

Image analysis

Quantitative measurements were made using a dedicated work-station (Dell Workstation precision 650, View Forum release 3.4 system). SI of the lesions and liver were measured by one of the radiologists (N.I) for each b factor (0, 500 and 1,000 sec/mm^2) using a region of interest (ROI). The ROI was placed centrally and the size of the ROI was kept as large as possible, covering at least two-thirds of the lesion, yet avoiding the interference from the

surrounding liver tissue and major blood vessels. In addition, the ADC maps were created automatically and the mean ADC values of lesions and liver were determined on images with b factor 0 and 1000 sec/mm^2. The average of three measurements was recorded as the final SI or ADC. SI of the lesions, lesion-to-liver SI ratio (SIR), ADC of the lesions, and lesion-to-liver ADC ratio (ADCR) were calculated.

Statistical analysis

SI, SIR, ADC, and ADCR were compared between the groups. The fitness of numeric data set to normal distribution was determined by Kolmogorov-Smirnov test. The data were normally distributed; hence the differences in SIs, SIRs, ADCs, and ADCRs were analyzed by the student-t test. A p value of less than 0.05 was considered statistically significant. To evaluate the diagnostic performance of the quantitative tests (SIR and ADCR) for differentiating metastases from haemangiomas and to describe the sensitivity and specificity of the tests, the receiver operating characteristic (ROC) analysis was performed. The areas and standard errors for each ROC curve were calculated by the method described by Metz.[6] The area under the ROC curve reflects the performance of the tests. The optimum cut-off point was determined as the value that best discriminates between the two groups in terms of maximum sensitivity and minimum number of false-positive results. All statistical analyses were performed using SPSS (Statistical Package for Social Science) software.

Results

The mean age was 66.9 ± 9.3 years and 45.5 ± 12.5 years for patients with metastases and haemangiomas, respectively. The mean size for metastases and haemangiomas were 44.7 ± 28.4 mm and 38.1 ± 23.2 mm, respectively. 80% of the metastases were found in the right lobe (segment 5 to 8) with the remaining in the left lobe (segments 1 to 4). 69% of the haemangiomas were found in the right lobe (segment 5 to 8) with the remaining in the left lobe (segments 1 to 4).

The results of the quantitative analysis of the DW imaging were reviewed in Table 1. With b factors of 0 and 500 sec/mm^2, no difference of statistical significance was achieved ($p > 0.05$). With the b factor of 1000 sec/mm^2, the SIs and SIRs of the metastases were significantly higher than those of the haemangiomas ($p < 0.001$) (Figures 1B, 2B). The area under the ROC curve was 0.891 ± 0.04 for SIR ($p < 0.001$). With a cut-off value of 2.6, SIR had a sensitivity of 86% and a specificity of 82% (Figure 3A). The ADCs and ADCRs of metastases were significantly lower than that of the haemangiomas ($p < 0.001$) (Figures 1C, 2C). The area under the ROC curve was 0.893 ± 0.04 for ADCR ($p < 0.001$). Setting the cut-off value at 1.7, we found a sensitivity of 88% and a specificity of 72% for ADCR.

TABLE 1. Quantitative analysis of DW imaging

	Metastases (n=36)	Haemangiomas (n=33)	p
SIR (b=1000 sec/mm^2)	3.4 ± 0.9	2.2 ± 0.5	< 0.001
ADC (x10^{-3} mm^2/sec)	1.9 ± 0.4	2.5 ± 0.3	< 0.001
ADCR	1.6 ± 0.3	1.9 ± 0.3	< 0.001

Note. Data are mean ± SD.
*SIR: lesion-to-liver SI ratio; ADC: apparent diffusion coefficients; ADCR: lesion-to-liver ADC ratio.

Discussion

For the differential diagnosis of haemangiomas from metastases of the liver, the sensitivity and specificity are generally superior with contrast enhanced MRI when compared to other imaging modalities.[2] MRI-based techniques are also useful to assess the other hepatic pathology.[7] However, the greatest clinical experience in the differential diagnosis was with non-specific extracellular gadolinium chelates contrasts because they are safe, relatively inexpensive and they also provide the characterization of most of these lesions.[3] However, sometimes non-specific extracellular gadolinium chelates may not allow us to recognize these lesions well. In these patients, new contrast agents (SPIO-enhanced MRI) or new MRI techniques (DWI) must be used, especially in patients with the known primary malignancy. In a report published by Nasu *et al.*[8], the authors compared accuracy of DWI with of SPIO-enhanced MRI in the evaluation of hepatic metastases. In that report it was shown that DWI has more sensitivity than SPIO-enhanced MRI. However, in their study ADC measurement was not performed. In two other reports, the authors compared accuracy of DWI with of SSh T2-W TSE sequences in the evaluation of hepatic metastases. In those reports,

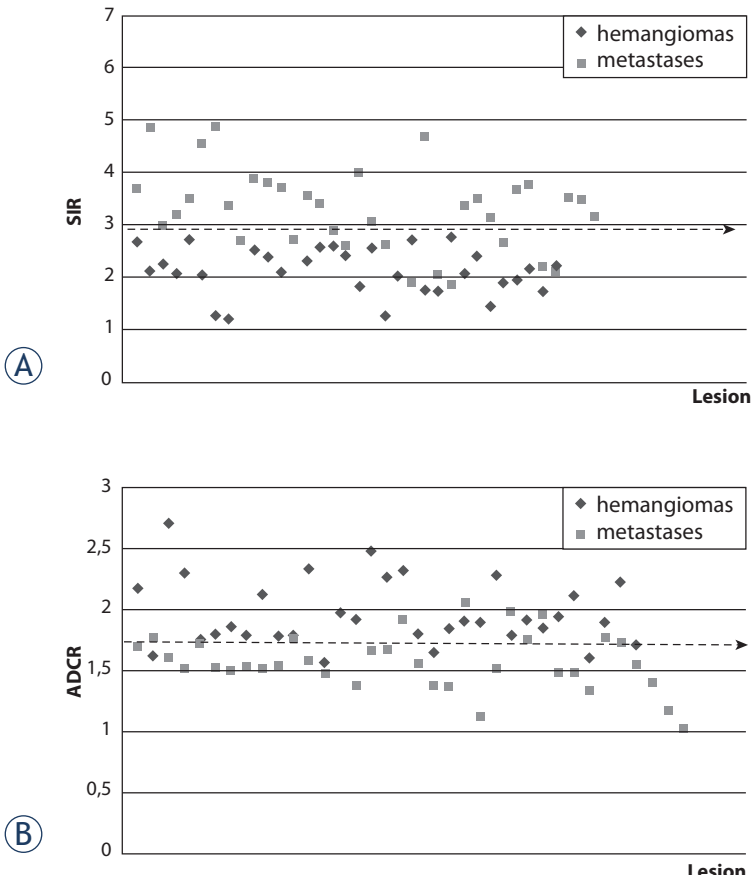

FIGURE 3. Scattergram distribution of lesion-to-liver SI ratios on DW images with b factor 1000 sec/mm² (A) and ADC ratios (B) of haemangiomas and metastases.

although image artifacts were lower with T2-W TSE than SSh-EPI, it is shown that DWI was more useful than SSh T2-W TSE sequences for the detection of lesions.[9,10] However, in daily practice the lesion characterization is as important as the lesion detection.

Recent reports have suggested that DWI with SS EPI may be helpful in the characterization of focal and diffuse liver lesions, with high specificity and sensitivity.[2,8,11-20] Those studies reported that the ADC values in benign lesions (such as haemangiomas and cysts) were significantly higher than those of the malignant lesions (hepatocellular carcinomas, metastases). This difference was attributed to the difference in cellular density. Since malignant tumors often have higher cellularity than benign lesions, the ADCs of most malignant tumors are lower than benign masses. In these previous studies, different imaging parameters were applied to evaluate a wide range of hepatic lesions, including metastases and haemangiomas. The ADC values of both the normal liver and the liver lesions were differed significantly at different b values.[18] Namimoto *et al.*[16] reported a low ADC of the liver with low and high b values (30 and 1200 sec/mm²). On the other hand, high ADCs were reported by Taouli *et al.*[15] using low and intermediate b values (0 and 500 sec/mm²). In a study of Yamada *et al.*[18] in which the b values of 30, 300, 900 or 1100 sec/mm² were used, high ADCs were obtained at low b values. In conclusion, when only a high b value is used, the ADC values reflect the true diffusion of the tissue. On the contrary, when only a low b value is used, the ADC may be influenced by the intravoxel perfusion.[21]

In our study, significant differences between the SIs and SIRs of haemangiomas and metastases were found only on images with a b factor of 1000 sec/mm². At higher b values, the contribution of the T2 shine-through to the signal intensity decreases, while tissue cellularity makes a greater contribution.[22] Hence, the hyperintensity of metastases on b 1000 sec/mm² images can not be totally attributed to the T2 shine-through effect. Diffusion can be quantitatively evaluated by ADC, which is free of the T2 shine-through effect.[23] In our series, the mean ADC of the metastases was significantly lower than that of the haemangiomas. Hence, at least a part of the increase in signal on DW images must have been caused by the reduced diffusion in metastases. Since the cavernous haemangiomas are mainly composed of liquid component which consists of fiber septation, scar, and hemorrhage the ADC of the haemangiomas is increased. On the contrary, the metastases have higher cellularity, hence the lower ADC.[1]

This study has several technical limitations. The main limitation was that the SSh-EPI sequence employed with a higher b value had a lower SNR, resulting in greater image distortion. In addition, the EPI sequence caused anatomic distortion due to susceptibility effects.[22] Although the best lesion conspicuity is achieved with low b value for detecting small focal liver lesions, the best lesion specificity is achieved with a high b value.[22] Because of that reason, we used a high b value for the characterization of lesions. Another important limitation was that there were not any atypical haemangiomas (such as calcified, hyalinised or sclerosed) and cystic metastases in our study. The necrotic metastasis may exhibit the pronounced hyperintensity on T2-W image and less restricted diffusion.

The differential diagnosis of most of the haemangiomas from metastases is usually possible with the combined use of specific radiologic features. However, sometimes the differential diagno-

sis of these lesions may still be difficult. Our preliminary data suggest that DWI with a high b value may be helpful in this setting and it can be easily added to routine liver imaging protocols.

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case report

Percutaneous transcatheter arterial embolization in haemodynamically stable patients with blunt splenic injury

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Background. The nonoperative management of the blunt splenic injury in haemodynamically stable patients has become an accepted treatment in recent years. We present a case of the blunt splenic injury successfully treated by supraselective embolization with microspheres.

Case report. A young hockey player was brought to the Emergency Department with the history of blunt abdominal trauma 2 h earlier. A Grade III splenic injury with haemoperitoneum was diagnosed on sonographic evaluation and the patient was treated with the selective distal splenic artery embolization with microspheres. Postprocedural ultrasound and computed tomography follow-up a year later revealed only a small area of parenchymal irregularity.

Conclusions. The percutaneous splenic arterial embolization has a major role in the management of traumatic splenic injuries. Embolization is particularly beneficial in injuries of grade III or higher.

Key words: splenic trauma; treatment; angiography; percutaneous transcatheter embolization

Introduction

Spleen injuries are most commonly associated with blunt abdominal trauma and represent a potentially life-threatening condition. The management of splenic trauma is still controversial, but there have been major changes over the last three decades. In the past, any damaged spleen was surgically removed to avoid a delayed rupture. The increased susceptibility of the patient to infection after splenectomy – in particular, the risk of overwhelming, potentially fatal postsplenectomy sepsis – motivated physicians to favour splenic preservation procedures.[1] Nonsurgical management (NOM) with bed rest and observation has traditionally been the treatment of choice for the splenic injury in paediatric patients. Although the nonsurgical management of stable blunt splenic injuries in adults has gained popularity in recent years, the initial choice of surgical versus nonsurgical management remains controversial. However, embolization is also wildly used for another indications with much less controversial results.[2,3] The controversy of the splenic arterial embolization has been attributed to the relatively high failure rate of such a treatment (10–31%), with a resultant need for secondary splenectomy, and to the potential of missing other intra-abdominal injures that require laparotomy. The splenic transcatheter arterial embolization (TAE) has been proposed to reduce the risk of nonsurgical management failure in adults and children. Sclafani *et al*. reported a series of cases in which NOM by means of the transcatheter arterial embolization was successful in 91% of hemodynamically stable patients, and the splenic function was preserved in all patients who underwent TAE.[1] The most widely accepted indication for TAE is evidence of the arterial injury on a computed tomography (CT) scan. In cases of the arterial injury, embolization is performed with microcoils or gelfoam particles as distally as possible, in a small arterial branch that supplies the segment

in which the extravasation is detected, to preserve perfusion to the remaining splenic parenchyma.[1,2] We report on a patient with blunt splenic trauma who was successfully treated by the supraselective embolization with microspheres.

Case report

A 20-year-old hockey player presented with blunt trauma to the left upper abdomen. The abdominal ultrasound revealed a small amount of free fluid around the spleen (haemoperitoneum). A small intraparenchimal splenic haematoma and a laceration 4 cm in depth were seen in the lower pole of the spleen. The repeated abdominal ultrasound (performed 4 h later) revealed an increased amount of free abdominal fluid (around 1000 cm^3). The splenic haematoma had also increased in size (measuring 5.5 cm in diameter). The patient was haemodynamically stable. His blood pressure was 110/85 mmHg with a heart rate of 90 beats/min. A multidisciplinary decision for the nonoperative treatment – percutaneous embolization – was reached. An urgent angiogram was performed to identify and possibly also treat the source of bleeding. The informed, written consent of the patient was obtained before the procedure for both diagnostic angiography and possible embolization. The selective catheterisation of the splenic artery with a 5F Sidewinder catheter (Cordis, Miami, FL, USA) demonstrated the extravasation of contrast media from the distal branch of the splenic artery (Figure 1A,B). A decision to perform the distal splenic artery embolization was made. Supraselective catheterisation and embolization with 500–700 μm Bead Block (Biocompatibles, Farnham, Surrey, UK) microspheres via a Progreat 2.8 Fr microcatheter (Terumo, Leuven, Belgium) were performed. A postprocedural splenic arteriogram showed the successful embolization (Figure 2). Follow-up ultrasound and CT examination revealed a small area of infarction at the site of the embolization (Figure 3). The patient was stable and discharged from hospital two weeks later. A postprocedural follow-up a year after the procedure revealed only a small area of parenchymal irregularity.

Discussion

The nonoperative management of blunt splenic injuries is the treatment modality of choice in haemodynamically stable adults and paediatric patients regardless of the severity of the injury. The embolization is a useful adjunct in the nonoperative management of patients who continue to bleed (Eastern Association for the Surgery of Trauma, Trauma Practice Management Guidelines, 2003). These guidelines are now accepted in most modern trauma centres. Contrast-enhanced CT has been shown to be highly accurate in diagnosing

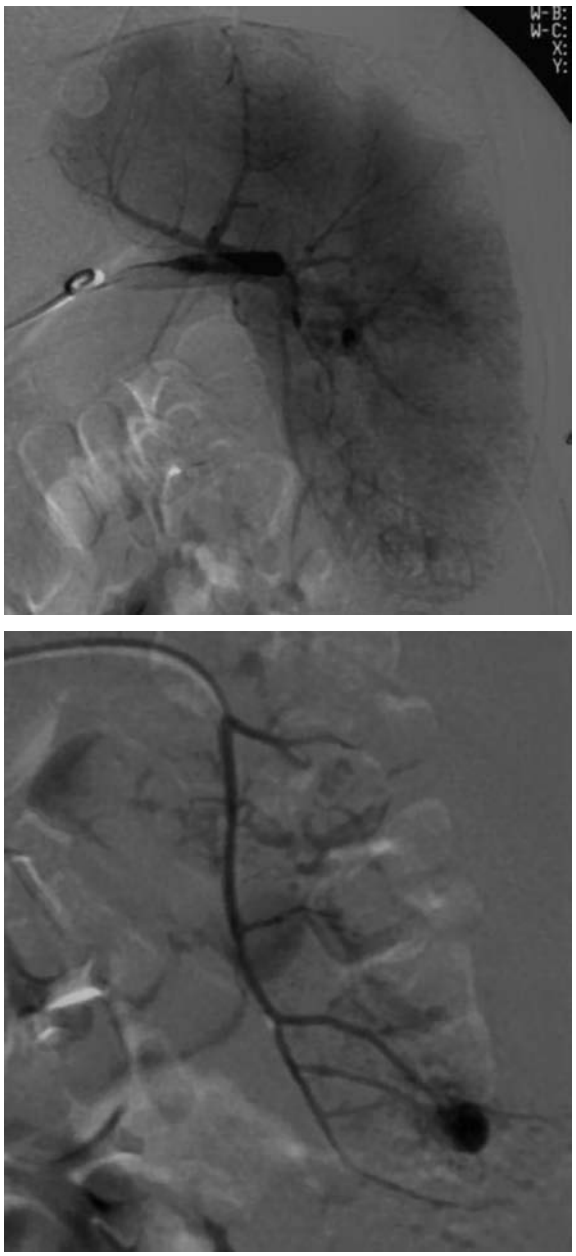

FIGURE 1. A. Selective splenic artery embolization in a 20-year-old man with blunt abdominal trauma. Splenic angiography obtained before the embolization procedure shows active extravasation from distal branch of splenic artery. B. Supraselective splenic angiography obtained with microcatheter – the extravasation is better seen.

acute splenic injuries. It enables the classification of the splenic injury according to severity, for which the Organ Injury Scale for the spleen (American Association for the Surgery of Trauma-AAST) is a widely accepted grading system.[4-6] The scale is as follows: Grade I – subcapsular haematoma of less than 10% of surface area or capsular tear of less than 1 cm in depth; Grade II – subcapsular hematoma of 10–50% of surface area or intraparenchymal haematoma of less than 5 cm in diameter or laceration of 1–3 cm in depth and not involving trabecular vessels; Grade III – subcapsular haematoma of greater than 50% of surface area (or expanding and ruptured subcapsular or parenchymal haematoma) or intraparenchymal hematoma of greater than 5 cm (or expanding) or laceration of greater than 3 cm in depth (or involving trabecular vessels); Grade IV – laceration involving segmental or hilar vessels with the devascularisation of more than 25% of the spleen; Grade V – shattered spleen or hilar vascular injury. Patients with AAST grade I or II splenic injuries and no associated splenic vascular injuries can be managed with just a simple observation. Those who are found to have one of the previously mentioned CT findings indicative of angioembolization — including AAST grade III–V splenic injury, active contrast extravasation or vascular injury of the spleen (pseudoaneurysm or A-V fistula) — should proceed to angiography and splenic embolization.[4,6]

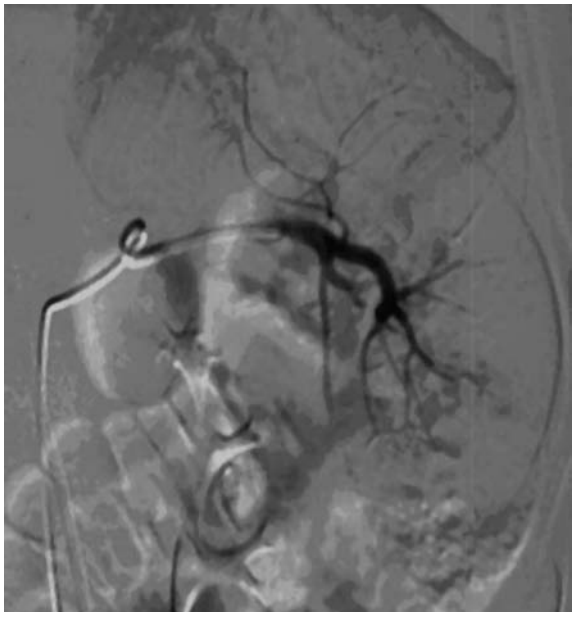

FIGURE 2. Splenic angiography obtained after the selective embolization with Bead Block microspheres (diameter range, 700-900 μm) via a Progreat 2.8 Fr microcatheter shows a complete haemostasis. No extravasation is seen.

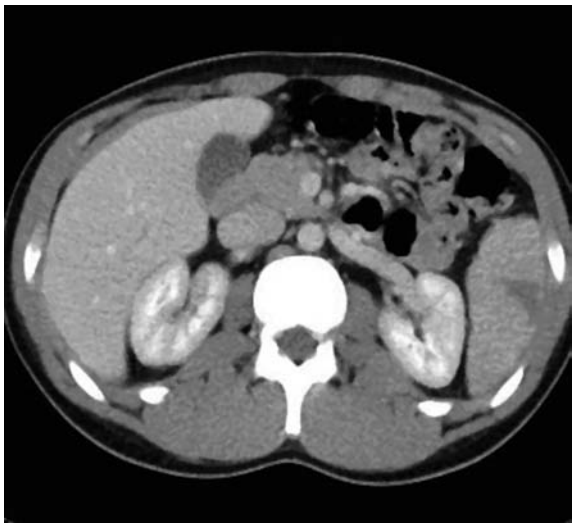

FIGURE 3. Transverse CT scan, obtained one month after the embolization shows small area of parenchymal infarct.

The objective of the splenic arterial embolization is to improve the results of the nonoperative management.[7] The embolization is performed via percutaneous access (usually via the common femoral artery). There are two methods regarding the splenic artery embolization. The decision about which method to use depends on angiographic findings. The distal splenic artery embolization is the method of choice for the management of a haemorrhage which originates from a distal branch of the splenic artery. This type of embolization is usually performed with microcoils and/or gelatin sponge pledgets that are injected through a microcatheter.[2,8-11] This technique achieves haemostasis to the injured parts while preserving the perfusion to the remainder of the spleen.

When a haemorrhage persists in spite of the distal embolization or the patient is at high risk of secondary spleen rupture (injury Grade III or higher), a more proximal splenic artery embolization is performed which reduces the pressure in the splenic parenchyma.[5,9-13] This type of embolization is usually performed with microcoils inserted in the middle segment of the splenic artery. Coils inserted at this site allow the reconstitution of blood supply through collateral vessels (short gastric and gastroepiploic, transgastric and transpancreatic arteries). The proximal embolization has been shown to be associated with less frequent and smaller infarcts than the distal embolization. It does not affect spleen anatomy or immune function.[14] The success of the splenic arterial embolization is defined by the splenic salvage rate.

The Quality Improvement Guidelines of the Society of Interventional Radiology reports a success rate between 87–100%.[4,11,13]

Major postprocedural complications are splenic abscess and infarct. Postembolization CT shows splenic infarcts in two thirds of patients after the proximal embolization and in all cases after the distal embolization.[14] The reported rate of the splenic abscess after the proximal or distal embolization is 3%.[10,13] Other relatively rare complications include coil migration, iatrogenic vascular injury and missed injuries to the diaphragm or pancreas.

Grading of the spleen injury in our case was not based on CT but on the sonographic evaluation. It was estimated as a Grade III injury (intraparenchymal haematoma greater than 5 cm and laceration greater than 3 cm in depth). The patient was haemodinamically stable but continued to bleed, and, therefore, the decision to perform embolization was made. Arteriography revealed bleeding from a small distal branch of the splenic artery. The successful distal splenic artery embolization with microspheres was performed. An early postprocedural follow up with CT and ultrasound was performed, revealing only a small area of parenchymal infarct (less than 2 cm in diameter). A postprocedural follow-up a year after the procedure revealed only a small area of parenchymal irregularity.

Conclusions

The percutaneous splenic arterial embolization has a major role in the management of traumatic splenic injuries. The embolization is particularly beneficial in injuries of AAST grade III or higher. Microspheres can be used as an alternative to microcoil or gelfoam particles for the distal splenic artery embolization.

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research article

Numerical study of the electroporation pulse shape effect on molecular uptake of biological cells

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Background. In order to reduce the side-effects of chemotherapy, combined chemotherapy-electroporation (electrochemotherapy) has been suggested. Electroporation, application of appropriate electric pulses to biological cells, can significantly enhance molecular uptake of cells due to formation of transient pores in the cell membrane. It was experimentally demonstrated that the efficiency of electroporation is under the control of electric pulse parameters. However, the theoretical basis for these experimental results is not fully explained. In order to predict the outcome of experiments and optimize the efficiency of electroporation before each treatment, we developed a model to investigate the effect of pulse shape on efficiency of electroporation.

Results. Our model is based on a developed chemical-kinetics scheme and trapezium barrier model, while self-consistency was taken into account. This model is further supplemented with a molecular transport model to acquire the molecular uptake of cells. The investigated pulse shapes in this study were unipolar rectangular pulses with different rise and fall times, triangular, sinusoidal and bipolar rectangular pulses and also sinusoidal modulated unipolar pulses with different percentages of modulation. The obtained results from our modelling and simulations are in good agreement with previously published experimental results.

Conclusions. We therefore conclude that this model can be used to predict the effects of arbitrarily shaped electroporation pulses on cell membrane conductivity and molecular transport across the cell membrane.

Key words: electrochemotherapy, optimization; membrane permeability; membrane conductivity

Introduction

Cancer is a leading cause of death around the world and for this reason it has drawn the attention of many researchers. Chemotherapy has been used for many years and is one of the most common treatments for cancer. Cytotoxic chemotherapeutic drugs are usually hydrophilic with very low transport through the cell membrane and thus high doses of these drugs are needed for treatment. Therefore, while chemotherapy can be quite effective in treating certain cancers by interfering with the cancer cell's ability to grow or reproduce, chemotherapeutic drugs reach all parts of the body, not just the cancer cells. Because of this, there may be many side-effects such as nausea, blood cell deficiency, fatigue and loss of hair during treatment.[1]

Two decades ago, electrochemotherapy was suggested for its use in clinical treatment of localized tumors.[2] Electrochemotherapy consists of electroporation and chemotherapy. Electroporation is a technique in which permeability of the plasma membrane increases transiently and reversibly with appropriate pulse parameters[3-6] and is nowadays widely used not only in electrochemotherapy,[7-9] but also in biotechnology[10,11] and in medical applications such as gene electrotransfer[12,13] and transdermal drug delivery.[14] Electroporation in combination with chemotherapy can increase drug delivery into the cells and consequently drug doses and thus side-effects of chemotherapy can be reduced.[10, 11,15,16]

It was experimentally demonstrated in a number of studies that the efficiency of electroporation is under the control of electric pulse parameters

such as pulse amplitude, duration, and shape.[17-22] Optimization of electric field parameters for successful electroporation requires time-consuming and costly experiments for different experimental criteria unless an appropriate model for this phenomenon can be suggested. Although the known models were proposed in previous studies[23-28], they are still unable to explain the effect of some parameters such as pulse shape, pulse repetition frequency and number of pulses on the molecular uptake enhancement of the cells under exposure.

The suggested mechanism for electroporation consists of structural changes resulting in formation of transient aqueous pores in the cell membrane. In order to reveal the exact mechanisms and dynamics of pore formation and closure and more importantly resealing of the membrane, theoretical models have drawn a great deal of attention.

In our present study, we investigated the effect of pulse shape on the efficiency of electroporation and, therefore, electrochemotherapy using modelling and simulation. Our model was based on a chemical-kinetics scheme with two types of pores[29] which have been recently confirmed.[30] We used developed equations with field-dependent rate coefficients in order to obtain the pore distribution on the membrane. Besides, the conductivity of pores was defined based on a trapezium barrier model for the image forces.[31] A self-consistent set of equations was used to consider all simultaneous changes. This model was supplemented with a molecular transport model for a single cell to acquire the molecular uptake of cells. The investigated pulse shapes in this study were unipolar rectangular pulses with different rise and fall times, triangular, sinusoidal and bipolar rectangular pulses and also sinusoidal modulated unipolar pulses with different percentages of modulation – all previously used in experimental studies.

Material and methods

Model description

Modified chemical-kinetics model for electroporation

When a cell is exposed to an external electric field, the induced transmembrane voltage (ITV) starts to increase based on the Laplace equation which leads to structural changes of the cell membrane. Based on a previously suggested[29], and recently confirmed[30] kinetic model, in the first step the intact closed lipids (C) transform to tilted lipid headgroups (C1). In the second step, the prepores (P1) are formed and finally, in the last step the final pores (P2) are formed. The sequential reaction can be described by:

$$C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P1 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} P2 \qquad [1]$$

The permeability of the P1 state is negligibly small and P2 is predominantly responsible for molecular uptake. Pore formation and closure are denoted by k_i and k_{-i} (i=1,2,3) rate coefficients, respectively. For simplicity, the rate coefficients k_1, k_2 and k_3 are considered equal ($k_1=k_2=k_3=k_p$).[29] The governed rate laws of constituting steps for the scheme [1] are:

$$\frac{d[C(\vec{r},t)]}{dt} = -k_p[C(\vec{r},t)] + k_{-1}[C1(\vec{r},t)]$$

$$\frac{d[C1(\vec{r},t)]}{dt} = -k_p([C1(\vec{r},t)] - [C(\vec{r},t)]) - k_{-1}[C1(\vec{r},t)] + k_{-2}[P1(\vec{r},t)]$$

$$\frac{d[P1(\vec{r},t)]}{dt} = -k_p([P1(\vec{r},t)] - [C1(\vec{r},t)]) - k_{-2}[P1(\vec{r},t)] + k_{-3}[P2(\vec{r},t)]$$

$$\frac{d[P2(\vec{r},t)]}{dt} = k_p[P1(\vec{r},t)] - k_{-3}[P2(\vec{r},t)] \qquad [2]$$

where t and $\vec{r}$ denote time and position, respectively. [C], [C1], [P1] and [P2] show normalized distribution of each membrane lipid state relative to the initial value of the closed state $[C(\vec{r},0)]$.[29]

Regarding the Van't Hoff relationship in electro-thermodynamics, the rate coefficient of pore formation can be obtained from:[29,31]

$$k_p = k_{p0} \exp\left(\frac{\Delta V_p \varepsilon_0 (\varepsilon_W - \varepsilon_L)}{2 k_B T d_m{}^2} ITV^2\right) \quad [3]$$

where ITV is the potential difference between the outer and inner layer of the membrane, ΔV_p is the mean volume change due to pore formation, ε_0 is the permittivity of the vacuum and ε_W and ε_L are dielectric constants of water and lipids, respectively. k_B is the Boltzmann constant, d_m is the thickness of the membrane and T is temperature. While the pore formation rate coefficient k_p is electric field-dependent, the closure rate coefficients (k_{-1}, k_{-2} and k_{-3}) are constant and independent of electric field strength.[29]

Whenever electroporation occurs, an increase in conductivity during the pulse is observed[32] which can be explained by the formation of pores in the cell membrane. Based on the trapezium barrier model for the image forces, the intrinsic pore conductivities $\sigma_{p,i}$ (i=1 and 2 represents P1 and P2 pores, respectively) are expressed as follows:[31,33]

$$\sigma_{p,i} = \sigma^0_{p,i} \exp\left(\alpha_{p,i} n |ITV| \frac{F}{RT}\right) \quad [4]$$

where

$$\sigma^0_{p,i} = \frac{\sigma_{ex} + \sigma_{in}}{2} \exp\left(\frac{-\varphi^0_{im,i} F}{RT}\right) \quad \text{and} \quad \alpha_{p,i} = 1 - \frac{RT}{F \varphi^0_{im,i}} \quad [5]$$

In the above equations, σ_{ex} and σ_{in} are the extracellular and intracellular conductivities respectively, n is the geometrical parameter of the trapezium model for energy barrier, F is the Faraday constant and $\varphi^0_{im,i}$ is the intrinsic pore barrier potential.

Therefore, conductivity of the membrane (σ_m) can be obtained by:

$$\sigma_m(\vec{r},t) = \sigma_{m0} + [P1(\vec{r},t)] \times \sigma_{p,1} + [P2(\vec{r},t)] \times \sigma_{p,2} \quad [6]$$

where σ_{m0} is the physiological/baseline conductivity of the membrane. Thus conductivity at each point on the membrane changes with time during and after the pulse, depending on pore distribution variations which affect ITV and in turn the distribution of pores.

Transmembrane molecular transport model

Based on previous studies[34-36], we defined two distinct phases for the electroporated membrane and two related transport mechanisms: the first one is the porated phase [P2] with relatively fast relaxation due to pore closure according to Eq. [1]. The second phase is the memory phase [M] due to enhanced membrane perturbation and ruffling with quite slow relaxation[34-36] which returns to its baseline value with a dual exponential decay function:[29]

$$[M] = [P2]_e \left(B \exp(-k_f t) + (1 - B) \exp(-k_s t)\right) \quad [7]$$

where $[P2]_e$ is the normalized distribution of [P2] pores at the end of the pulse, k_f and k_s are decay rate coefficients for this second phase and B is a constant.

The considered transport mechanisms for these two phases were interactive diffusion through the pores and endocytotic-like transport through the permeabilized area of the membrane.[34-37] Thus, the permeability of the membrane can be written as the sum of two distinct contributions:

$$P_m(\vec{r},t) = \left([P2(\vec{r},t)] D_p / d_m\right) + \left([M(\vec{r},t)] D_r / d_m\right) \quad [8]$$

where D_p and D_r are the attributed diffusion coefficients for interactive transport and endocytotic-like transport, respectively.

While the membrane is being permeabilized due to the electric field, the molecules pass through the membrane due to a concentration gradient. A quantitative description of diffusion is contained in Fick's first law. The total flux can be approximated by $j = P_m(c^{out} - c^{in})$, where c^{out} and c^{in} are the outer and inner concentrations adjacent to the membrane. The total number of molecules transported through the mem-

TABLE 1. Values of parameters used in simulations

Parameter	Symbol	Value	Ref.
Membrane thickness	d_m	5e-9 m	29
Extracellular conductivity	σ_{ex}	0.14 S/m [a]	29, 38
Intracellular conductivity	σ_{in}	0.3 S/m [b]	29, 39
Initial conductivity of membrane	σ_{m0}	5e-7 S/m	40
Extracellular permittivity	ε_o	7.1e-10 As/Vm	29
Intracellular permittivity	ε_i	7.1e-10 As/Vm	29
Membrane permittivity	ε_m	4.4e-11 As/Vm [c]	29
Water relative dielectric constant	ε_w	80 As/Vm	29
Lipid relative dielectric constant	ε_l	2 As/Vm	29
Free diffusion coefficient	D_0	5e-10 m^2/s	29
Zero-field equilibrium constant	K_0	2e-2	29
Mean average aqueous pore volume	ΔV_p	9e-27 m^3	29
Intrinsic barrier potential of P1 state	φ_{im1}^0	0.13 V	31
Intrinsic barrier potential of P2 state	φ_{im2}^0	0.084 V	31
A geometrical parameter	n	0.12	33
Decay rate coefficient for C1	k_{-1}	10^5 s^{-1}	41-43
Decay rate coefficient for P1 pores	k_{-2}	2000 s^{-1}	41-43
Decay rate coefficient for P2 pores	k_{-3}	2 s^{-1}	41
Decay rate coefficients for endocytotic-like process	k_f, k_s	0.044, 0.003 s^{-1}	29

[a] This is for SMEM. The range of extracellular medium is quite large. [b] Reported between (0.2-0.55) S/m [c] Reported between (4.4-5)*10^{-11} As/Vm

brane (N) was computed with integration of transported molecules through the cell membrane over time and the cell surface:

$$N = N_A \int_{t=0}^{\tau} \int_S j dS dt \qquad [9]$$

where S is the surface of the cell membrane, τ is the time at which the quantity of transported molecules is to be determined and N_A is Avogadro's number.

Construction of the model

The simulations in this study were performed using the COMSOL 3.3 package (COMSOL Inc., Burlington, MA) based on the finite element method. To construct the geometrical model, a spherical cell with radius of 5.6 μm was located between two virtual electrodes. Since incorporating an extremely thin membrane is problematic in meshing and solving the problem, we assigned the boundary condition to the membrane.[38] We neglected the resting transmembrane voltage. The initial intracellular and extracellular concentrations of probe were set to 0 and 10 mM, respectively. The diffusion coefficients for interactive diffusion and for an induced endocytotic-like process are considered as $D_0/5$ and $D_0/10000$. These two values, however, depend considerably on the type and size of the transported molecules. The necessary parameters used in our simulations are given in Table 1. Our simulation was designed to solve the Laplace equation considering all related equations in this model (Eq. [2], [3], [6]) taking into account self-consistency of parameters to find the distribution of pores on the cell membrane, spatially and temporally, and all related parameters such as ITV, cell membrane conductivity and permeability. Afterwards, the uptake of the cells for each different pulse shape was obtained. All simulations were performed on a PC (2.8 GHz Pentium IV processor, 3 GB RAM) and

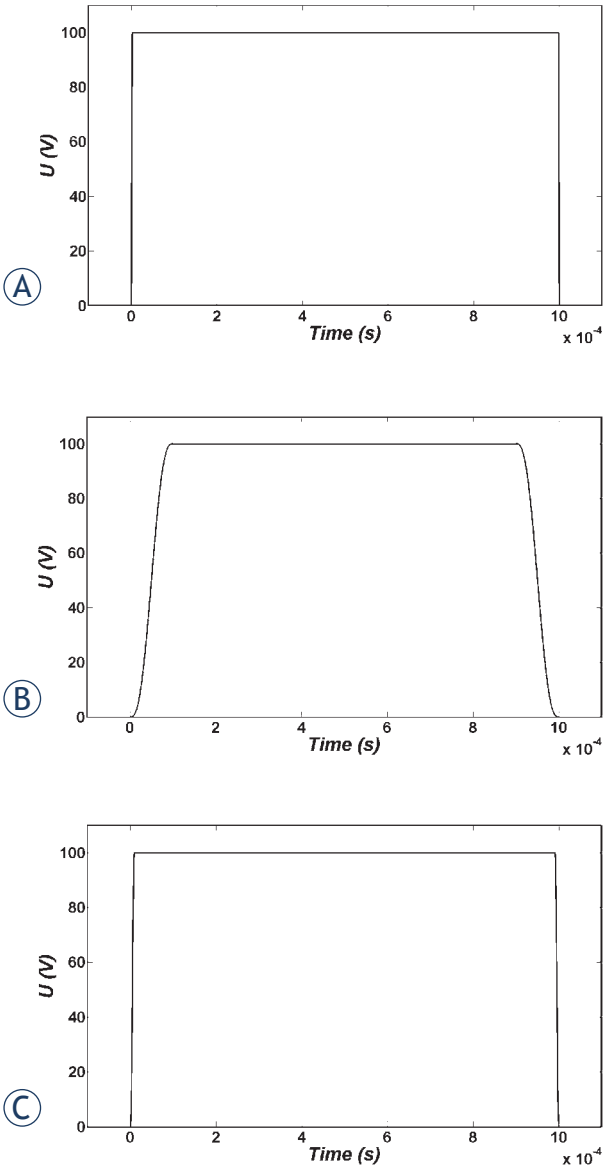

FIGURE 1. The investigated rectangular pulse shapes in this study with rise and fall times of (A) 2, (B) 10 and (C) 100 µs, respectively.

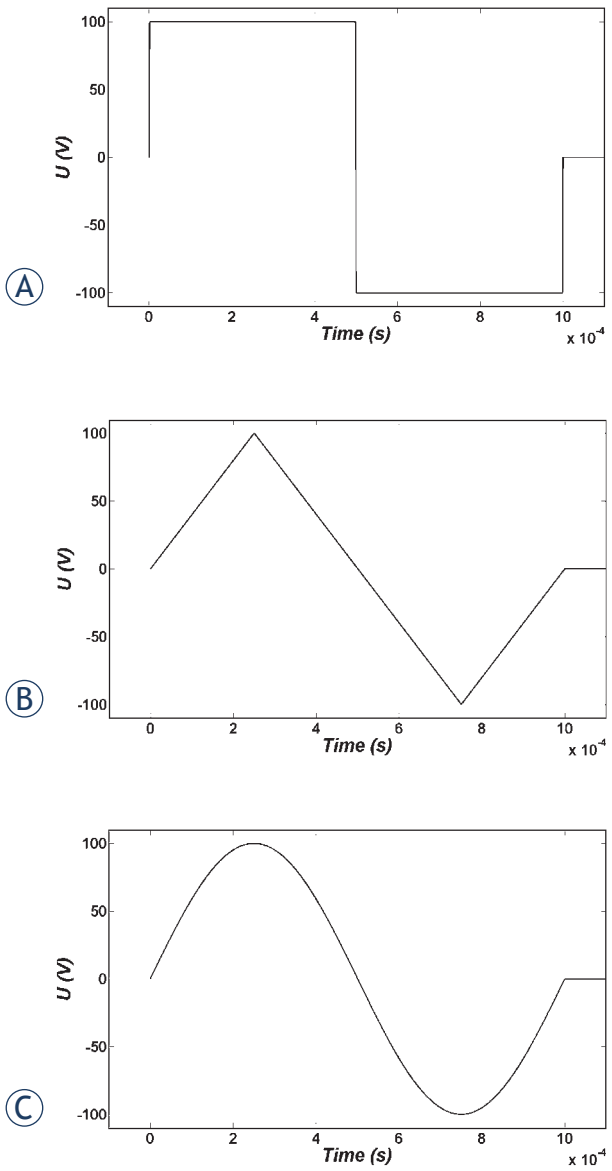

FIGURE 2. The (A) bipolar rectangular, (B) triangular and (C) sinusoidal pulses considered in this study.

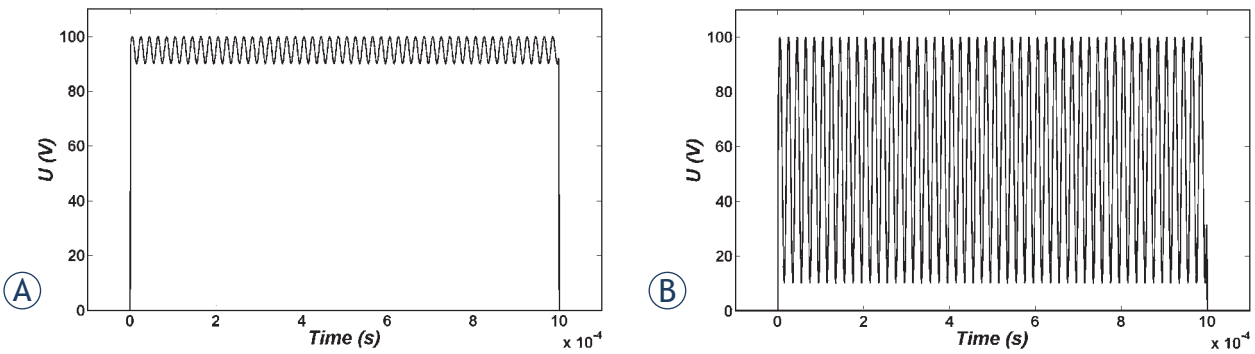

FIGURE 3. The sine-modulated 50 kHz unipolar pulses with (A) 10% and (B) 90% modulation investigated in this paper.

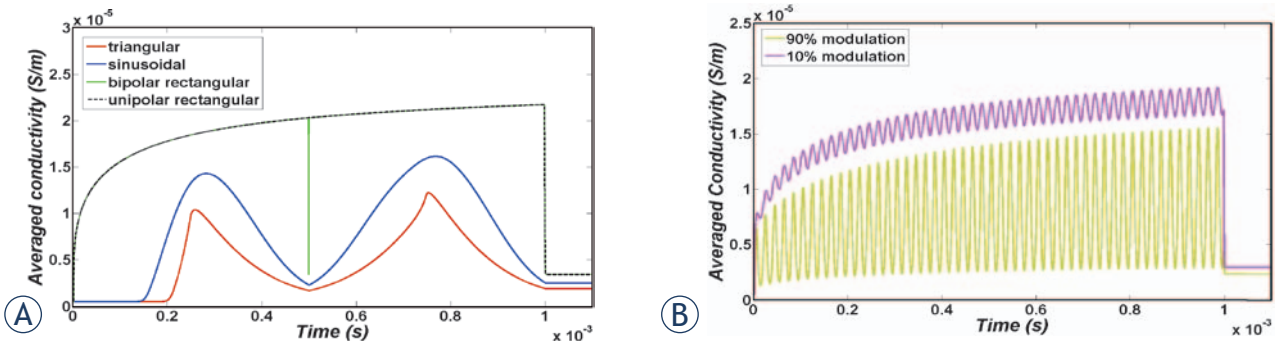

FIGURE 4. Temporal evolution of the overall membrane conductivity during the pulse for (A) unipolar and bipolar rectangular, triangular and sinusoidal pulses and (B) 10% and 90% sine-modulated unipolar pulses.

each simulation lasted 3-25 minutes depending on the considered pulse shape and number of pulses in each train of pulse.

The investigated pulse shapes in this study were unipolar rectangular pulses with different rise and fall times of 2, 10 and 100 μs (Figure 1); triangular, sinusoidal and bipolar rectangular pulses (Figure 2); and also sinusoidal modulated unipolar pulses with different percentages of modulation of 10% and 90% with 50 kHz frequency (Figure 3).

Results

Immediately after the smoothed step pulse is switched on, ITV starts to increase based on the Laplace equation and causes membrane structural changes initiation, which in turn results in the membrane conductivity increase according to Eq. [6]. The temporal behaviour of average conductivities over the cell membrane due to application of the considered pulse shapes (Figures 1, 2 and 3) are shown in Figure 4. All pulses were considered to have a peak of 1 kV/cm and total duration of 1 ms.

It can be observed in Figure 4 that the overall conductivity changes for unipolar and bipolar pulses have negligible differences. The reason for this fact is a very quick switch between positive and negative voltage, as well as ignoring the resting voltage in this model. Besides, a comparison between conductivity increases due to rectangular, triangular and sinusoidal pulses was performed. Figure 4A shows that the largest and smallest changes were due to rectangular and triangular pulse shapes, respectively. Figure 4B shows that a conductivity change due to 10% modulation is higher than the 90% one but both are still lower than the rectangular pulse.

The temporal behaviour of averaged cell membrane permeability for pulses in Figures 1 to 3 is illustrated in Figure 5. Permeability changes occur slowly. Therefore, for bipolar pulses and modulated pulses in which the fall and rise is very fast, there is not enough time for resealing of permeability which causes different behaviour for membrane permeability related to membrane conductivity. Based on Figure 5, we expect the order of efficiency of pulses of the same peak as follows: unipolar

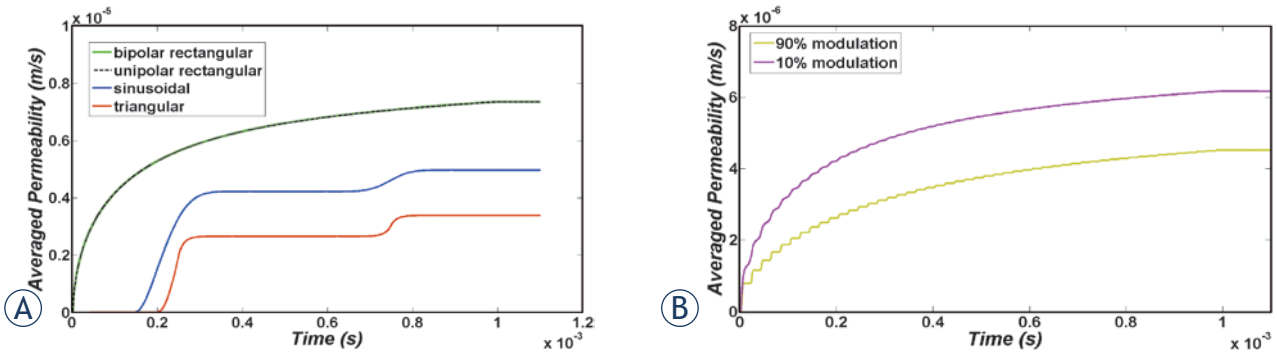

FIGURE 5. Temporal evolution of the overall membrane permeability during the pulse for (A) unipolar and bipolar rectangular, triangular and sinusoidal pulses and (B) 10% and 90% sine-modulated unipolar pulses.

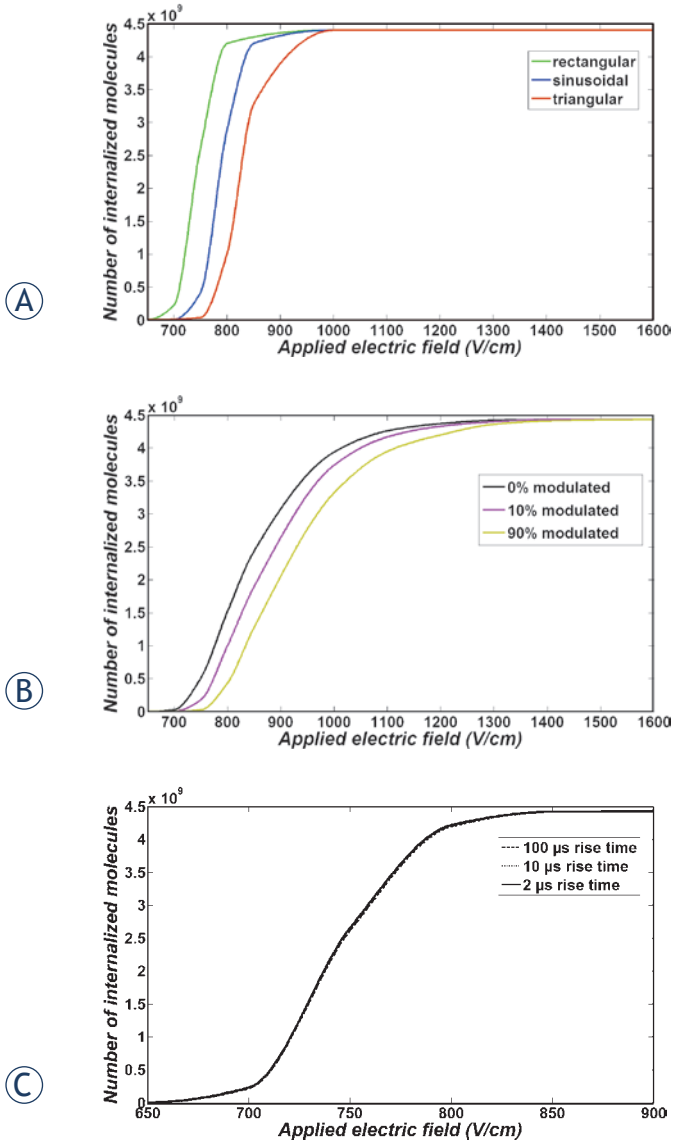

FIGURE 6. Dye uptake 16 minutes after pulse cessation versus electric field amplitude for (A) 8 pulses of 1 ms and 1 Hz unipolar and bipolar rectangular, triangular and sinusoidal pulses, (B) one 1 ms pulse of 0%, 10% and 90% sine-modulated and (C) 8 pulses of 1 ms rectangular pulses with rise and fall times of 2, 10 and 100 µs.

and bipolar rectangular, 10% modulated, sine, 90% modulated and finally bipolar triangular pulses. Note that unlike membrane conductivity in Figure 4, membrane permeability does not recover as fast after the pulse ceases.

To be able to check the validity of our simulation results, the uptake enhancement of the cell was calculated for the same pulse parameters of previously obtained experimental results.[19] The chosen parameters were 8 pulses of 100 µs duration and 1 Hz pulse repetition frequency with different pulse strengths for each pulse shape.

Figure 6A shows the results of simulation for 8 pulses of bipolar rectangular, sine and triangular pulses. It shows that the rectangular pulses are more efficient than sinusoidal pulses which in turn are more efficient than triangular pulses. These results are in good agreement with experimentally obtained results.[19]

As can be seen in Figure 2A for the bipolar pulses, the pulse switch from positive to negative takes place very fast. During the switch time, the pore creation rate and, therefore, membrane conductivity decrease. But due to very short time of switching related to pulse duration, these changes are negligible in comparison to the conductivity change related to the unipolar pulse (Figure 4A). Consequently, the uptake due to unipolar pulses is larger than bipolar pulses but this difference is negligible and not observable (data not shown). While our simulation shows no significant difference between these two pulse types, in experimental results bipolar pulses are significantly more efficient than unipolar pulses. The reason for this inconsistency is most probably due to neglecting resting voltage in the simulations.

In addition, Figure 6B demonstrates the comparison between unipolar pulses of 0, 10 and 90% modulation. The results are also in good qualitative agreement with previously obtained experimental results.[19] The uptake enhancement results for 8 pulses of unipolar trapezoidal pulses of 1 ms duration with 2, 10 and 100 µs rise and fall times are shown in Figure 6C. It can be seen from the figure that there is no significant difference between these pulses which is again in good agreement with previously published experimental results.[19]

Conclusions

The described model enables determination and prediction of all electrical and diffusion parameters for different pulse shapes. Thus, knowing electrical and diffusion properties of the cells and the specific dye, optimization of the electroporation protocol can be performed before the treatment. Our results show that rectangular pulses are more effective than the sinusoidal and triangular pulses. Besides, our results indicate that the higher the percentage of unipolar pulses modulation with sine shape pulses of 50 kHz, the lower the uptake enhancement of the cells. Moreover, the rise and fall times of unipolar rectangular pulses do not significantly affect the uptake of molecules by the cells. Our simulation results are consistent with experimental observations.

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research article

Development of human cell biosensor system for genotoxicity detection based on DNA damage-induced gene expression

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Background. Human exposure to genotoxic agents in the environment and everyday life represents a serious health threat. Fast and reliable assessment of genotoxicity of chemicals is of main importance in the fields of new chemicals and drug development as well as in environmental monitoring. The tumor suppressor gene *p21*, the major downstream target gene of activated *p53* which is responsible for cell cycle arrest following DNA damage, has been shown to be specifically up-regulated by genotoxic carcinogens. The aim of our study was to develop a human cell-based biosensor system for simple and fast detection of genotoxic agents.

Methods. Metabolically active HepG2 human hepatoma cells were transfected with plasmid encoding Enhanced Green Fluorescent Protein (EGFP) under the control of the *p21* promoter (p21HepG2GFP). DNA damage was induced by genotoxic agents with known mechanisms of action. The increase in fluorescence intensity, due to *p21* mediated EGFP expression, was measured with a fluorescence microplate reader. The viability of treated cells was determined by the colorimetric MTS assay.

Results. The directly acting alkylating agent methylmethane sulphonate (MMS) showed significant increase in EGFP production after 48 h at 20 μg/mL. The indirectly acting carcinogen benzo(a)pyren (BaP) and the cross-linking agent cisplatin (CisPt) induced a dose- dependent increase in EGFP fluorescence, which was already significant at concentrations 0.13 μg/mL and 0.41 μg/mL, respectively. Vinblastine (VLB), a spindle poison that does not induce direct DNA damage, induced only a small increase in EGFP fluorescence intensity after 24 h at the lowest concentration (0.1 μg/mL), while exposure to higher concentrations was associated with significantly reduced cell viability.

Conclusions. The results of our study demonstrated that this novel assay based on the stably transformed cell line p21HepG2GFP can be used as a fast and simple biosensor system for detection of genetic damage caused by chemical agents.

Key words: HepG2 cells; biosensor system; green fluorescent protein; reporter gene assay; genotoxicity; p21 promoter

Introduction

Genotoxicity data play an important role in evaluating health hazards associated with exposure of humans and living organisms to chemical substances. Genotoxicity assays are needed for screening compounds that are candidate drugs, food additives, or cosmetics to assess whether the compound of interest induces DNA damage. The methods for detecting genotoxic agents are also needed to monitor contamination of water supplies with genotoxic pollutants. In addition, genotoxicity screening should be introduced to monitor environmental pollution through industrial and municipal waste disposal. Regulatory requirements for genotoxicity testing of chemicals and products such as pharmaceuticals, pesticides, food additives, and cosmetics rely on a battery of genotoxicity tests, which generally consist of an *in vitro* test for gene mutations in bacteria and mammalian

cells, an *in vitro* test for chromosomal damage and an *in vivo* test for chromosomal damage in rodent hematopoetic cells.[1] However these same methods are unsatisfactory for rapid screening for several reasons: testing can take many weeks, when it is desirable to obtain genotoxic data in a shorter time frame, or large quantities of a tested compound are needed, when only limited quantities are available, such as during drug development or in environmental monitoring when concentrated samples are tested. Here we have developed a method suitable for primary genotoxicity screening.

Genotoxic agents cause different types of damage to the DNA molecule. To counteract the consequences of DNA damage, cells have evolved complex defense mechanisms resulting in cell cycle arrest, DNA damage repair and apoptosis, which positively contribute to genomic stability. In bacteria, DNA damage or inhibition of its replication invokes a well-characterized SOS response with the induction of about 20 different genes.[2] An even larger number of genes are involved in the cellular response to DNA damage in yeasts[3], and in mammalian cells.[4] Alteration in expression of these genes can be used as a surrogate for early detection and quantification of DNA damage caused by genotoxic agents. Reporter gene expression systems that measure changes in expression of DNA damage response-associated genes as the markers of DNA damage have been shown to be suitable as high-throughput screens for genotoxicity. The most widely used are bacterial systems in which genotoxic effects are identified based on the changes in expression of SOS response genes.[5-6] Recently, yeast *Saccharomyces cerevisiae* DNA reporter assays in which the *RAD54* promoter is fused to green fluorescent protein (GFP)[7] and *RAD51* promoter fused to *Renilla* luciferase[8], have been developed.

In mammalian cells, the most prominent pathway of cellular response to DNA damage is activation of the tumor suppressor and transcription factor p53 through phosphorylation by DNA damage-responsive kinases.[9] Activated p53 then induces the expression of genes involved in DNA repair, cell cycle arrest, or apoptosis.[10] The cyclin-dependent kinase 1A (CDKN1A) inhibitor *p21* (*Waf1/ Cip1*) is the major downstream target gene of activated p53 and is responsible for causing cell cycle arrest following DNA damage.[11] The activated p53 protein directly stimulates expression of *p21* which, through its negative effect on various CDKs, inhibits both the G1 to S and the G2 to mitosis transition.[12] In addition, by binding to the proliferating cell nuclear antigen (PCNA), p21 interferes with PCNA-dependent DNA polymerase activity, thereby inhibiting DNA replication and modulating various PCNA-dependent DNA repair processes.[13] Up-regulation of *p21*expression upon exposure to irradiation or genotoxic chemicals has been reported in several *in vitro* and *in vivo* studies.[14-17]

Here we describe a new genotoxicity test system based on a *p21*-dependent GFP reporter gene assay with stably transformed human hepatoma HepG2 cells. The HepG2 cells were chosen because of their human origin and their retained activities of xenobiotic-metabolizing enzymes, which make them a better model for reflecting the processes in an intact liver than other *in vitro* test systems.[18] In addition, HepG2 cells express wild-type tumor suppressor p53[19], making them an appropriate model for development of the test system based on the p53-mediated DNA damage response. The results showed that this test could be used for a high throughput screening for genotoxic agents.

Materials and methods

Chemicals and reagents

Methyl methane sulphonate (MMS), benzo[a] pyrene (BaP) and dimethyl sulphoxide (DMSO) were purchased from Sigma, St. Louis, USA). Cisplatin (CisPt) was obtained from Medac, Hamburg, Germany, and vinblastine sulphate (VLB) from Lilly France S.A., Fagersheim, France.

Cell line

The human hepatoma HepG2 cell line was obtained from ECACC (Wiltshire, UK), and was grown in minimum essential medium (MEM, advanced, GIBCO, Invitrogen, Paisley, UK) without phenol red supplemented with 10% heat inactivated fetal calf serum (FCS, SIGMA, St. Louis, MO, USA). Cells were routinely subcultured twice per week and were maintained in a humidified atmosphere with 5% CO_2 at 37°C.

Construction of plasmids

The plasmid pEGFP-N1, encoding Enhanced Green Fluorescent Protein (EGFP) controlled by the CMV promoter (Clontech, Basingstoke, UK) was used as a source of the coding sequence of the EGFP gene.

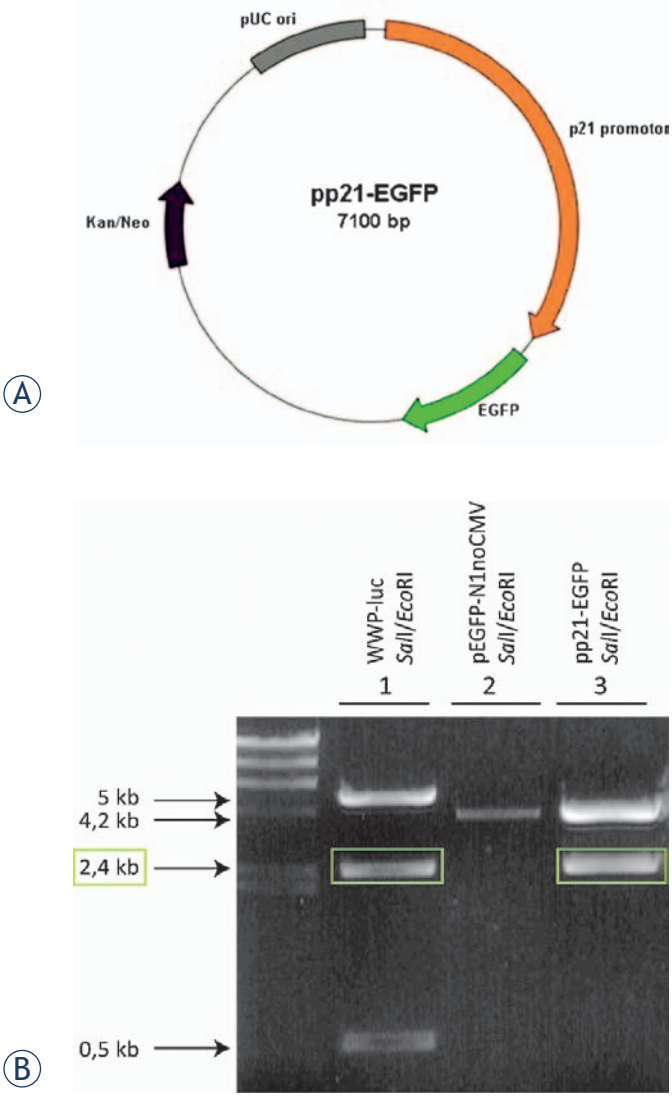

FIGURE 1. pp21-EGFP plasmid (A) and confirmation of successful construction of the plasmid (B). The identity of the plasmid was confirmed with SalI and EcoRI restriction. Sample 1 is WWP-Luc from which the p21 promoter (marked yellow) was isolated. Sample 2 is linearized pEGFP-N1 without CMV plasmid from which CMV was cut out with the same restriction enzymes to form blunt ends. Sample 3 is pp21-EGFP plasmid resulting from ligation of a 2.4 kB p21 promoter from WWP-Luc and sample 2 restricted with SalI and EcoRI.

The source of the coding sequence of the *p21* promoter was the WWP-LUC plasmid, which was a gift from Prof. Bert Vogelstein (Johns Hopkins Oncology Center, Baltimore, Maryland, USA). The construction of a recombinant vector containing the *p21* promoter reporter cassette and EGFP was done in several steps using the Clontech pEGFP-N1 plasmid as a backbone and standard molecular biology techniques of restriction and ligation. In addition, the gene for neomycin resistance was included into the plasmid, which enabled the isolation of HepG2 cells with stable expression of the reporter gene under pressure of Geneticin® (neomycin, GIBCO). The constructed plasmid pp21-EGFP was cloned into *E. coli* (strain DH5α, Invitrogen, UK), and isolated using the Qiagen Maxi Endo-Free kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Purified plasmid DNA was subjected to quality control and quantity determinations, performed by agarose gel electrophoresis and by means of spectrophotometry.

Transfection of HepG2 cell line

The HepG2 cells were transfected with the pp21-EGFP plasmid using electroporation as described.[20] 40 µl of cell suspension (2.5 x10^7 cells/ml) were mixed with 10 µg of plasmid DNA and placed between two flat parallel stainless steel electrodes with a 2-mm gap and subjected to 8 square-wave shaped electric pulses of 5 ms duration, repetition frequency 1 Hz. Different electric field intensities were tested: 400 V/cm, 600 V/cm, 700 V/cm, 800 V/cm and 1000 V/cm. The electric pulses were generated by an electroporator (GT-1, electroporator, Faculty of Electrical Engineering, University of Ljubljana, Slovenia). After exposure to electric pulses, the cells were incubated for 5 min at room temperature. Thereafter, cells were maintained in non-selective medium for 1-2 days after transfection. The selection of stably transfected clones was performed by culturing the cells in medium containing 1 mg/ml Geneticin®. Cultivation in the selective medium was continued for 2-3 weeks. During this period, the cells without plasmid died while the cells containing stably incorporated plasmid were able to replicate and form colonies.

Separate colonies were picked and transferred into wells of 96-well microtiter plates and cultivated under pressure of 0.5 mg/ml Geneticin®. After reaching a sufficient number, the cells were transferred to larger plates for further propagation to obtain a sufficient number of cells for further selection of the most responsive clones. The clones with visible morphological and/or replication changes were discharged.

Cell treatment with model genotoxic agents and EGFP measurement

Model genotoxic agents with known mechanisms of action were used to test and validate the cell biosensor system. Stock solutions were pre-

pared prior to testing: MMS, and CisPt were dissolved in distilled water at concentrations 50 mg/mL (454 mM) and 2 mg/mL (6.7 mM), respectively. BaP was dissolved in DMSO at a concentration 2.52 mg/mL (10 mM) and VLB in 0.9% NaCl at a concentration 1 mg/mL (1.1 mM). Further dilutions were made in cell culture media.

A suspension of exponentially growing p21-HepG2GFP cells ($3x10^5$ cells/mL) in minimum essential medium without phenol red with 10% fetal calf serum was distributed in 3 mL aliquots to plastic test tubes. 30 µL of test chemical of appropriate concentration (100-fold higher concentrations than final treatment concentrations) or 30 µL of vehicle for controls were added to each tube . The following final concentrations were used: MMS: 5, 10, 20, 40, 50 µg/mL; CisPt: 0.4125, 0.825, 1.65, 3.3, 6.6 µg/mL; BaP: 0.05, 0.13, 0.25, 0.5, 1.26 µg/mL, and VBL 0.1, 0.5, 1.0, 2.5, 5.0 mg/mL. For the EGFP fluorescence measurements, 100 µL aliquots from each tube were distributed to 6 wells of 96-well black microtiter plates with a clear bottom (Greiner BIO-ONE, Nuernberg, Germany). The plates were incubated at 37°C, 5% CO_2 for 7 days, and the EGFP fluorescence was determined after 24, 48, 72, 120 and 168 h. The intensity of EGFP fluorescence was measured at 485 nm excitation and 535 nm emission wavelengths with a fluorescence microplate reader (Tecan Infinite 200). The experiments were repeated three times.

From fluorescence intensity measurements, a relative EGFP induction ratio was calculated. Fluorescence intensity of the treated cells was divided by the fluorescence intensity of control cells and normalized to the relative cells viability determined with the MTS assay.

Determination of cell viability (MTS assay)

The cell viability was determined by the colometric (3-(4.5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) MTS assay with the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, USA) according to the manufacturer's instructions. The 100 µL aliquots from each test tube of treated or control cells were distributed into 4 wells of normal 96 well microtiter plates and incubated for 24, 48, 72 120 or 168 h. For each of the 5 time point measurements, a separate microtiter plate was prepared. At the end of the incubation period with chemical agents, 20 µL of

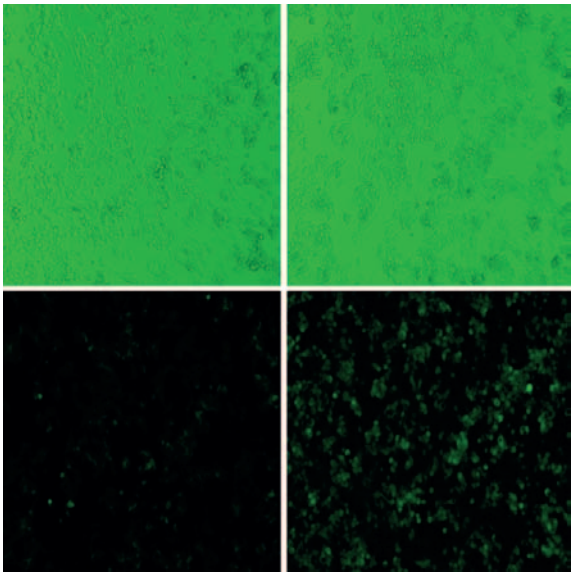

FIGURE 2. Photomicrographs of control (A, C) and p21HepG-2GFP cells exposed to 50 µg/ml MMS for 48 hours (B, D). Images taken under visible light condition (A, B) and images taken fluorescence epi-illumination (C, D).

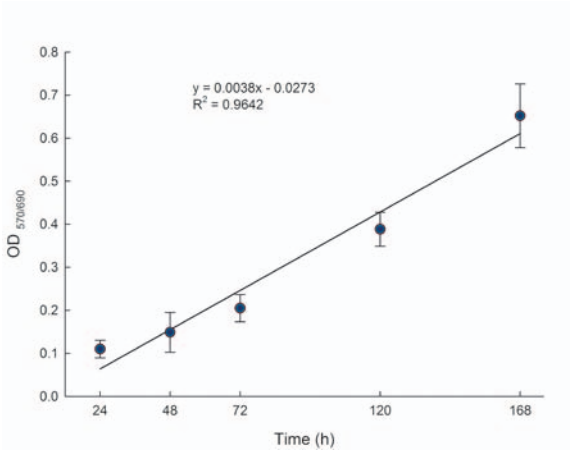

FIGURE 3. Proliferation of p21HepG2GFP cells measured with the MTS assay. 5000 cells per well were plated on 96-well microtitre plates in triplicate and incubated for 24, 48, 72, 120 and 168 h. The values represent means of four independent experiments ± SD.

MTS solution were added to each well of 96-well microtiter plates and incubated for 2 h in a humidified atmosphere with 5% CO_2 at 37°C. After the incubation with MTS, the microtiter plates were shaken for 30 s and the absorbance of the resulting solution was measured at 492 nm using a Labtec HT2 microplate reader (Anthos, Wals, Austria). Relative survival of cells was calculated by dividing the absorbance of the treated cells with the absorbance of the control cells. The experiments were performed in quadruplets and repeated 3-times.

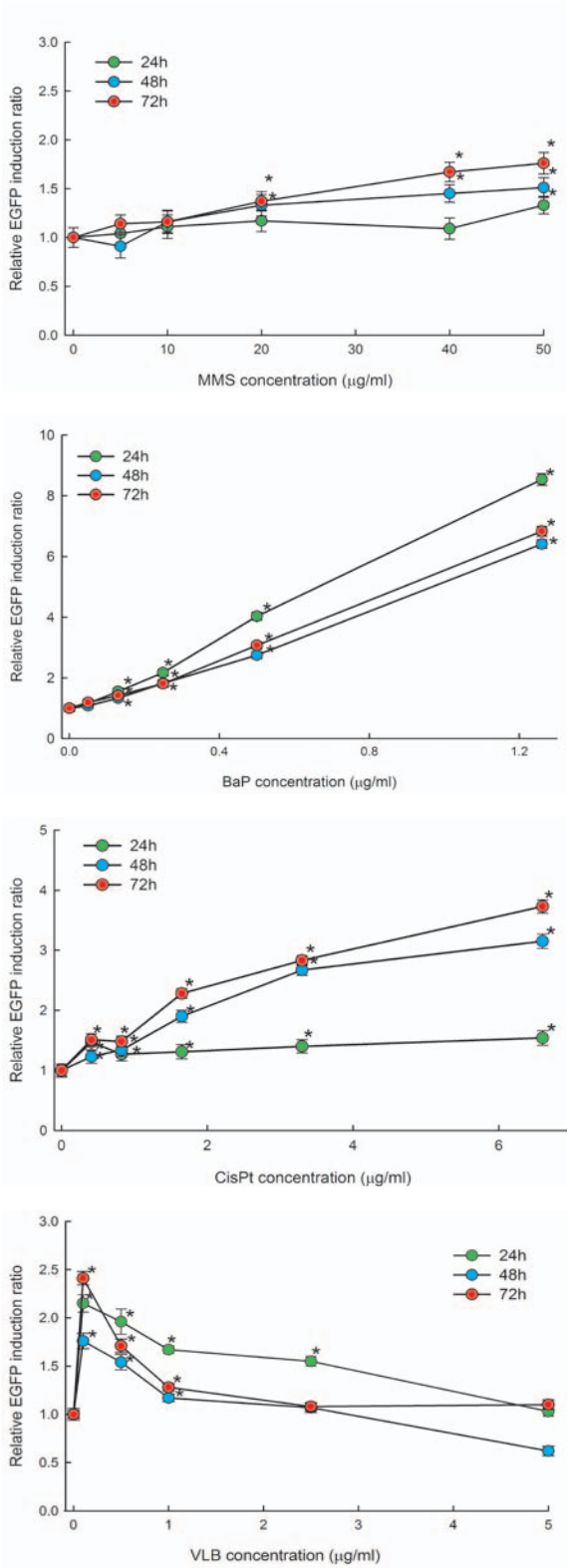

FIGURE 4. Dose- and time-dependent induction of EGFP expression in p21HepG2GFP cells treated with graded doses of MMS (A), BaP (B), CisPt (C) and VBL (D) after 24, 48 and 72 h of exposure. The response is presented as the relative EGFP induction ratio which is the ratio between EGFP fluorescence of the treated cells and the background fluorescence of control cells normalized to the relative cell viability. The values represent means of four independent experiments ± SD; *p<0.05

Statistical analysis

Statistical analysis was performed using SigmaStat software (Systat Software, Inc., Richmond CA). All data were first tested for normality with the Kolmorogov-Smirnov normality test. Significance tests were carried out using analysis of variance (ANOVA) and two-tailed Student's t-test. Values of $p<0.05$ were considered significant. Data were presented as the arithmetic mean (AM) ± standard deviation of the mean (SD).

Results

Construction of reporter gene plasmid and stably transformed HepG2 cells

For this genotoxicity screening system, a plasmid pp21-EGFP with the *p21* promoter inserted in front of the EGFP reporter gene was constructed (Figure 1A). Successful construction and isolation of the pp21-EGFP plasmid was confirmed with restriction analysis (Figure 1B). The pp21-EGFP plasmid was then transfected to HepG2 cells. In the final step, HepG2 cell clones expressing low basal and high inducible EGFP expression were isolated.

For the isolation of DNA damage-responsive clones we used MMS. After measuring the basal and MMS induced EGFP levels in 36 independent clones the one with the highest inducible and the lowest basal level of EGFP expression was selected for further propagation and characterization and for the experiments with the known model genotoxic compounds. The clone was named p21HepG-2GFP. Microscopic observations of p21HepG2GFP cells demonstrated a clear increase of EGFP fluorescence intensity induced by 50 μg/mL MMS after 48 h exposure (Figure 2).

Cell viability as an internal standard

Since it is known that genotoxic chemicals are toxic at certain concentrations and thus suppress cell growth during exposure which was continued for up to 7 days, it was necessary to normalize the observed level of EGFP to the number of viable cells. The induction of EGFP fluorescence was measured after 24, 48, 72, 120 and 168 h on the same population, while this was not possible for determination of cell viability, since no appropriate method that would allow for determination of cell viability without termination of cell cultur-

ing is available. The MTS assay that measures the conversion of MTS to the formazan product by dehydrogenase enzymes of the intact mitochondria of living cells correlates with the number of viable cells. We therefore used this assay to indirectly determine the relative changes in cell numbers during the exposure to tested chemicals. For each treatment, we prepared five plates for the measurement of cell viability (one plate for each time point) in parallel to the plate for EGFP fluorescence measurements. The correlation analysis of the proliferation of p21HepG2GFP cells showed that absorbance of the formed formazan product correlated to cell proliferation (r = 0.94) (Figure 3). The data also indicate that during the exponential growth phase the doubling time of the p21HepG-2GFP cells is about 48 h. At each time point, the relative cell viability compared to non-treated control cells was calculated and the factor was used for normalization of the relative EGFP induction ratio to the number of viable cells. A reduction of relative cell viability by more than 30% (reduction factor 0.7) was considered as cytotoxic.

Responses of p21HepG2GFP cells to exposure to model genotoxic agents

To demonstrate the sensitivity of this bioassay for detection of genotoxic agents, we tested several genotoxic agents with known mechanisms of action. To determine the optimal exposure conditions, a time and dose dependence of *p21*-dependent EGFP fluorescence induced by model genotoxic agents was investigated.

Methyl methane sulphonate (MMS), a direct acting genotoxic agent that induces alkylation of DNA bases, induced a statistically significant increase in EGFP fluorescence after 24 h to 50 μg/mL and after 48 h exposure to 20, 40 and 50 μg/mL (Figure 4A, Table 1). The MMS-induced increase of EGFP fluorescence was time- and dose-dependent, which is clearly reflected in the increasing values of relative EGFP induction ratio (Figure 4A, Table 1). After 120 and 168 h exposure, a significant increase in EGFP fluorescence associated with the increase in relative EGFP induction ratio was observed at all concentrations (Table 1). The parallel measurement of cell viability during the exposure to MMS showed that it was not significantly affected during the initial 72 h of exposure, while after 120 and 168 h it was reduced by more than 30% compared to non-treated control cells (Table 1).

Benzo[a]pyrene (*BaP)* is a mutagenic and carcinogenic indirectly-acting genotoxic agent which forms BaP diolepoxide (BPDE)-DNA adducts after metabolic activation. BaP induced a significant dose-dependent increase in EGFP fluorescence at all exposure times and all concentrations except the lowest one (0.05 μg/mL). However, the relative EGFP induction ratio did not increase with prolonged exposure indicating that the EGFP induction reached a plateau (Figure 4B, Table 1). BaP did not significantly reduce the cell viability during the exposure up to 72 h (Table 1), while with further exposure the viability was reduced by more than 30% at all doses of BaP (Table 1).

Cisplatin (CisPt), a well known chemotherapeutic, is a directly-acting genotoxic agent that induces alkylation of DNA and DNA cross-links. CisPt induced significant increase of EGFP fluorescence already after 24 h exposure at all concentrations. With further exposure, the relative EGFP induction ratio tended to increase with the time of exposure (Figure 4C, Table 1). In cells exposed to 3.3 μg/mL CisPt, the relative EGFP induction ratio increased from 1.40, determined after 24 h, to 2.83 determined after 72 h of exposure (Figure 4C, Table 1). CisPt did not reduce cell viability after 24 h of exposure. After 48 and 72 h exposure, the viability of the cells was significantly reduced at the two highest concentrations (3.3 and 6.6 μg/mL) while after 120 and 168 h exposure, CisPt reduced cell viability by more than 30% at all tested concentrations (Table 1) *Vinblastine (VLB)* is a chemotherapeutic that does not induce DNA damage but induces disturbances in cell replication due to its interference with mitotic spindle formation. This compound induced significant increase of EGFP fluorescence after 24 h exposure to all concentrations, except the highest (5.0 μg/μL). After 48 h exposure, a significant increase of EGFP fluorescence was detected at the lowest three concentrations (0.1, 0.5 and 1.0 μg/mL), while at higher concentrations and with prolonged exposure the EGFP fluorescence intensity was reduced (Figure 4D, Table 1). The viability measurements showed that VBL was highly cytotoxic. Although after 24 and 48 h exposure cell viability was not reduced by more than 30%, except at the highest concentration, after prolonged exposure it rapidly decreased. After 72 h exposure the viability was reduced by more than 40% at all concentrations and after 168 h exposure it decreased by more than 90% compared to the viability of non-treated control cells (Table 1).

TABLE 1. Cell viability and induction of EGFP fluorescence in p21 HepG2 GFP cells exposed to Methylmethane sulphonate (MMS), Benzo[a]pyrene (BaP), Cisplatin (CisPt) and Vinblastine (VLB) for 24, 48, 72, 120 and 168 h.

MMS	24 hours			48 hours			72 hours			120 hours			168 hours		
Conc. µg/ml	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]
0	100 ± 0,05	8,8 ± 0,87	1,00 ± 0,10	100 ± 0,02	12,8 ± 1,29	1,00 ± 0,10	100 ± 0,03	15,4 ± 1,58	1,00 ± 0,10	100 ± 0,06	10,5 ± 1,42	1,00 ± 0,14	100 ± 0,06	13,4 ± 1,94	1,00 ± 0,14
5,00	102 ± 0,04	9,4 ± 0,98	1,04 ± 0,10	117 ± 0,04	13,8 ± 1,79	0,92 ± 0,12	91 ± 0,04	16,1 ± 1,34	1,14 ± 0,09	79 ± 0,06	12,9 ± 1,03	1,56 ± 0,12	67 ± 0,08	16,7 ± 1,11	1,85 ± 0,11
10,00	108 ± 0,03	10,6 ± 1,17	1,11 ± 0,12	106 ± 0,02	15,8 ± 1,48	1,16 ± 0,11	104 ± 0,03	18,7 ± 2,08	1,16 ± 0,12	87 ± 0,05	18,5 ± 2,16	2,02 ± 0,17	63 ± 0,05	20,6 ± 1,13	2,43 ± 0,11
20,00	104 ± 0,04	10,8 ± 1,06	1,17 ± 0,11	109 ± 0,02	18,6 ± 1,47	1,33 ± 0,11	107 ± 0,03	22,6 ± 1,58	1,37 ± 0,10	79 ± 0,06	23,3 ± 0,87	2,82 ± 0,11	60 ± 0,06	25,1 ± 2,47	3,13 ± 0,16
40,00	110 ± 0,04	10,6 ± 1,13	1,09 ± 0,11	110 ± 0,02	20,5 ± 1,07	1,45 ± 0,09	102 ± 0,04	26,3 ± 1,58	1,67 ± 0,10	59 ± 0,05	24,8 ± 1,56	4,04 ± 0,14	37 ± 0,05	25,3 ± 2,17	5,03 ± 0,15
50,00	88 ± 0,04	10,4 ± 0,75	1,33 ± 0,09	104 ± 0,02	20,1 ± 1,35	1,51 ± 0,10	94 ± 0,03	25,5 ± 1,78	1,76 ± 0,11	51 ± 0,04	23,4 ± 0,81	4,34 ± 0,11	27 ± 0,04	24,5 ± 0,81	6,74 ± 0,10

BaP	24 hours			48 hours			72 hours			120 hours			168 hours		
Conc. µg/ml	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]
0	100 ± 0,05	9,8 ± 1,12	1,00 ± 0,11	100 ± 0,01	14,3 ± 0,98	1,00 ± 0,07	100 ± 0,08	17,6 ± 1,60	1,00 ± 0,09	100 ± 0,04	13,0 ± 1,30	1,00 ± 0,10	100 ± 0,04	17,1 ± 1,60	1,00 ± 0,09
0,05	110 ± 0,04	12,5 ± 1,42	1,16 ± 0,13	100 ± 0,02	15,5 ± 1,38	1,08 ± 0,08	91 ± 0,12	19,1 ± 1,87	1,19 ± 0,10	66 ± 0,04	16,2 ± 2,06	1,89 ± 0,13	55 ± 0,05	22,2 ± 1,77	2,36 ± 0,10
0,13	119 ± 0,03	18,1 ± 1,06	1,55 ± 0,11	109 ± 0,01	20,7 ± 1,73	1,33 ± 0,09	99 ± 0,11	24,7 ± 2,40	1,42 ± 0,11	58 ± 0,05	21,0 ± 1,30	2,79 ± 0,10	32 ± 0,04	26,2 ± 1,69	4,79 ± 0,10
0,25	112 ± 0,04	23,8 ± 1,04	2,17 ± 0,11	104 ± 0,02	27,1 ± 1,88	1,82 ± 0,10	98 ± 0,10	31,2 ± 2,50	1,81 ± 0,12	50 ± 0,03	28,0 ± 1,15	4,31 ± 0,09	23 ± 0,03	33,6 ± 0,56	8,54 ± 0,06
0,50	101 ± 0,04	39,9 ± 1,46	4,03 ± 0,13	105 ± 0,02	41,3 ± 2,13	2,75 ± 0,11	84 ± 0,08	45,4 ± 2,38	3,07 ± 0,11	46 ± 0,03	43,0 ± 1,50	7,19 ± 0,11	20 ± 0,03	45,7 ± 2,98	13,36 ± 0,13
1,26	96 ± 0,04	80,3 ± 2,55	8,54 ± 0,19	99 ± 0,02	90,8 ± 3,18	6,41 ± 0,14	76 ± 0,11	91,4 ± 4,14	6,83 ± 0,16	41 ± 0,03	80,0 ± 4,39	15,01 ± 0,22	17 ± 0,02	79,3 ± 2,01	27,28 ± 0,11

CisPt	24 hours			48 hours			72 hours			120 hours			168 hours		
Conc. µg/ml	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]
0	100 ± 0,05	13,5 ± 1,50	1,00 ± 0,11	100 ± 0,02	21,0 ± 1,89	1,00 ± 0,09	100 ± 0,08	24,1 ± 1,25	1,00 ± 0,05	100 ± 0,01	16,4 ± 2,26	1,00 ± 0,04	100 ± 0,11	11,3 ± 0,97	1,00 ± 0,02
0,41	90 ± 0,04	17,8 ± 2,28	1,47 ± 0,14	98 ± 0,02	25,4 ± 2,75	1,23 ± 0,11	92 ± 0,07	33,5 ± 3,48	1,51 ± 0,10	70 ± 0,01	26,8 ± 2,63	2,33 ± 0,05	41 ± 0,08	18,6 ± 0,79	4,01 ± 0,02
0,83	103 ± 0,04	17,6 ± 1,55	1,27 ± 0,11	97 ± 0,02	27,2 ± 2,56	1,34 ± 0,11	102 ± 0,08	36,3 ± 2,87	1,48 ± 0,09	71 ± 0,01	30,8 ± 2,46	2,65 ± 0,04	37 ± 0,06	20,6 ± 0,59	4,93 ± 0,02
1,65	105 ± 0,05	18,6 ± 1,85	1,31 ± 0,12	89 ± 0,02	35,6 ± 2,26	1,90 ± 0,10	89 ± 0,07	48,8 ± 2,59	2,28 ± 0,08	56 ± 0,01	38,2 ± 1,34	4,16 ± 0,03	27 ± 0,06	25,7 ± 0,90	8,42 ± 0,02
3,30	100 ± 0,05	18,9 ± 1,57	1,40 ± 0,11	71 ± 0,02	39,8 ± 1,83	2,67 ± 0,09	80 ± 0,09	54,5 ± 2,48	2,83 ± 0,08	34 ± 0,01	41,5 ± 1,23	7,44 ± 0,03	22 ± 0,06	31,7 ± 0,85	12,75 ± 0,02
6,60	96 ± 0,05	20,0 ± 1,63	1,54 ± 0,12	51 ± 0,02	33,7 ± 3,35	3,15 ± 0,12	48 ± 0,09	43,2 ± 3,97	3,73 ± 0,11	21 ± 0,02	38,2 ± 2,43	11,09 ± 0,04	14 ± 0,07	32,8 ± 1,90	20,73 ± 0,03

VLB	24 hours			48hours			72 hours			120 hours			168 hours		
Conc. µg/ml	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]	Viab. (%)± SD[a]	GFP int.± SD[b]	GFP ind.± SD[c]
0	100 ± 0,01	13,0 ± 0,45	1,00 ± 0,03	100 ± 0,00	18,2 ± 0,78	1,00 ± 0,04	100 ± 0,04	20,8 ± 1,27	1,00 ± 0,06	100 ± 0,03	18,5 ± 1,06	1,00 ± 0,06	100 ± 0,10	20,8 ± 1,17	1,00 ± 0,06
0,10	94 ± 0,01	26,3 ± 1,89	2,15 ± 0,09	81 ± 0,01	25,9 ± 2,01	1,76 ± 0,08	57 ± 0,01	28,7 ± 1,50	2,41 ± 0,07	18 ± 0,02	18,2 ± 1,12	5,46 ± 0,06	5 ± 0,01	17,2 ± 1,22	16,55 ± 0,05
0,50	95 ± 0,01	24,3 ± 2,87	1,96 ± 0,13	80 ± 0,01	22,3 ± 2,27	1,54 ± 0,08	58 ± 0,01	20,7 ± 1,50	1,71 ± 0,07	14 ± 0,00	12,2 ± 0,92	4,70 ± 0,05	4 ± 0,00	11,2 ± 1,22	13,45 ± 0,05
1,00	90 ± 0,00	19,6 ± 0,49	1,67 ± 0,04	85 ± 0,00	18,0 ± 0,75	1,17 ± 0,04	56 ± 0,01	14,9 ± 0,58	1,28 ± 0,04	16 ± 0,02	9,6 ± 1,01	3,24 ± 0,06	5 ± 0,01	8,3 ± 0,57	8,03 ± 0,04
2,50	85 ± 0,01	17,2 ± 0,82	1,55 ± 0,05	77 ± 0,01	15,0 ± 1,08	1,07 ± 0,05	52 ± 0,00	11,7 ± 0,47	1,08 ± 0,04	16 ± 0,01	7,3 ± 0,78	2,45 ± 0,05	5 ± 0,01	7,3 ± 0,80	7,07 ± 0,05
5,00	78 ± 0,04	10,4 ± 0,76	1,03 ± 0,05	70 ± 0,01	7,9 ± 1,07	0,62 ± 0,05	42 ± 0,01	9,6 ± 0,75	1,10 ± 0,05	15 ± 0,00	3,3 ± 0,56	1,17 ± 0,04	6 ± 0,01	3,7 ± 0,67	2,95 ± 0,04

[a] Cell viability was measured with the MTS assay and is expressed as % of viable p21 HepG2 GFP cells treated with MMS, BaP, CisPt and VLB compared to control, non-treated cells.
[b] Intensity of EGFP fluorescence measured at 485 nm excitation and 535 nm emission wavelengths.
[c] Relative EGFP induction expressed as the ratio between the EGFP fluorescence intensity of the treated cells and non-treted control cells, normalized to cell viability.
Light grey areas represent significantly different values compared to control (P<0.001)
Dark grey areas represent values of cell viability below 70% of control.

Discussion

We developed a novel microplate genotoxicity assay test system using EGFP as the reporter that enables simple and rapid detection of genotoxic agents. The assay is based on a p21HepG2GFP cell line that contain the EGFP reporter under the control of the *p21* promoter. In response to DNA damage, the transcription of the *p21* promoter is activated leading to concurrent accumulation of EGFP that is detected in intact cells with the fluorescence microplate reader.

Several reporter genotoxicity assays using mammalian cells and DNA damage responsive genes as the biomarkers of genotoxic injury have been described. Todd *et al.*[21] were the first who exploited DNA damage responsive genes: *p53R2*, *GADD45a* and *GADD153* for construction of a chloramphenicol acetyl transferase (CAT) reporter that was stably integrated into HepG2 cells. However, there is very little data published from this assay. The *p53R2*, one of the p53 target genes that encode a subunit of ribonucleotide reductase, which is expressed mainly in response to DNA damage[22, 23], has been used more recently for construction of a reporter assay with MCF7 and HepG2 cells using luciferase as the reporter gene.[24,25] The growth arrest and DNA damage (*GADD*)-inducible gene family is another group of target genes regulated by p53 that are expressed in response to various environmental stresses including DNA damage. In response to DNA-damage *GADD* genes induce arrest in cell cycle progression at G1/S or G2/M checkpoints.[26] Hastwell *et al.*[27] developed an assay that exploits a reporter system in which the expression of EGFP is controlled by regulatory elements of the *GADD45a* gene hosted in the p53-competent human lymphoblastoid TK6 cell line. A thorough validation of this assay showed its high sensitivity and specificity.[28] The assay is commercially available as GreenScreen HC assay provided by Gentronics Ltd (UK). Recently Zhang *et al.*[29] developed a stably transfected HepG2 cell line containing *GADD153* promoter regions coupled to the luciferase reporter gene.

p21 belongs to p53 mediated DNA damage responsive genes that has not been previously used as an indicator of genotoxic injury. For the construction of our reporter system, we selected the *p21* promoter to drive EGFP expression since recently Ellinger-Ziegelbaure *et al.* [17] reported that *p21* was up-regulated only by genotoxic carcinogens in the liver of rats exposed to genotoxic and non-genotoxic carcinogens. The *GADD45a* gene was up-regulated by both, genotoxic and non-genotoxic carcinogens. Therefore, it could be that our test system will allow for discrimination of the two types of carcinogens.

We evaluated the sensitivity of the assay and established optimal exposure conditions for induced EGFP fluorescence data collection using four model genotoxic agents with known mechanisms of action. The results showed that the optimal exposure time for detection of EGFP expression is 48 h. Although the EGFP fluorescence in cells exposed to MMS, BaP and CisPt increased with the time of exposure, the lowest effective concentration (LOEC) at which a significant increase in EGFP fluorescence was observed did not change. Longer exposures lead to reduced cell viability, resulting either from cytotoxicity or inhibition of cell division that may interfere with the reliability of EGFP fluorescence detection and calculation of the relative EGFP induction ratio as a quantitative measure of genotoxic activity. When measurements of EGFP fluorescence are performed in wells with a very different number of control vs. treated cells, interference with the optical measurements due to changes in the background reflectance and absorbance of the microplate is possible. The half-life of EGFP in mammalian cells has been reported to be in the range of 24 – 48 h.[30,31] As the relative EGFP induction ratio is normalized to the cell viability, which was significantly reduced after prolonged exposure, normalization might give unreliable high values of the EGFP induction ratio due to the accumulated EGFP. The reason for unreliable results can also be cytotoxicity per se. The breakdown of cell integrity can lead to non-specific DNA damage and thus to p21 activation, which does not lead to genetic consequence if cells are dying or dead. Therefore, only the EGFP measurements at which cell viability was not reduced by more than 20% were considered as relevant for genotoxicity evaluation while reduction of cell viability by more than 30% was considered as cytotoxic.

The alkylating agent MMS is a known mutagen and rodent carcinogen.[32,33] Recently, it has been reported that MMS induces phosphorylation of the p53 protein and increases its DNA-binding properties to cause an increased expression of *p21*.[34] MMS induced a dose- dependent increase of EGFP fluorescence with a LOEC of 20 μg/mL. The sensitivity of our system for MMS genotoxicity detection is similar to that of the GreenScreen HC assay with the *GADD45a* promoter fused to an EGFP gene, in which the LOEC was 25 μg/mL[27], and to that with the *p53R2* promoter fused to the luciferase reporter

in MCF-7 cells in which the LOEC was around 10 μg/mL.[25]

BaP is an indirectly-acting genotoxic carcinogen that is metabolized by cytochrome P450 enzymes to diol epoxide BPDE, which binds covalently to guanine bases.[35] Exposure to BaP is known to induce activation of the p53 protein and its downstream regulated genes including *p21*.[36,37] The LOEC for BaP was at 0.13 μg/mL (0.5 μM), and at the highest tested concentration 1.26 μg/mL (5 μM) the relative EGFP induction ratio was 8.54 after 24 h exposure. HepG2 cells transfected with GADD153 fused to luciferase were significantly more sensitive for BaP genotoxicity detection; the LOEC was 0.0025 μg/mL (10 nM).[29] The authors ascribed high sensitivity of their assay compared to other reporter systems to the sensitivity of luciferase, which seems to be higher than that of EGFP.[29] In MCF-7 cells transfected with *p532R* coupled to the luciferase reporter gene, the LOEC for BaP was 0.26 μg/mL when tested without metabolic activation and 0.12 μg/mL in the presence of metabolic activation.[24] The lower sensitivity of MCF-7 cells in the absence of metabolic activation compared to HepG2 cells can be ascribed to their lower expression of metabolic enzymes. When using metabolically incompetent cells, the indirectly-acting genotoxic agents have to be tested in the presence of exogenous metabolic activation, usually S9 liver extracts. However, S9 is light-absorbing and fluorescent that can confound spectrophotometric measurements of fluorescence, which is the main limitation of reporter systems based on EGFP. For the GreenScreen HC test system, a protocol based on flow cytometry (FCM) has been developed for the detection of indirectly-acting genotoxic chemicals, and the LOEC for BaP was 1.25 μg/mL.[38] Thus, our test system with HepG2 cells represents great potential for direct detection of the indirectly-acting genotoxic agents.

A DNA cross-linker CisPt induces bulky lesions, which block DNA transcription *in vitro*.[39] The response to CisPt-induced DNA damage activates p53 through the ATR-Chk2 pathway.[40] The bulky DNA damage induced by different genotoxic chemicals such as DNA cross-linkers or BaP are repaired by nucleotide excision repair (NER). The studies showed that triggering of the signal transduction cascade that leads to phosphorylation of p53 or Chk1 requires recognition and processing of the lesions by NER.[41] In p21HepG2GFP, CisPt induced a dose-dependent induction of EGFP fluorescence. The LOEC was 0.41 μg/mL, which is more sensitive compared to the response observed with the GreenScreen HC assay in which the LOEC was 1 μg/mL.[27] The MCF-7 cells carrying the *p53R2* promoter linked to the luciferase reporter were less sensitive; the LOEC was around 10 μg/mL.[25]

VBL belongs to spindle poisons that block polymerization of tubulin into microtubules and inhibit cell division without directly damaging DNA.[42] These chemicals induce activation of p53 and cell cycle arrest mediated by p21[43], although the details of this process are not clear. VBL induced a significant increase of EGFP fluorescence at the lowest tested concentration of 0.1 μg/mL, which decreased at higher concentrations. VBL showed a cytostatic effect, which is reflected in rapid decrease of relative cell viability during prolonged exposure. At all tested concentrations, the relative cell viability was reduced by 20% or more already after 48 h exposure. Therefore, only the effect observed after 24 h exposure was considered. Lower induction of *p21*-mediated EGFP expression at higher concentrations may be explained by its toxicity. In MCF-7 cells with the *p53R2*-mediated luciferase reporter, VBL induced comparable cytotoxicity and induction of the reporter gene[25] as we observed in our test system. VBL was highly cytotoxic also in the GreenScreen HC test with LOEC for growth inhibition and GFP induction at 0.02 μg/mL.[27]

In conclusion, our study showed that the new biosensor system with the human hepatoma cell line p21HepG2GFP efficiently detects different types of genotoxic agents. Its main advantages are the use of metabolically competent human cells that allow for direct detection of indirectly-acting genotoxic chemicals and spectrofluorimetric measurement of reporter genes on a microplate format ensuring easy handling and rapid data acquisition. After further validation of the test system, which is currently in progress, this genotoxicity assay based on *p21* gene expression can become a valuable tool with potential applications in the fields of chemical and drug safety evaluation as well as for environmental and occupational monitoring of exposure to chemical agents.

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research article

Attitudes of midwifery students towards teaching breast-self examination

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Background. The purpose of this study was to asses the attitude of undergraduate midwifery students towards teaching other women in methods of breast self-examination (BSE).

Participants and methods. The study was performed at the beginning and at the end of students' study at the Faculty of Health Sciences in Ljubljana, Slovenia. It was carried out during the academic year 2002/2003 and involved 28 first and 25 third year undergraduate midwifery students. The data were gathered from questionnaires and processed with the use of descriptive and inferential statistics.

Results. All study participants were of the opinion that teaching other women in methods of BSE is of great importance for an early detection of breast cancer (BC) and that this task ought to be one of their duties. There were no significant differences between the two groups when the readiness to upgrade their own knowledge of BSE or when the optimism regarding the progress in breast cancer detection and therapy in the future were concerned.

Conclusions. The readiness of midwifery students to pass the knowledge of BSE to other women could help to increase their breast health awareness and thus improve their willingness and ability to detect early changes, associated with BC.

Key words: midwifery students; breast cancer, teaching breast self-examination; breast health awareness; specific education

Introduction

Breast cancer is the most common form of cancer and the cause of death from cancer in women in Slovenia (population two million), where it was diagnosed in 1112 women in the year 2006 and has shown a fivefold increase in its incidence in the last five decades.[1-3] Breast cancer incidence may be low in less developed countries, but survival rates of those suffering from this disease are also low, thus making it an important health care problem all over the world.[4,5]

The efficacy of BC screening with mammography has been confirmed in a number of randomized and observation studies.[6,7] However, small palpable tumours can also be detected with the clinical breast examination (CBE), performed in clinical surroundings, and with BSE, which can be done by properly trained women themselves.[6-8] Despite many controversies, it has been accepted that BSE increases the awareness of normal appearance and structure of breasts and the consequent ability to detect subtle changes that don't correspond to the normal features of healthy breasts. Breast health awareness may, therefore, increase the ability of women to find small palpable tumours early, leading them to consult their physician immediately after discovering undue changes in their breasts.[6,9,10] Unfortunately, few women perform BSE and there is little information on the attitude of individual

TABLE 1. The attitudes of midwifery students (Faculty of Health Sciences, Ljubljana, Slovenia, academic year 2002/2003) towards teaching BSE, receiving additional BSE education and breast towards BC detection and treatment in the future

		1. year (n = 28)	3. year (n = 25)	Chi-square	p-value
Teaching BSE helps early BC detection	Yes No	28 (100%) 0 (0%)	25 (100%) 0 (0%)	/	/
Teaching BSE should be one of midwives' duties	Yes No	28 (100%) 0 (0%)	25 (100%) 0 (0%)	/	/
I am willing to receive further BSE education	Yes No	26 (92.86%) 2 (7.14%)	22 (88%) 3 (12%)	0.362	0.5469
I favour practical demonstration of BSE	Yes No	22 (78.56%) 6 (21.44%)	16 (64%) 9 (36%)	0.9327	0.3341
I am optimistic about BC detection and treatment in the future	Yes No	24 (85.71%) 4 (14.29%)	23 (92%) 2 (8%)	0.5311	0.4664

n = number; BSE = breast self-examination; BC = breast cancer

women and specific groups of women towards this way of breast change detection.[3,6]

In this study, we, thus, assessed the attitude of midwifery students towards the teaching other women in methods of BSE. We also assessed how the specific education of midwifery students, gained during the course of their three year study, affects their attitude towards BSE. Finally, we examined their attitude towards the progress in BC detection and treatment in the future.

Participants and methods

Twenty eight first year and 25 third year midwifery students were included in the study carried out in the academic year 2002/2003 at the Faculty of Health Sciences in Ljubljana, Slovenia. There were no males among the midwifery students in both study groups in that academic year. The part of the study involving the first-year students was carried out at the end of their first term and the part involving third-year students at the end of their sixth term. A written consent was obtained from all the involved participants before enrolling them in the study and all were asked to complete the questionnaires with data about their personal characteristics. Questionnaires also included five statements concerning their attitudes whether teaching other women in methods of BSE helps in BC detection, whether teaching other women about BSE ought to be one of their duties, about their attitude regarding additional BSE training and the form of such training. Finally, they were asked to express their attitude about the development and successfulness of the BC treatment in the future.

The acquired data were sorted and analyzed with the help of Microsoft Excel 97 and SPSS 11.0 for Windows programs. Most answers were assessed with the use of Chi-square test for 2X2 contingency tables with which the differences in frequencies were verified. The results were considered as statistically significant if $p<0.05$.[11]

Results

The mean age of the first-year midwifery students in the study was 19.86 ± 1.18 (SD) years (range 18-23 years), the mean age of the third-year students was 21.76 ± 2.26 (SD) years (range 20-32 years). None of them had undergone a mammography or ultrasound breast examination prior to participating in the study.

All the participants, regardless of the year of study, were of the opinion that teaching BSE to other women helps in the early detection of BC. All were of the opinion that teaching and informing women of the advantages and disadvantages of BSE ought to be one of their duties. Most of the participants from both groups would be willing to receive additional training in how to perform BSE. The number of the participants willing to receive this training was slightly smaller in the group of the third-year students, but the difference was not statistically significant. In the group of the first-year students more participants were in favour of practical BSE lessons given live by experts and demonstrators as compared to a brochure on BSE obtained by mail (Table 1). The majority of the participants from both groups were optimistic in their opinion regarding the progress in the develop-

ment and successfulness of the BC detection and the treatment in the future. The number of participants holding such opinion was slightly larger in the group of the third-year students, the difference between both groups was not statistically significant (Table 1).

Discussion

In this study, we found that midwifery students could be regarded as having a positive attitude towards teaching other women in methods of BSE. They were of opinion that this type of teaching helps in the BC detection and that it ought to be one of their duties. Most of the participants were also willing to receive the additional training in BSE themselves. The opinion that they should play an active part in educating other women and that they should additionally educate themselves probably makes them optimistic in their views of the development and successfulness of the BC detection and the treatment in the future.

A favourable attitude of midwifery students towards BSE may come from an inherent motivation to become involved in problems associated with BC and from the specific education they gain during the course of their study. This knowledge makes them more informed than other women[5,12-18], giving them a profound insight into the anatomy, histology and physiology of healthy breasts, as well as into the significance of the incidence, mortality and pathogenic characteristics of BC.[19] Midwifery students are at the end of their study in possession of an important amount of information about BC and know that it is an important public health problem. In this context, it is safe to conclude that their specific education is of utmost importance.

A special emphasis should be given to the attitude of all the participants in the study that BSE is important in the early detection of BC. All of them would fully participate in teaching other women about BSE that certainly must include its advantages and disadvantages.[6,15] Their willingness to teach BSE should receive the additional support by giving the results of the studies investigating BSE some further consideration. According to some of the studies, BSE does not decrease the mortality caused by BC.[20-22] However, the results of one of such studies also show that the compliance of one of the study groups to BSE program (BSE every month or every two months) helped to achieve a higher rate of 15 year survival.[22] Some of the studies also show that BSE reduces the size of newly discovered primary breast tumours[20,23,24], which may lead to a prompter use of modern diagnostic procedures and enables carrying out more conservative surgical procedures.[10,25] Even when examining the negative results from some of these studies, a careful consideration should be given to local cultural values, level of readiness of participants to perform BSE and the likelihood that all of them were properly informed about BC issues.[20,26-29]

Despite some of the doubts mentioned above, the possibility that BSE is used for achieving the increased breast health awareness in women should be examined carefully.[6,30-32] The breast health awareness means accepting health responsibilities to a greater extent by being able to recognize a normal appearance and structure of breasts during different cycle periods and with regard to age, by being able to recognize undue changes and to inform the physician immediately.[6,23,30,31,33] If the breast health awareness in women is to increase, the easiest way to achieve this would probably be by teaching them how to perform BSE. In some opinions the breast health awareness should take the role of BSE[30], with BSE itself being not much different from what the concept of the breast health awareness includes.[23,34]

The majority of midwifery students in our study were willing to receive the additional education in relation to BSE. Most of them favoured the practical demonstration of the BSE procedures. A smaller interest in the additional training and practical demonstrations is understandable with the students finishing their study and, on the other hand, this smaller interest again proves the importance of knowledge gained during the study of midwifery.[19] A slightly bigger number of those who hold an optimistic view of the future of the BC treatment in the group of the third-year students probably also points to the importance of this knowledge.[18,35,36]

In this study we found that female midwifery students included in the study have a favourable attitude towards teaching BSE, although it is undoubtedly hard to learn and teach.[37-39] All considered BSE to be of great importance for an early breast cancer detection and all were willing to teach it to other women, including those with previously treated malignant disease.[40] As a consequence of their attitude towards BSE and specific contents of their study, midwifery students could towards the end of their study probably also perform CBE quite efficiently and relieve other women of difficult endeavour of learning all the steps of the appropriate and effective BSE.[41,42] In Slovenia and elsewhere, this application of their knowledge would be es-

pecially important when younger women are in question, because in their cases mammography screening is not so efficient.[9,23] On the other hand, the participants in this study could, due to their knowledge and other characteristics, represent a specific group continuing with quality and regular BSE. Their attitude towards and knowledge of BSE could, in due course, be evaluated again. However, in some of the less developed parts of the world, the knowledge of BSE could enable similarly educated midwives to have an important impact on the early detection of breast cancer and, along with other nurses, an important role in health promotion, prevention and education activities associated with breast cancer.[43,44]

In conclusion, in this study we show that midwifery students, a highly motivated and specifically educated group, are ready to pass the knowledge of BSE to other women, to receive the further BSE education themselves and are generally optimistic about the BC detection and the treatment in the future. In this way, they could have an important role in increasing the breast health awareness of other women and thus improve their willingness and ability to detect early changes associated with BC.

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case report

Genetic testing for young-onset colorectal cancer: case report and evidence-based clinical guidelines

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Background. Young-onset colorectal cancer is clinicopathologically different from older-onset colorectal cancer and tends to occur in patients with hereditary germline conditions such as Lynch syndrome and familial adenomatous polyposis.

Case report. We describe the case of a 44-year-old man with a paternal history of colon polyps, a personal 2-year history of hematochezia, and a diagnosis of rectal cancer. Further clinical evaluation of the patient at our institution determined the cancer to be stage IIIA. The patient underwent genetic counseling and testing, which indicated he was negative for the most common familial cancer syndromes. After treatment with neoadjuvant chemoradiotherapy, surgery, and adjuvant chemotherapy, the patient has done well. We review the hereditary cancer syndromes and genetic tests to consider for patients with early-onset colorectal cancer.

Conclusions. This case underscores the importance of following cancer-screening guidelines.

Key words: adenomatous polyposis coli; attenuated familial adenomatous polyposis; colorectal cancer; familial adenomatous polyposis; microsatellite instability; *MYH*-associated polyposis

Introduction

Colorectal cancer (CRC) is a common malignancy in North America and Europe; most patients have sporadic disease without a known genetic predisposition to the illness. With the exception of the hereditary germline conditions Lynch syndrome, familial adenomatous polyposis (FAP), and attenuated FAP, which are marked by an early age of onset and familial CRC clustering, most cases of CRC do not develop until age 65 years or older. Still, up to 20% of all cases of CRC arise in persons aged 50 years or younger who do not have Lynch syndrome or FAP.[1-3]

Lynch syndrome, formerly known as hereditary nonpolyposis colorectal cancer, is caused by a germline mutation in 1 of several DNA mismatch repair (MMR) genes and is the most common single-gene–related cause of hereditary colon cancer in North American and European populations.[4,5] CRC in Lynch syndrome has unique histopathologic and clinical findings. These cancers tend to be more responsive to treatment despite being poorly differentiated.[2]

One report based on the experience from 2 cancer registries in the United States, the National Program of Cancer Registries and the Surveillance, Epidemiology and End Results studies, emphasized that CRC is of concern for young adults: it is among the top 10 cancers in persons aged 20 to 49 years of all races.[6] Young-onset and older-onset CRC are clinicopathologically different in that young-onset CRC usually presents at a later stage and is more poorly differentiated.[6] Here, we present the case of a young middle-aged man with rectal cancer.

Case report

A 44-year-old man sought medical care in 2001 for a 2-year history of hematochezia. Colonoscopy performed at an outside institution in September 2001 showed a lesion within the rectum. Biopsy performed at that time confirmed a poorly differentiated, grade 3 adenocarcinoma, which measured 2×1.3×1 cm, with invasion through the muscularis mucosa. He came to our institution in October 2001 for further evaluation.

The patient's family history of cancer was primarily limited to a brother who received a diagnosis of chronic myelogenous leukemia at age 47 years. His father had colon polyps removed in his mid 50s, and a maternal aunt had colon polyps at age 35 years. Distant paternal relatives may have had CRC in old age.

The patient had been a smoker for the previous 16 years and had a history of gastroesophageal reflux disease. He had a history of sebaceous cysts on the left scapula and the back of the thighs and a lipoma on the inferior border of the right scapula. He did not have supernumerary teeth.

Evaluation of the patient included genetics counseling and testing because of his young age at diagnosis (Figure 1). Immunohistochemical testing (IHC) for MLH1, MSH2, and MSH6 showed intact MMR. The patient was not tested for the *APC* gene mutation because he was not suspected to have FAP (Figure 2). He was also not tested for *MYH*, which was not discovered until later.[7]

Endorectal ultrasonography with guided fine-needle aspiration biopsy of suspicious lymph nodes confirmed an ulcerated lesion extending from the anal verge to 3.5 cm proximally along the anterior wall of the rectum. Fine-needle aspirates from 2 enlarged perirectal lymph nodes were positive for adenocarcinoma. In the ascending colon, a 5-mm hyperplastic polyp was removed with cold snare.

Abdominal and pelvic computed tomography detected a 0.6-cm sclerotic lesion within the left iliac bone, which was considered benign after a bone scan demonstrated no bony metastases. Results of upper endoscopy were normal. On the basis of the clinical evaluations, the patient's cancer was determined to be stage IIIA (T2N1M0) by the American Joint Committee on Cancer 6th edition criteria.

Neoadjuvant chemoradiotherapy was recommended on the basis of the tumor stage and the proximity of the tumor to the prostate. From November through December 2001, the patient was given a continuous infusion of radiosensitizing 5-fluorouracil (5-FU) (225 mg/m^2). He was treated with 180 cGy in 28 fractions, for a total of 5040 cGy. He tolerated the therapy well, with some mild diarrhea, weight loss, decreased energy, and perianal irritation. The cancer in the rectum showed complete response to therapy.

In January 2002, the patient underwent surgery to have an abdominoperineal resection and permanent colostomy. He tolerated the procedure well, and no residual tumor was identified. Two of 31 regional lymph nodes were positive for grade 3 mucinous adenocarcinoma. In the following month, the patient had several perineal drain site infections and was treated for depression.

In April 2002 the patient began his first of 4 cycles of adjuvant 5-FU and leucovorin systemic chemotherapy. Prolonged neutropenia and gastrointestinal tract bleeding occurred in the middle of the first cycle of chemotherapy; therefore, the dosage of 5-FU (425 mg/m^2) was decreased by 10% (to 380 mg/m^2). He completed chemotherapy in August 2002.

Surveillance colonoscopies in the following years have been negative. The patient was last seen at our institution in January 2007. He has had no new gastrointestinal tract or genitourinary symptoms or problems. His appetite has returned to normal, and he feels strong. He has returned to work as a carpenter and is now being monitored with colonoscopy every 3 years.

Discussion

According to the Fearon and Vogelstein model of carcinogenesis, the accumulation of multiple mutations is required for the transformation of normal colonic mucosa into dysplastic adenomas and then into invasive carcinomas.[4] CRC can be classified broadly as exhibiting either chromosomal instability through gain-of-function mutations (APC/β-catenin pathway) or microsatellite instability (MSI) (defects in DNA MMR). In sporadic CRC, mutations are acquired in a stepwise fashion. In the case of single-gene hereditary colorectal cancer syndromes, all DNA-containing cells have a germline mutation in one allele of the involved gene and thus are a step closer to the accumulation of additional acquired mutations necessary to lead to CRC.

In human CRC, 80% of the tumors are microsatellite stable, which means they have intact DNA MMR and can correct single-base and small-loop base-pair mismatches present throughout the non-

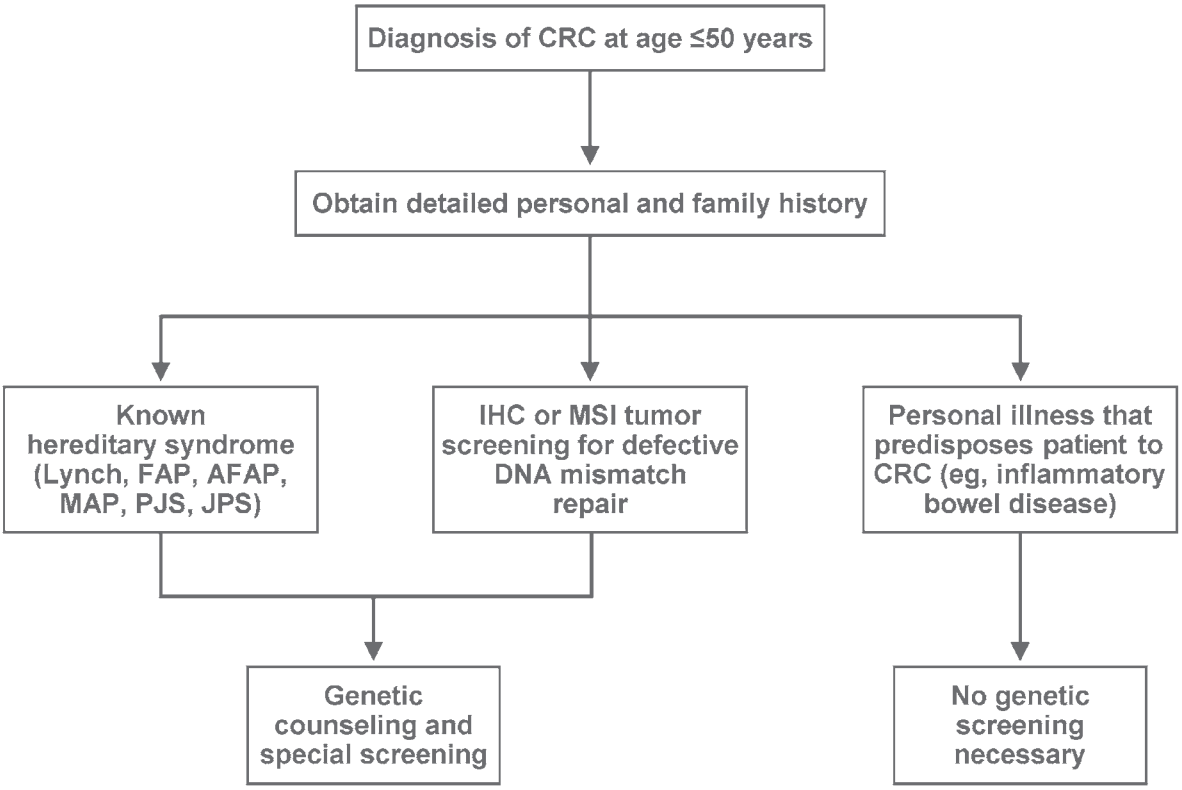

FIGURE 1. Scheme describing the recommended initial evaluation of a patient aged 50 years or younger with a diagnosis of colorectal cancer (CRC). AFAP indicates attenuated familial adenomatous polyposis; FAP, familial adenomatous polyposis; IHC, immunohistochemistry; JPS, juvenile polyposis syndrome; MAP, *MYH*-associated adenomatous polyposis; MSI, microsatellite instability; PJS, Peutz-Jeghers syndrome.

coding and coding regions of the genome. The remaining 20% of CRC tumors exhibit MSI due to defects in this DNA MMR pathway that corrects small base-pair mistakes in mononucleotide, dinucleotide, and trinucleotide repeat regions throughout the genome and are classified as having high or low MSI (MSI-H or MSI-L, respectively).[5] A small fraction of MSI-H tumors result from germline mutations in 1 of 4 DNA MMR genes—*MLH1*, *MSH2*, *MSH6*, and *PMS2*—and result in the hereditary CRC condition called Lynch syndrome. However, the greater proportion of MSI-H tumors arises via impairment of DNA MMR through hypermethylation of the *MLH1* gene.

Although only 15% to 20% of sporadic cancers are MSI-H, 90% of patients who meet the Amsterdam criteria for Lynch syndrome have MSI-H CRC.[8] Tumor DNA can be evaluated for MSI using polymerase chain reaction to amplify a panel of DNA sequences with nucleotide repeats.

Lynch syndrome, an autosomal dominant disorder, is the most common hereditary colon cancer syndrome. Mutations in 1 of the MMR genes usually result in truncated or lost protein product. Thus, tumors can be screened for defective DNA MMR by using polymerase chain reaction to test for MSI or IHC to test for loss of MMR protein expression. The results of IHC may then be used to direct germline sequencing toward a specific DNA MMR gene in young-onset cases or in persons with clinical or family history criteria suggestive of Lynch syndrome. Hypermethylation assays and *BRAF* V600E mutation testing of tumor DNA can be used to distinguish an MSI-H tumor with absent *MLH1* expression caused by hypermethylation of the *MLH1* promoter from a tumor caused by a germline *MLH1* mutation. Tumors with hypermethylation of *MLH1* and with the *BRAF* V600E mutation nearly always represent sporadic CRC not caused by a germline *MLH1* mutation and not associated with Lynch syndrome.

In the case presented here, the patient was tested for a familial syndrome because of his relatively young age at presentation (44 years); however, he lacked many of the features of either FAP or Lynch syndrome. The genetic diagnosis of Lynch

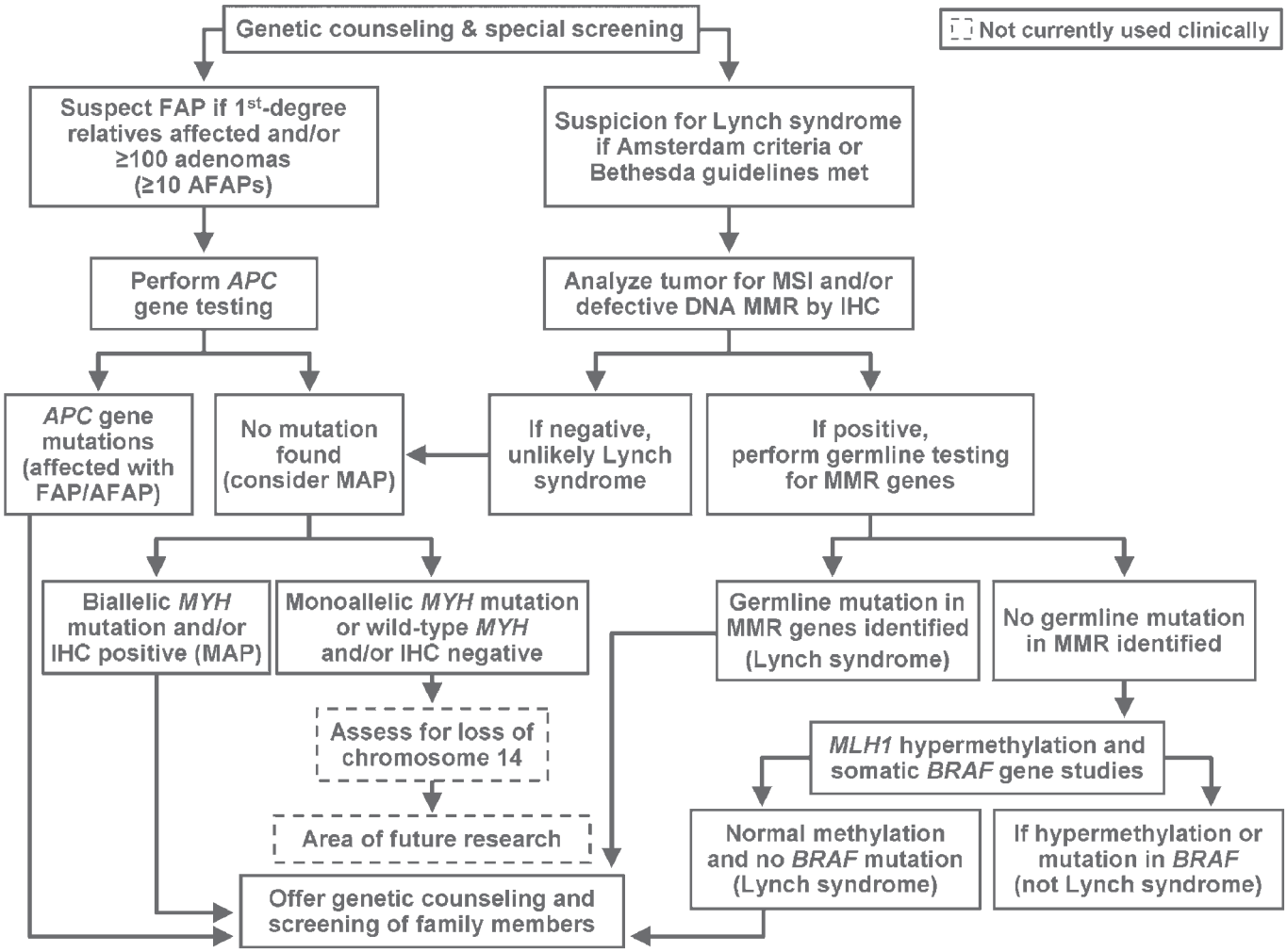

FIGURE 2. Scheme describing the recommended genetic testing for a patient with a diagnosis of colorectal cancer. AFAP indicates attenuated familial adenomatous polyposis; *APC*, adenomatous polyposis coli; *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; FAP, familial adenomatous polyposis; IHC, immunohistochemistry; MAP, *MYH*-associated adenomatous polyposis; *MLH1*, MutL homolog 1; MMR, mismatch repair; MSI, microsatellite instability; *MYH*, MutY homolog.

syndrome requires a germline mutation in 1 of the MMR genes. The patient's tumor showed normal expression of the MMR genes *MLH1*, *MSH2*, and *MSH6* by IHC. MSI testing was not performed. A previous study from our institution showed that a normal IHC test for MLH1 and MSH2 has a 96.7% positive predictive value for a microsatellite stable/MSI-L phenotype.[9] On the basis of the IHC data alone, it is highly unlikely that the patient has a germline mutation in an MMR gene, which would lead to Lynch syndrome with MSI.[9]

Another hereditary CRC syndrome that can be considered is *MYH*-associated polyposis (MAP). MAP has a phenotypic overlap with FAP, attenuated FAP, and Lynch syndrome; biallelic carriers have an 80% cumulative lifetime risk of CRC by age 70 years.[10] In several studies, among patients with early-onset CRC (diagnosed before age 50 years) who tested negative for Lynch syndrome, 1% to 2% were biallelic carriers of the *MYH* mutation.[11-13]

CRC screening and testing recommendations

Although CRC does not usually develop until age 65 years or older, up to 20% of CRC cases will arise in persons 50 years or younger who do not have either of the known hereditary CRC conditions.[1-3,6,14] The American Gastroenterological Association has published guidelines for CRC screening for aver-

age-risk and higher-risk patients.[15,16] Persons with a family history of CRC or adenomatous polyps (a first-degree relative with CRC or adenomatous polyps diagnosed before age 60 years, or 2 first-degree relatives with CRC diagnosed at any age) should have screening colonoscopy starting at age 40 years, or 10 years younger than the earliest diagnosis, whichever comes first, with repeat colonoscopy every 5 years.

If testing for MMR is negative, patients with early-onset CRC may be tested for *MYH* mutations, regardless of their family history or the number of colon polyps. IHC can be used in clinical practice to test for MAP regardless of the specific *MYH* mutations.[17]

Conclusions

The case presented here highlights that CRC can occur at an age younger than the cancer-screening guidelines suggest for average-risk patients and also shows the importance of using family history to determine the timing of the first CRC screening. Had our patient undergone his first screening colonoscopy at age 40 years as recommended by the American Gastroenterological Association—given that the patient's father had colon polyps removed before age 60 years—his CRC might have been diagnosed at an earlier stage. Similarly, the patient's 2-year history of hematochezia warranted a colon examination. We stress the importance of acknowledging and pursuing these symptoms, even in younger patients.

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research article

A neutron track etch detector for electron linear accelerators in radiotherapy

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Background. Electron linear accelerators in medical radiotherapy have replaced cobalt and caesium sources of radiation. However, medical accelerators with photon energies over 10 MeV generate undesired fast neutron contamination in a therapeutic X-ray photon beam. Photons with energies above 10 MeV can interact with the atomic nucleus of a high-Z material, of which the target and the head of an accelerator consist, and lead to the neutron ejection.

Results and conclusions. Our neutron dosimeter, composed of the LR-115 track etch detector and boron foil BN-1 converter, was calibrated on thermal neutrons generated in the nuclear reactor of the Josef Stefan Institute (Slovenia), and applied to dosimetry of undesirable neutrons in photon radiotherapy by the linear accelerator 15 MV Siemens Mevatron. Having considered a high dependence of a cross-section between neutron and boron on neutron energy, and broad neutron spectrum in a photon beam, as well as outside the entrance door to maze of the Mevatron, we developed a method for determining the effective neutron detector response. A neutron dose rate in the photon beam was measured to be 1.96 Sv/h. Outside the Mevatron room the neutron dose rate was 0.62 μSv/h. PACS: 87.52. Ga; 87.53.St; 29.40.Wk.

Key words: electron linear accelerator; photoneutron; track etch detector; neutron dose equivalent

Introduction

Nowadays, cobalt and caesium teletherapy machines in medical radiotherapy are being replaced by linear accelerators.[1] The great advantage of this new equipment is that it has no attached radioactive source, which makes them safer from the radiological point of view. However, medical accelerators with photon energies over 10 MeV generate undesired fast neutron contamination in a therapeutic beam. Photons with energies above 10 MeV can interact with the atomic nucleus of a high-Z material, of which the target and the head of the accelerator consist, and lead to the neutron ejection. Consequently, this can increase the patient dose and pose a problem in room shielding dosimetry, which is essential for the quality assurance in radiotherapy.[2,3]

Neutrons are principally produced through giant dipole resonance in a nuclear reaction (γ, n) between photons and target nuclei.[4] The giant resonance process produces two groups of neutrons; the first and the largest group has a spectrum, which can be described by a Maxwellian distribution, with the most probable energy, typically between 0.4 and 1 MeV; the second group of neutrons is produced by direct emission and is somewhat higher in energy (up to 10-20% of the total neutrons in general).[5] The mean energy of the neutron spectrum generated by the (γ,n) reaction is around a few MeV, but, at the patient plane, neutrons have a more complex distribution and a less mean energy. As beam energies increase (>10 MeV), an undesirable photoneutron dose also increases. Otherwise one can expect, that inside the area treated by linear accelerator, the neutron dose in a tissue will not exceed 1% of the photon dose.[6]

Material and methods

The neutron dosimeter consisted of the LR-115 track detector and boron foil BN-1 (Kodak-Pathe,

France) or ^{10}B converter for reaction (n, α); it was calibrated on neutrons generated in the nuclear reactor of the J. Stefan Institute (Slovenia).[7] Neutron irradiation was carried out in the thermal column of the TRIGA Mark II reactor where the neutron flux was $\Phi = 3.33 \times 10^5$ cm^{-2} s^{-1}; for irradiation time, t = 240 s, we got the fluence, $f = \Phi t = 7.99 \times 10^7$ cm^{-2}.

The LR-115 detectors, 2x3 cm^2, were etched in a 10% NaOH aqueous solution at 60 °C for 120 min and afterwards counted visually using a microscope of (10x16) magnification.

The irradiated neutron track etch detector had a net track density $D_t = (63394 - B) \cong 63370$ cm^{-2}, where the background, B, was 24 cm^{-2}; the standard deviation of the D_t was $s_{Dt} = 570$. The response, r_t, of the neutron dosimeter for thermal neutrons was:

$$r_t = D_t/f = (7.930 \pm 0.071) \times 10^{-4}, \quad [1]$$

where the error was given as a standard deviation, $s_{rt} = s_{Dt}/f = 7.1 \times 10^{-6}$.

The electron linear accelerator Siemens Mevatron 15 MV has been used as an X-ray radiotherapy source in the Clinical Hospital Osijek. The same accelerator was used to perform experiments for determining a dose equivalent of undesirable photoneutrons by using the neutron track etch detector.

Results and discussion

Linear accelerator neutrons - track detector in the beam

In order to determine a dose equivalent of photoneutrons produced by linear accelerator, operating in a photon production mode at an acceleration potential of 15 MV, we used the LR-114 track etch detector, which was positioned in the beam at 1 m from the accelerator head.

For fast neutrons with higher energy, a detector response should be lower; having considered a total cross-section of neutrons on boron, ^{10}B (n, α) ^7Li, depending on neutron energy (σ(E), Figure 1)[8], as well as a neutron fluence spectrum on energies by the linear accelerator (Figure 2)[2], we divided neutron energy E_a (MeV) in the two areas as follows: the first area , $0 < E_{a1} < 0.5$, and the second one, $0.5 \leq E_{a2} \leq 7.5$.

Afterwards, we estimated the respective mean cross-sections $\sigma_t = 1000$ b (for thermal neutrons), $\sigma_{a1} = 1.33$ b and $\sigma_{a2} = 0.25$ b (for energies E_{a1} and E_{a2}, respectively) from the curve σ(E) in Figure 1, and

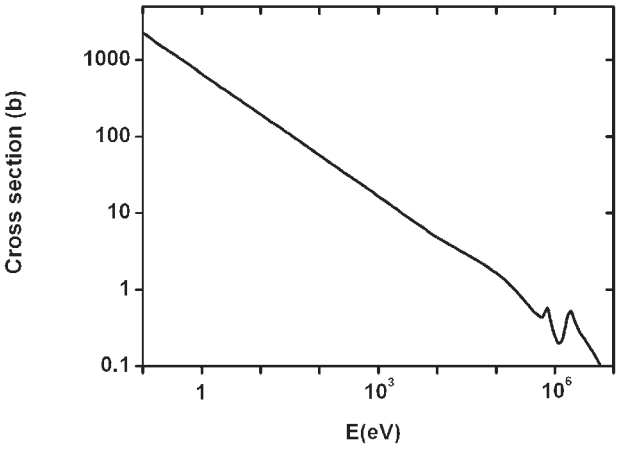

FIGURE 1. The total cross-sections of neutrons on boron (σ/b) versus neutron energy (E/eV).[8]

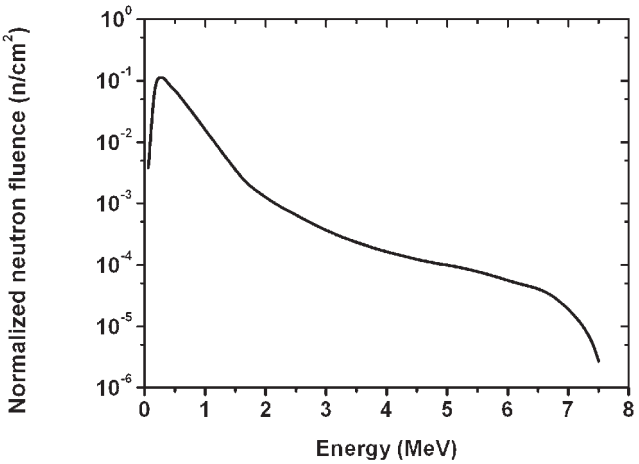

FIGURE 2. Neutron spectrum produced in 15 MV linear accelerator after crossing the tungsten head; fluence (f/n cm^{-2}) versus neutron energy (E/MeV).[2]

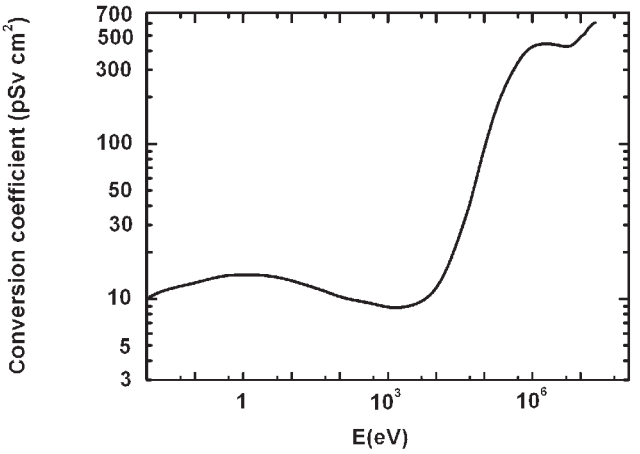

FIGURE 3. Conversion coefficient (k/pSv cm^2) from neutron fluence to personal dose equivalent versus neutron energy (E/eV).

we determined the neutron detector responses for the tow energy areas as follows: $\sigma_t/\sigma_{a1} = r_t/r_{a1}$, $\sigma_t/\sigma_{a2} = r_t/r_{a2}$, where the r_t was the response that had already been calculated by using equation [1]. Thus we got $r_{a1} = 1.06 \times 10^{-6}$ and $r_{a2} = 2.00 \times 10^{-7}$, with the standard deviations of 9.1×10^{-9} and 1.8×10^{-9}, respectively, and we were able to determine an average or effective value of the neutron detector response r_{ae}, but as a weighted or pondered mean.[9]

Therefore, we took the surfaces under the neutron fluence spectrum curve (Figure 2; f(E)) for the two energy areas as above and we got the relative surfaces: $s_{a1} = 0.58$ and $s_{a2} = 0.42$, those had the meaning of the relative frequencies in the calculation of the pondered mean (for instance, s_1 is the ratio of the surface under the curve between 0 and 0.5 MeV to the total surface under the curve between 0 and 7.5 MeV). The mean or effective detector response was: $r_{ae} = r_{a1}s_{a1} + r_{a2}s_{a2} = (6.97 \pm 0.07) \times 10^{-7}$.

For a measured detector density D_a, the respective fluence was (like in equation [1]): $f_{ae} = D_a / r_{ae}$. When the neutron fluence is known, a conversion coefficient (k) from neutron fluence to dose equivalent, depending on neutron energy, gives a personal dose equivalent (H_a), as follows:[10] $H_a = k_{ae} f_{ae}$.

Having considered a great dependence of k on neutron energy (Figure 3), we calculated the average or effective k_{ae} for the two energy areas, like above, and we used the same relative frequencies s_{ai} (i = 1, 2); taking the average values of k_a for the neutron energies E_{a1} and E_{a2} as $k_{a1} = 200$ and $k_{a2} = 430$ pSv cm^2, respectively, we calculated the effective conversion factor as follows:

$$k_{ae} = k_{a1}s_{a1} + k_{a2}s_{a2} = 296.4 \text{ pSv cm}^2.$$

Thus, the measured detector net density $D_a = (383.1 \pm 0.04)$ cm^{-2} corresponded to the following dose equivalent:

$$H_a = k_{ae}f_{ae} = k_{ae}/r_{ae}\, D_a = 425.5 D_a \text{ (μSv)} , \qquad [2]$$

$$H_a = (0.163 \pm 0.002) \text{ Sv}$$

The dose rate was calculated as a ratio of the dose equivalent and exposure time, t, or:

$\dot{H} = H/t$, and for t = 5 min, we got the dose rate $\dot{H} = (1.96 \pm 0.02)$ Sv/h.

The measurement errors were determined as variances or standard deviations for track densities in the following way (according to the Poisson distribution):

$$s_D^2 = s_{Db}^2 + s_B^2 = D_b + B ;$$

The dose equivalent variance was calculated as total differential of the function of the form like in equitation [2], which led to the following expression:

$$s_H^2 = (kD/r^2)^2 s_r^2 + (k/r)^2 s_D^2 .$$

Linear accelerator neutrons - track detector behind the wall

The neutron track etch detector was positioned outside the entrance door to the accelerator maze. The neutron spectrum in the same position was measured by Schraube *et al*.[11], hereby presented in Figure 4. We used the given neutron spectrum in the procedure for determining a neutron dose equivalent by the track detector, as above. Otherwise, neutron spectrum can vary depending on the wall construction of a room.

We divided neutron energy, E_b, in two areas as follows: the first area, $0 < E_{b1} < 100$ eV, and the second one, 100 eV $\le E_{b2} \le$ 3750 eV. Because the E_{b1} was the area of thermal neutrons, we estimated the respective mean cross-sections $\sigma_t = \sigma_{b1} = 120$ b and $\sigma_{b2} = 14.75$ b (for energies E_{b1} and E_{b2}, respectively) from the curve σ(E) in Figure 1, and we determined the neutron detector responses for the low energy areas as follows: $\sigma_{b1}/\sigma_{b2} = r_{b1}/r_{b2}$, where $r_{b1} = r_t$ was already assessed response by using equation [1]. Thus we got $r_{b1} = 7.94 \times 10^{-4}$ and $r_{b2} = 9.75 \times 10^{-5}$, and were able to determine the average or effective value of the neutron detector response r_{be} for neutrons in the energy area E_b.

As in the previous case, we took the surfaces under the neutron fluence spectrum curve (Figure 4; f(E)) for the two energy areas and we got the relative surfaces: $s_{b1} = 0.18$ and $s_{b2} = 0.82$, those had the meaning of the relative frequencies in the calculation of the pondered mean. The mean or effective detector response was: $r_{be} = r_{b1}s_{b1} + r_{b2}s_{b2} = 2.2 \times 10^{-4}$.

For the measured detector density D_b, the respective fluence is (like in equation [1]): $f_{be} = D_b / r_{be}$. When the neutron fluence is known, a conversion coefficient (k) from neutron fluence to dose equivalent, depending on neutron energy, gives a personal dose equivalent (H_a), as follows: $H_b = k_{be} f_{be.}$

Having considered a great depending k on neutron energy (Figure 3), we calculated the average or effective k_{be} for the two energy regions, like above, and we used the same relative frequencies s_{bi} (i = 1, 2); taking the average values of k_b for the neutron energies E_{b1} and E_{b2} as $k_{b1} = 11.58$ and $k_{b2} = 9$ pSv

cm^2, respectively, we calculated the effective conversion factor as follows:

$k_{be} = k_{b1}s_{b1} + k_{b2}s_{b2} = 9.45$ pSv cm^2.

Thus, the measured detector net density D_b = 1.2 ± 0.01 cm^{-2} corresponded to the following dose equivalent:

$H_b = k_{be}f_{be} = k_{be}/r_{be}\, D_b = 42.9\text{x}10^{-3}\, D_b$ (μSv),

$H_b = (0.050 \pm 0.0006)$ μSv.

The dose rate was calculated as a ratio of the dose equivalent and exposure time of 5 min, and we got the neutron dose rate outside the entrance door to the maze of the 15 MV Mevatron, $\dot{H}_b = (0.62 \pm 0.007)$ μSv/h. The linac room 1.7 m walls were constructed of barite concrete, with density of 3200 kg/m^3.

Although the obtained neutron dose rate outside the accelerator room was 3165 times smaller than the neutron dose rate in the photon beam, the measured dose rate $\dot{H}_b$ was not negligible from the aspect of personal dosimetry.

Some considerations of neutron energy attenuation after crossing the treatment room walls were performed for different concrete barrier thickness and materials. A neutron spectrum attenuation from the 15 MV linear accelerator, after passing a conventional 1 m concrete barrier, with density of 2260 kg/m^3, was measured by Facure *et al*., hereby presented in Figure 5.[2]

Observing the neutron spectra in Figure 5 and Figure 4, one can notice a broad neutron energy area in Figure 5 (from 0.1 eV to 10 MeV), that contributes to the neutron dose outside the Mevatron treatment room; neutrons that crossed the 1 m concrete barrier had higher energies than those behind the 1.7 m concrete wall (with energy below 1 keV).

In order to compare the neutron detection parameters r_e and k_e, we divided neutron energy E_c (MeV) of the spectrum (Figure 5) in the two areas as follows: first area $E_{c1} < 2$ MeV, and the second one 2 MeV ≤ E_{c2} ≤ 7.5 MeV.

Afterwards, we estimated the respective mean cross-sections $\sigma_t = \sigma_{c1} = 0.619$ b (for thermal neutrons, like above), $\sigma_{c2} = 0.203$ b (for energies E_{c1}, E_{c2}, respectively) from the curve σ(E) in Figure1, and we determined the neutron detector responses for the three energy areas as follows: $\sigma_{c1}/\sigma_{c2} = r_{c1}/r_{c2}$, where $r_{c1} = r_t = 7.94\text{x}10^{-4}$ was the response that had already been calculated by using the equation [1]. Thus we got $r_{c2} = 2.6 \times 10^{-4}$ and we were able

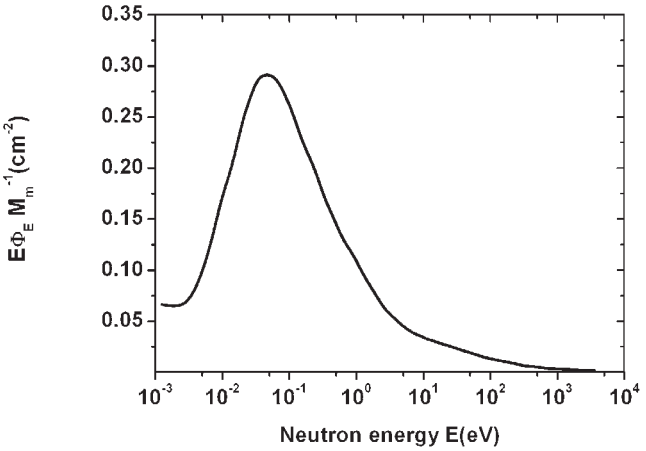

FIGURE 4. Neutron spectrum measured at position outside the entrance door to the maze of the 15 MV Mevatron.[11]

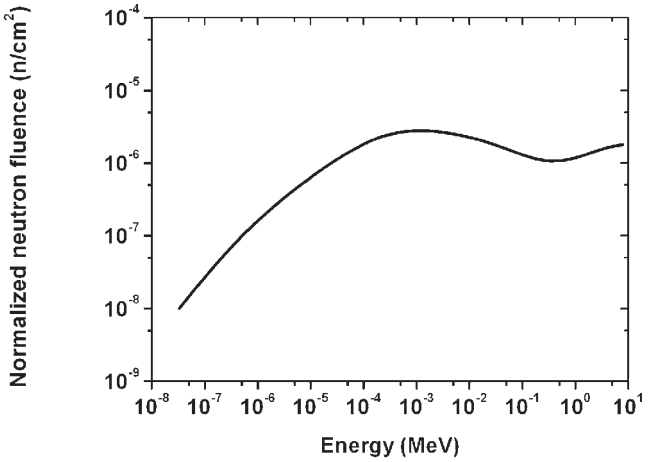

FIGURE 5. Neutron spectrum attenuation from a 15 MV linear accelerator, after concrete barrier of 1 m.

to determine the average or effective value of the neutron detector response r_{ce}, but as a weighted or pondered mean.

Therefore, we took the surfaces under the neutron fluence spectrum curve (Figure 5; f(E)) for the two energy regions, like above, and we got the relative surfaces: $s_{c1} = 0.22$, $s_{c2} = 0.78$, which had the meaning of the relative frequencies in the calculation of the pondered mean. The mean or effective detector response was: $r_{ce} = r_{c1}s_{c1} + r_{c2}\, s_{c2} = (3.78 \pm 0.03)\ \text{x}10^{-4}$.

Having considered a great depending *k* on neutron energy (Figure 3), we calculated the average or effective k_{ce} for the three energy areas, and we used the same relative frequencies s_{ci} (i = 1, 2, 3); taking the average values of k_c for the neutron energies E_{c1} and E_{c2} as $k_{c1} = 366$, $k_{c2} = 433$ pSv cm^2, respectively,

we calculated the effective conversion factor as follows:

$$k_{ce} = k_{c1}s_{c1} + k_{c2}s_{c2} = 418 \text{ pSv cm}^2.$$

Thus, since $k_c/r_c = 25{,}75\ k_b/r_b$, and according to equation [2], one can see that neutrons of the given distribution in Figure 5 (behind the 1 m concrete barrier) contribute to a neutron dose 26 times more than neutrons from the distribution in Figure 4 (behind the 1.7 m concrete wall).

Conclusions

The neutron dosimeter, consisting of the LR-115 track etch detector and boron foil BN-1, was calibrated on thermal neutrons generated in the nuclear reactor of the J. Stefan Institute (Slovenia), and was applied to dosimetry of undesirable neutrons in photon radiotherapy with the Siemens Mevatron 15 MV electron linear accelerator.

Having considered a broad neutron spectrum of energies in the photon beam and high dependence of the track detector response on neutron energy, we divided the spectrum in the two energy areas, below and over 2 MeV. Afterwards we determined the detector responses (r) for the energy areas using corresponding cross-sections for neutron and boron, and then we calculated the pondered or effective response depending on surfaces under the respective neutron spectrum areas. Using the empirical curve k(E), we performed the similar procedure for determining an effective conversion coefficient (k) from neutron fluence to dose equivalent, depending on neutron energy (E).

The relative measurement errors made by track etching method with the LR-115 detector were about 1%. It is to mention that we took the data from the empirical curves (e.g. σ(E), k(E)) as average values without respective experimental errors.

The measurement of the neutron dose equivalent by the track etch detector, positioned outside the Siemens Mevatron 15 MV room (room wall of 1.7 m, density of 3200 kg/m^3), gave the dose rate of 0.62 μSv/h.

Observing the neutron spectrum attenuation from 15 MV Mevatron behind the 1m concrete barrier, one was able to notice that the neutrons (Figure 5) had higher energies and contributed to a neutron dose 26 factor times more than neutrons from the distribution in Figure 4 (behind the 1.7 concrete wall).

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Vloga radioterapije pri zdravljenju melanoma

Strojan P

Izhodišča. V primeru melanoma je bila radioterapija dolgo časa obravnavana kot paliativna možnost zdravljenja in kot taka indicirana le za zdravljenje napredovalih ali sistemsko razširjenih primerov bolezni. V 70-ih letih preteklega stoletja je tehnološki napredek v radioterapiji, ki je bil tesno povezan z napredkom računalništva, prispeval k obujenemu zanimanju za radioterapijo tudi pri zdravljenju melanoma. Čeprav je pomanjkanje dobro zasnovanih prospektivnih in/ali randomiziranih kliničnih raziskav pomembno zaznamovalo vključevanje radioterapije v obstoječe načine zdravljenja melanoma, je v zadnjem obdobju radioterapija prepoznana kot nepogrešljiv del multidisciplinarne obravnave bolnikov s to boleznijo. Kar približno 23% bolnikov z melanomom naj bi bilo med svojo boleznijo obsevanih vsaj enkrat. V tem pregledu so opisane radiobiološke značilnosti melanoma, ki narekujejo načine frakcionacije, ki so v rabi pri tej bolezni. Poleg tega je podan pregled indikacij za obsevanje in pregled rezultatov kliničnih raziskav iz literature, ki so dale osnovo za uporabo radioterapije pri melanomu, ter kratek opis radioterapevtskih tehnik.

Zaključki. Temeljno zdravljenje pri melanomu je kirurško. Vendar takšno zdravljenje potrebuje dopolnilo, ko kirurški poseg ni radikalen ali je pri histopatološkem pregledu odstranjenega tkiva ugotovljena prisotnost neugodnih napovednih dejavnikov. Tudi pri bolnikih z neresektabilno boleznijo ali pri takih, ki niso sposobni za večji kirurški poseg oziroma ga odklanjajo, obstaja potreba po drugih učinkovitih načinih zdravljenja. Iz tega gledišča predstavlja radioterapija pomembno možnost: je učinkovita in varna, tako pri zdravljenju z namenom ozdravitve kot v paliativne namene, kar potrjujejo klinične izkušnje, kot tudi podatki iz strokovne literature.

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doi:10.2478/v10019-010-0007-y

Genetski označevalci pri oligodendrogliomih

Velnar T, Smrdel U, Popović M, Bunc G

Izhodišča. Oligodendrogliomi so po pogostosti pojavljanja med možganskimi tumorji na tretjem mestu. Zajemajo 2,5% primarnih možganskih tumorjev in 5-20% vseh gliomov. Sestavljajo jih celice, podobne oligodendrocitom.

Zaključki. Mutacije lokusov 1p in 19q se v oligodendrogliomih pogosto pojavljajo in so v histološko nejasnih primerih pomembne za točno diagnozo. So neodvisen ugodni napovedni dejavnik poteka bolezni in hkrati napovedujejo večjo občutljivost na kemoterapevtsko zdravljenje. Ugotavljanje omenjenih mutacij je izjemnega pomena pri načrtovanju zdravljenja, ker imajo bolniki z mutacijami lokusov 1p in 19q znatno daljše preživetje.

Radiol Oncol 2010; 44(1): 19-23.
doi:10.2478/v10019-010-0012-1

Odkrivanje raka debelega črevesja in danke z računalniško tomografsko kolonografijo

Sofić A, Bešlić Š, Kocijančič I, Šehović N

Izhodišča. Diagnostične metode pri odkrivanju in presejanju kolorektalnega raka so: rektalni klinični pregled, test blata na okultno krvavitev, rektoskopija, analiza DNA blata, irigografija, kolonoskopija in zadnjih nekaj let CT kolonografija. Namen raziskave je bil ugotoviti diagnostično zanesljivost CT kolonografije in počutje bolnikov ob preiskavi v primerjavi s klasično kolonoskopijo in irigografijo.

Bolniki in metode. V prospektivno raziskavo smo vključili 231 bolnikov, pri vseh smo naredili omenjene tri preiskave, ki smo jih želeli primerjati. Po opravljenih preiskavah so bolniki izpolnili vprašalnik, kako so se počutili med preiskavami. Diagnostično pozitivne rezultate smo potrdili s patohistološkimi vzorci, odvzetimi pri kolonoskopiji. Za vsako preiskavo smo izračunali občutljivost in specifičnost ter določili njihovo pozitivno in negativno napovedno vrednost.

Rezultati. Z vsemi tremi preiskavami smo odkrili 95 patoloških sprememb, 56 (59%) tumorjev in 39 (41%) polipov. Izmed polipov je patohistološka preiskava pokazala 34 adenomov, 3 tubuloviloznih adenomov in 2 lipoma. Med malignimi tumorji je bilo 55 adenokarcinomov in 1 limfom. Za odkrivanje polipov je CT kolonografija pokazala 89,7% občutljivost, irigografija 48,7%, in kolonoskopija 94,9% občutljivost. Za tumorje sta bili CT kolonografija in irigografija 100% občutljivi, irigografija pa 94.6%. Specifičnost in pozitivna napovedna vrednost sta bili za vse preiskave 100%. Vprašalnik o počutju med preiskavami je pokazal, da je CT kolonografija za bolnike izrazito najbolj udobna preiskava.

Zaključki. CT kolonografija je statistično značilno za bolnike najbolj udobna preiskava debelega črevesja in je značilno bolj občutljiva za odkrivanje polipov kot irigografija. Ob intraluminalnih spremembah lahko s CT kolonografijo prikažemo tudi spremembe ob lumnu debelega črevesja in nekatere druge naključno odkrite spremembe v abdomnu. Metoda je zelo uporabna za presejanje raka debelega črevesja in za oceno stadija pred zdravljenjem.

Radiol Oncol 2010; 44(1): 24-29.
doi:10.2478/v10019-010-0001-4

Razlikovanje hemangiomov od zasevkov v jetrih z magnetnoresonančnim difuzijskim slikanjem

Inan N, Kilinc F, Sarisoy T, Gumustas S, Akansel G, Demirci A

Izhodišča. Namen raziskave je bil ugotoviti pomen magnetnoresonančnega difuzijskega slikanja pri razlikovanju hemangiomov od zasevkov v jetrih.

Bolniki in metode. V retrospektivni raziskavi smo pri 38 bolnikih analizirali 69 žariščnih sprememb v jetrih (33 hemangiomov in 36 zasevkov). Slikanje z difuzijo je bilo narejeno med zadržanim vdihom s t.i. tehniko *single-shot-echo-shot echo-planar spin eho* sekvenco s tremi različnimi b vrednostmi (0, 500 in 1000 sek/mm^2). Izračunali smo pripadajoče difuzijske koeficiente. Za kvantitativno oceno smo med skupinama primerjali intenzitete signala v žariščnih spremebah, razmerja v intenziteti signala med žariščnimi spremembami in jetri, difuzijske koeficiente žariščnih sprememb ter razmerja difuzijskih koeficientov med žariščnimi spremembami in jetri. Za statistično analizo smo uporabili Studentov test.

Rezultati. Pri b vrednosti 500 sec/mm^2 nismo dokazali statistično značilne razlike ($p>0,05$). Pri b vrednosti 1000 sek/mm^2 sta bili obe – intenziteta signala ter razmerje med intenziteto signala med žariščnimi spremembami in jetri – pri zasevkih značilno višji kot pri hemangiomih ($p<0,001$). Pri mejni vrednosti 2,6 za razmerje intenzitet med žariščnimi spremembami in jetri sta bili občutljivost 86% in specifičnost 82%. Difuzijski koeficienti ter razmerje difuzijskih koeficientov med žariščnimi spremembami in jetri so bili za zasevke značilno nižje kot za hemangiome ($p<0,001$). Pri mejni vrednosti 1,7 za razmerje difuzijskih koeficientov med žariščnimi spremembami in jetri je bila občutljivost 88% in specifičnost 72%.

Zaključki. Magnetnoresonančno difuzijsko slikanje z visoko b vrednostjo pomaga razlikovati zasevek od hemangioma v jetrih.

Radiol Oncol 2010; 44(1): 30-33.
doi:10.2478/v10019-010-0011-2

Perkutana transkateterska arterijska embolizacija tope poškodbe vranice pri hemodinamsko stabilnem pacientu

Popovič P, Stanisavljević D, Jeromel M

Izhodišča. Neoperativno zdravljenje tope poškodbe vranice pri hemodinamsko stabilnem bolniku je v zadnjih letih postalo sprejemljiv način obravnave bolnika. Predstavljamo primer tope poškodbe vranice uspešno zdravljene s superselektivno embolizacijo z majhnimi delci.

Prikaz primera. Na Urgentni oddelek je bil pripeljan mlad igralec hokeja z anamnezo tope poškodbe trebuha, ki je nastala pred dvema urama. Z ultrazvočno preiskavo smo ugotovili poškodbo vranice III. stopnje s hematoperitonejem. Bolnika smo zdravili s selektivno embolizacijo distalne vranične arterije z majhnimi delci. Kontrolna ultrazvočna in računalniško tomografska preiskava po posegu in po enem letu sta pokazali zgolj majhno področje nepravilnosti v parenhimu.

Zaključki. Perkutana arterijska embolizacija vranice ima pomembno vlogo pri zdravljenju mehanskih poškodb vranice. Embolizacija je zlasti koristna pri poškodbah III. ali višje stopnje.

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doi: 10.2478/v10019-010-0002-3

Numerično modeliranje vpliva oblike elektroporacijskega električnega pulza na vnos molekul v celico

Miklavčič D, Towhidi L

Izhodišče. Elektrokemoterapijo – kombinacijo elektroporacije in kemoterapije – so uvedli zaradi želje, da bi povečali lokalno učinkovitost nekaterih kemoterapevtikov in zmanjšali stranske učinke. Elektroporacija, ki jo izzovemo z uporabo ustreznih električnih pulzov, povzroči znantno povečan vnos nekaterih molekul, ki sicer ne prehajajo plazmaleme, v celico. Elektroporacija in učinkovitost transmembranskega molekulskega transporta sta odvisni od parametrov električnih pulzov. To odvisnost so že pred časom eksperimentalno pokazali različni avtorji, pri čemer pa je treba poudariti, da teoretične osnove še niso v celoti znane in potrjene. V pričujoči študiji smo razvili model, s katerim smo raziskali vpliv oblike elektroporacijskega pulza na vnos molekul v celico z namenom napovedovanja izida elektroporacije celic.

Rezultati. Predstavljeni model temelji na že prej znanem kemično-kinetičnem opisu elektroporacije ob upoštevanju trapezne prepreke pore in samokonsistentnosti. Model smo nadgradili s transportom molekul prek plazmaleme, da smo tako lahko pridobili podatek o količini vnosa molekul v celico. Raziskali smo elektroporacijo celic z različnimi oblikami elektroporacijskih pulzov – monopolarnih pravokotnih pulzov z različnimi časi vzpona in upada pulza, bipolarnimi pulzi trikotne, pravokotne in sinusne oblike, ter uniporalne pulze z amplitudno modulacijo različnih stopenj s sinusnim signalom. Rezultati, ki smo jih dobili z uporabo opisanega modela in elektroporacijskih pulzov različnih oblik, se dobro ujemajo z že pred časom objavljenimi ekperimentalnimi rezultati dobljenimi v *in vitro* pogojih z enakimi oblikami elektroporacijskih pulzov.

Zaključki. Predstavljeni model je uporaben za opis in napovedovanje učinka elektroporacijskega pulza poljubne oblike na plazmalemo, spremembo njene prevodnosti in transporta molekul preko plazmaleme ter posledičnim vnosom molekul v celico.

Radiol Oncol 2010; 44(1): 42-51.
doi:10.2478/v10019-010-0010-3

Razvoj celičnega biosenzorskega sistema za zaznavanje genotoksičnosti na osnovi izražanja gena, ki se odziva na poškodbe DNA

Žager V, Čemažar M, Hreljac I, Lah TT, Serša G, Filipič M

Izhodišča. Izpostavljenost človeka genotoksičnim snovem v okolju in vsakodnevnem življenju resno groža zdravje ljudi. Hitra in zanesljiva ocena genotoksičnosti kemikalij je ključnega pomena pri razvoju novih kemikalij in zdravil, kot tudi pri opazovanju okolja. Tumorski supresorski gen *p21* je glavni ciljni gen aktiviranega proteina p53, ki je odgovoren za ustavitev celičnega ciklusa po poškodbi DNK. Ugotovili so, da genotoksični karcinogeni specifično povečajo njegovo izražanje. Cilj naše raziskave je bil razviti celični biosenzorski sistem za enostavno in hitro odkrivanje genotoksičnih snovi.

Metode. Metabolno aktivne človeške jetrne celice (HepG2) so bile genetsko modificirane (transfecirane) s plazmidom. Plazmid pa pod kontrolo promotorja za gen *p21* (p21HepG2 EGFP) nosi zapis za zeleno fluorescirajoči protein - Enhanced Green Fluorescent Protein (EGFP). Poškodbe DNA smo povzročili z genotoksičnimi snovmi z znanimi mehanizmi delovanja. Povečano intenzivnost fluorescence zaradi povečanega izražanja EGFP pod vplivom *p21* smo merili s spektrofluorimetrom za mikrotitrske plošče. Živost tretiranih celic smo določili s kalorimetričnim MTS testom.

Rezultati. Neposredno delujoča alkilirajoča snov methylmethane sulphonate (MMS) je povzročila statistično značilno povečanje tvorbe EGFP po 48 urah pri 20 µg/mL. Posredno delujoč rakotvoren benzo(a)pyren (BaP) in navzkrižno povezovalna snov cisplatin (CisPt) sta povzročila dozno odvisno povečanje EGFP fluorescence. To povečanje je bilo statistično značilno že pri koncentracijah 0,13 µg/mL oziroma 0,41 µg/mL. Vinblastin (VLB), inhibitor delitvenega vretena, ki ne inducira direktnih poškodb DNK, je povzročil le majhen porast intenzivosti EGFP fluorescence po 24 urah pri najmanjši koncentraciji (0,1 µg/mL), medtem ko je bila izpostavljenost višjim koncentracijam povezana z značilno zmanjšanim preživetjem celic.

Zaključki. Rezultati naše raziskave so pokazali, da je nov test, ki temelji na stabilno preoblikovani celični liniji p21HepG2EGFP, primeren za hitro in enostavno odkrivanje poškodb genetskega materiala, ki jih povzročajo kemične snovi.

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doi:10.2478/v10019-010-0009-9

Odnos študentk babištva do poučevanja samopregledovanja dojk

Plesničar A, Goličnik M, Kirar Fazarinc I, Kralj B, Kovač V, Kores Plesničar B

Izhodišča. Namen raziskave je bil ugotoviti, kakšen je odnos študentk dodiplomskega študija babištva do poučevanja samopregledovanja dojk.

Udeleženke in metode. Raziskava je potekala ob začetku in na koncu študija na Fakulteti za zdravstvene vede v Ljubljani, v akademskem letu 2002/2003. Vanjo je bilo vključenih 28 študentk prvega in 25 študentk tretjega letnika dodiplomskega študija. Podatki z izpolnjenih vprašalnikov so bili zbrani in analizirani s pomočjo deskriptivnih in inferenčnih statističnih metod.

Rezultati. Vse udeleženke raziskave so bile mnenja, da je poučevanje drugih žensk o samopregledovanju dojk zelo pomembno za zgodnje odkrivanje raka dojk in da bi tako poučevanje moralo biti ena od njihovih dolžnosti. Med skupinama študentk ni bilo statistično značilnih razlik glede pripravljenosti za izpopolnjevanje njihovega znanja samopregledovanja dojk ali glede optimizma v zvezi z napredkom odkrivanja in zdravljenja raka dojke v prihodnosti.

Zaključki. Pripravljenost študentk babištva, da svoje znanje samopregledovanja dojk posredujejo drugim ženskam, je povezano z njihovo ozaveščenostjo o zdravju dojk. S tem bodo lahko prispevale k zgodnjemu odkrivanju sprememb v dojkah ter omogočile njihovo zgodnjo diagnostiko in učinkovito zdravljenje.

Radiol Oncol 2010; 44(1): 57-61.
doi:10.2478/v10019-010-0005-0

Genetsko testiranje za ugotavljanje raka debelega črevesa in danke pri mladih: prikaz primera in na izsledkih temelječe smernice

Zhou Y, Boardman LA, Miller RC

Izhodišča. Rak debelega črevesa in danke, ki nastane pri mlajših, se kliničnopatološko razlikuje od takšnega raka pri starejših. Odkrivamo ga pri bolnikih z dednimi boleznimi, kot sta sindrom Lynch in familiarna adenomatoidna polipoza.

Prikaz primera. Opisujemo primer 44-letnega bolnika. Njegov oče je imel polipe debelega črevesa, bolnik pa je 2 leti odvajal svetlordeč krvav feces. Diagnosticirali smo rak debelega črevesa, stadij IIIA. Ob genetskem svetovanju smo bolnika tudi genetsko testirali, vendar nismo ugotovili najpogostejših familiarnih sindromov povezanih z rakom. Bolnika smo zdravili z neoadjuvantno kemoradioterapijo, operacijo in adjuvantno kemoterapijo. Po šestih letih nismo ugotovili ponovitve bolezni.

Zaključki. Pri mlajših bolnikih z rakom debelega črevesa in danke svetujemo ugotavljanje morebitnih familiarnih sindromov povezanih z rakom in genetsko testiranje. Opisani primer kaže na pomembnost presejalnih testov, ki morajo temeljiti na izsledkih.

Radiol Oncol 2010; 44(1): 62-66.
doi: 10.2478/v10019-010-0003-2

Detektor sledi za dozimetrijo nevtronov iz linearnih elektronskih pospeševalnikov v radioterapiji

Vuković B, Faj D, Poje M, Varga M, Radolić V, Miklavčić I, Ivković A, Planinić J

Izhodišča. Linearni elektronski pospeševalniki so v radioterapiji zamenjali kobaltove in cezijeve izvore sevanja. Vendar pospeševalniki s fotonskimi energijami nad 10 MeV proizvajajo neželeno kontaminacijo fotonskega snopa s hitrimi nevtroni. Med fotoni z energijami nad 10 MeV lahko prihaja do interakcije z atomskimi jedri snovi z visokim vrstnim številom. Ti sestavljajo tarčo in glavo pospeševalnika, kar vodi do izsevanja nevtronov.

Rezultati in zaključki. Nevtronski dozimeter sestavljata detektor sledi LR-115 in pretvornik BN-1 in so ga umerili s termalnimi nevtroni iz jedrskega reaktorja v Inštitutu Jožef Stefan (Slovenija). Uporabili smo ga za dozimetrijo neželenih nevtronov v fotonskem snopu iz linearnega pospeševalnika 15 MV Siemens Mevatron. Upoštevali smo visoko odvisnost preseka reakcije med nevtronom in borom od energije nevtrona ter širok spekter nevtronov tako v fotonskem žarku kot tudi pred vhodnimi vrati labirinta k Mevatronu. Na ta način smo razvili metodo določanja efektivnega odziva nevtronskega detektorja. Izmerjena nevtronska hitrost doze v fotonskem žarku je bila 1.96 Sv/h. Zunaj sobe z Mevatronom je bila nevtronska hitrost doze 0.62 μSv/h. PACS: 87.52. Ga; 87.53.St; 29.40.Wk

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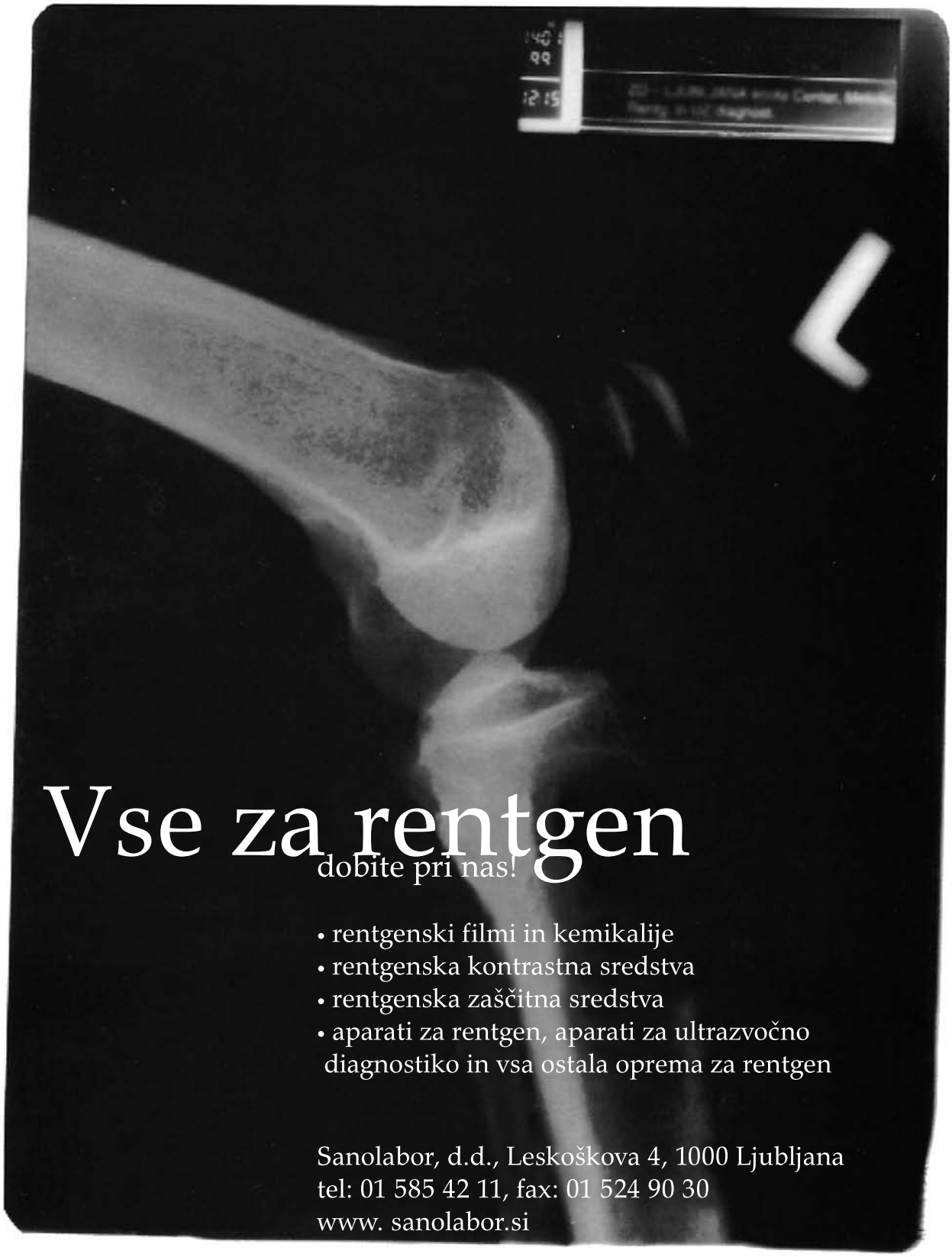
Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – a report for the first quarter of 2010

One of the main activities of Dr. J. Cholewa Foundation for Cancer Reasearch and Education is The Dr. J. Cholewa Foundation for Cancer Research and Education is of the opinion that the spread of knowledge about cancer among health professionals and population in general through cancer research, information about new approaches in cancer therapy and cancer education should be regarded as its main activity. Among the activities mentioned above, the Foundation continues to support the publication of "Radiology and Oncology" international medical scientific journal that is edited, published and printed in Ljubljana, Slovenia. "Radiology and Oncology" is an open access journal, available free of charge on its own website. In this way, the Foundation indirectly complements the approaches mentioned previously, with the spread of information supporting the ever increasing number of patients with various types of cancer in Slovenia. In addition, the Foundation is therefore also active in increasing the amount of scientific information about various aspects of cancer among the scientists in different fields with a budding interest in cancer research.

One of the main activities of Dr. J. Cholewa Foundation for Cancer Research and Education is to carefully asses the requests for research grants and scholarships submitted by experts in oncology and other scientific activities in Slovenia. The Foundation's opinion is that the advances in cancer research, therapy and education must not be hindered for the simple lack of financial support. Within its possibilities, the Foundation supports the implementation of all advances in cancer therapy and education into everyday hospital, ambulatory and health promotion practice. Hopefully, the results of cancer research will thus find their way into the practical application across Slovenia and elsewhere as quickly as possible.

The Foundation hopes to successfully continue with its activities in 2010, leading to greater application of the latest cancer diagnostic, therapy and education methods and knowledge to everyday research, clinical and public environment in Slovenia.

Borut Štabuc, MD, PhD
Andrej Plesničar, MD
Tomaž Benulič, MD



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SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

1. Povzetek glavih značilnosti zdravila Iressa (gefitinib). Junij 2009.

Sestava: Filmsko obložene tablete vsebujejo 250 mg gefitiniba. **Indikacije:** zdravljenje odraslih bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim rakom z aktivacijskimi mutacijami EGFR-TK **Odmerjanje in način uporabe:** Zdravljenje z gefitinibom mora uvesti in nadzorovati zdravnik, ki ima izkušnje z uporabo zdravil proti raku. Priporočeno odmerjanje zdravila IRESSA je ena 250-mg tableta enkrat na dan. Tableto je mogoče vzeti s hrano ali brez nje, vsak dan ob približno istem času. **Kontraindikacije:** preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov, dojenje **Opozorila in previdnostni ukrepi:** Pri 1,3 % bolnikov, ki so dobivali gefitinib, so opažali intersticijsko bolezen pljuč (IBP). Ta se lahko pojavi akutno in je bila v nekaterih primerih smrtna. Če se bolniku poslabšajo dihalni simptomi, npr. dispneja, kašelj in zvišana telesna temperatura, morate zdravljenje z zdravilom IRESSA prekiniti in bolnika takoj preiskati. če je potrjena IBP, morate terapijo z zdravilom IRESSA končati in bolnika ustrezno zdraviti. čeprav so bile nepravilnosti testov jetrnih funkcij pogoste, so jih redko zabeležili kot hepatitis. Zato so priporočljive redne kontrole delovanja jeter. V primeru blagih do zmernih sprememb v delovanju jeter je treba zdravilo IRESSA uporabljati previdno. Če so spremembe hude, pride v poštev prekinitev zdravljenja. Zdravilo IRESSA vsebuje laktozo. Bolniki z redko dedno intoleranco za galaktozo, laponsko obliko zmanjšane aktivnosti laktaze ali malabsorpcijo glukoze2galaktoze ne smejo jemati tega zdravila. Bolnikom naročite, da morajo takoj poiskati zdravniško pomoč, če se jim pojavijo kakršnikoli očesni simptomi, huda ali dolgotrajna driska, navzea, bruhanje ali anoreksija, ker lahko vse te posredno povzročijo dehidracijo. **Medsebojno delovanje zdravile:** Induktorji CYP3A4 lahko povečajo presnovo gefitiniba in zmanjšajo njegovo koncentracijo v plazmi. Zato lahko sočasna uporaba induktorjev CYP3A4 (npr. fenitoina, karbamazepina, rifampicina, barbituratov ali zeliščnih pripravkov, ki vsebujejo šentjanževko/Hypericum perforatum) zmanjša učinkovitost zdravljenja in se ji je treba izogniti. Pri posameznih bolnikih, ki imajo genotip slabih metabolizatorjev s CYP2D6, lahko zdravljenje z močnim zaviralcem CYP3A4 poveča koncentracijo gefitiniba v plazmi. Na začetku zdravljenja z zaviralcem CYP3A4 je treba bolnike natančno kontrolirati glede neželenih učinkov gefitiniba. Pri nekaterih bolnikih, ki so jemali varfarin skupaj z gefitinibom, so se pojavili zvišanje internacionalnega normaliziranega razmerja (INR) in/ali krvavitve. Bolnike, ki sočasno jemljejo varfarin in gefitinib, morate redno kontrolirati glede sprememb protrombinskega časa (PČ) ali INR. Zdravila, ki občutno in dolgotrajno zvišajo pH v želodcu npr. zaviralci protonske črpalke in antagonisti H2, lahko zmanjšajo biološko uporabnost gefitiniba in njegovo koncentracijo v plazmi in tako zmanjšajo učinkovitost. Redno jemanje antacidov, uporabljenih blizu časa jemanja zdravila IRESSA, ima lahko podoben učinek. **Neželeni učinki:** V kumulativnem naboru podatkov kliničnih preskušanj III. faze so bili najpogosteje opisani neželeni učinki, ki so se pojavili pri več kot 20 % bolnikov, driska in kožne reakcije (vključno z izpuščajem, aknami, suho kožo in srbenjem). Neželeni učinki se ponavadi pojavijo prvi mesec zdravljenja in so praviloma reverzibilni. Ostali pogostejši neželeni učinki so: anoreksija, konjunktivitis, blefaritis in suho oko, krvavitev, npr. epistaksa in hematurija, intersticijska bolezen pljuč (1,3 %), navzea, bruhanje, stomatitis, dehidracija, suha usta, nepravilnosti testov jetrnih funkcij, bolezni nohtov, alopecija, asimptomatično laboratorijsko zvišanje kreatinina v krvi, proteinurija, astenija, pireksija. **Vrsta in vsebina ovojnine:** škatla s 30 tabletami po 250 mg gefitiniba **Način izdajanja zdravila:** samo na recept **Datum priprave besedila:** junij 2009 **Imetnik dovoljenja za promet:** AstraZeneca AB, S-151 85, Sodertalje, Švedska Pred predpisovanjem, prosimo, preberite celoten povzetek glavnih značilnosti zdravila. **Dodatne informacije so na voljo pri:** AstraZeneca UK Limited, Podružnica v Sloveniji, Verovškova 55, 1000 Ljubljana, telefon: 01/51 35 600.

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Erbitux 5 mg/ml raztopina za infundiranje (skrajšana navodila za uporabo)

Cetuksimab je monoklonsko IgG_1 protitelo, usmerjeno proti receptorju za epidermalni rastni faktor (EGFR). **Terapevtske indikacije:** Zdravilo Erbitux je indicirano za zdravljenje bolnikov z metastatskim kolorektalnim rakom in nemutiranim tipom KRAS; v kombinaciji s kemoterapijo in kot samostojno zdravilo pri bolnikih, pri katerih zdravljenje z oksaliplatinom in irinotekanom ni bilo uspešno. Zdravilo Erbitux je indicirano za zdravljenje bolnikov z rakom skvamoznih celic glave in vratu; v kombinaciji z radioterapijo za lokalno napredovalo bolezen in v kombinaciji s kemoterapijo na osnovi platine za ponavljajočo se in/ali metastatsko bolezen. **Odmerjanje in način uporabe:** Zdravilo Erbitux pri vseh indikacijah infundirajte enkrat na teden. Začetni odmerek je 400 mg cetuksimaba na m^2 telesne površine. Vsi naslednji tedenski odmerki so vsak po 250 mg/m^2. **Kontraindikacije:** Zdravilo Erbitux je kontraindicirano pri bolnikih z znano hudo preobčutljivostno reakcijo (3. ali 4. stopnje) na cetuksimab. **Posebna opozorila in previdnostni ukrepi:** Če pri bolniku nastopi blaga ali zmerna reakcija, povezana z infundiranjem, lahko zmanjšate hitrost infundiranja. Priporočljivo je, da ostane hitrost infundiranja na nižji vrednosti tudi pri vseh naslednjih infuzijah. Če se pri bolniku pojavi huda kožna reakcija (≥ 3. stopnje po kriterijih *US National Cancer Institute, Common Toxicity Criteria;* NCI-CTC), morate prekiniti terapijo s cetuksimabom. Z zdravljenjem smete nadaljevati le, če se je reakcija pomirila do 2. stopnje. Priporoča se določanje koncentracije elektrolitov v serumu pred zdravljenjem in periodično med zdravljenjem s cetuksimabom. Po potrebi se priporoča nadomeščanje elektrolitov. Posebna previdnost je potrebna pri oslabljenih bolnikih in pri tistih z obstoječo srčno-pljučno boleznijo. **Neželeni učinki:** Zelo pogosti (≥ 1/10): dispneja, blago do zmerno povečanje jetrnih encimov, kožne reakcije, blage ali zmerne reakcije povezane z infundiranjem, blag do zmeren mukozitis. Pogosti (≥ 1/100, < 1/10): konjunktivitis, hude reakcije povezane z infundiranjem. Pogostost ni znana: Opazili so progresivno zniževanje nivoja magnezija v serumu, ki pri nekaterih bolnikih povzroča hudo hipomagneziemijo. Glede na resnost so opazili tudi druge elektrolitske motnje, večinoma hipokalciemijo ali hipokaliemijo. **Posebna navodila za shranjevanje:** Shranjujte v hladilniku (2 °C - 8 °C). Ne zamrzujte**. Vrsta ovojnine in vsebina:** 1 viala po 20 ml ali 100 ml. Imetnik dovoljenja za promet: Merck KGaA, 64271 Darmstadt, Nemčija. Podrobne informacije o zdravilu so objavljene na spletni strani Evropske agencije za zdravila (EMEA) http://www.emea.europa.eu.

Dodatne informacije so vam na voljo pri: Merck d.o.o., Dunajska cesta 119, 1000 Ljubljana, tel.: 01 560 3810, faks: 01 560 3831, el. pošta: info@merck.si

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PM-ONC-02/09/10.04.2009

Povzetek glavnih značilnosti zdravila

Ime zdravila: Temodal 20 mg, 100 mg, 140mg, 180 mg, 250 mg, Temodal 2,5 mg/ml prašek za raztopino za infundiranje **Kakovostna in količinska sestava:** Vsaka kapsula zdravila Temodal vsebuje 20 mg, 100 mg, 140 mg, 180 mg ali 250 mg temozolomida. Ena viala vsebuje 100 mg temozolomida Po rekonstituciji 1 ml raztopine za infundiranje vsebuje 2,5 mg temozolomida. Pomožna snov: Ena viala vsebuje 2,4 mmol natrija. **Terapevtske indikacije:** Zdravilo Temodal 2,5 mg/ml je indicirano za zdravljenje: odraslih bolnikov z novo diagnosticiranim multiformnim glioblastomom, sočasno z radioterapijo (RT) in pozneje kot monoterapija in otrok, starih 3 leta in več, mladostnikov in odraslih bolnikov z malignimi gliomi, npr. multiformnimi glioblastomi ali anaplastičnimi astrocitomi, ki se po standardnem zdravljenju ponovijo ali napredujejo. **Odmerjanje in način uporabe:** Zdravilo Temodal 2,5 mg/ml smejo predpisati le zdravniki, ki imajo izkušnje z zdravljenjem možganskih tumorjev. **Odrasli bolniki z novo diagnosticiranim multiformnim glioblastomom** Zdravilo Temodal 2,5 mg/ml se uporablja v kombinaciji z žariščno radioterapijo (faza sočasne terapije), temu pa sledi do 6 ciklov monoterapije (monoterapijska faza) z temozolomidom (TMZ). **Faza sočasne terapije** TMZ naj bolnik jemlje v odmerku 75 mg/m2 na dan 42 dni, sočasno z žariščno radioterapijo (60 Gy, danih v 30 delnih odmerkih). Zmanjševanje odmerka ni priporočeno, vendar se boste vsak teden odločili o morebitni odložitvi jemanja TMZ ali njegovi ukinitvi na podlagi kriterijev hematološke in nehematološke toksičnosti. TMZ lahko bolnik jemlje ves čas 42-dnevnega obdobja sočasne terapije (do 49 dni), če so izpolnjeni vsi od naslednjih pogojev:

- absolutno število nevtrofilcev (ANC – Absolute Neutrophil Count) ≥ 1,5 x 109/l;
- število trombocitov ≥ 100 x 109/l;
- skupna merila toksičnosti (SMT) za nehematološko toksičnost ≤ 1. stopnje (z izjemo alopecije, navzee in bruhanja).

Med zdravljenjem morate pri bolniku enkrat na teden pregledati celotno krvno sliko.

Faza monoterapije Štiri tedne po zaključku faze sočasnega zdravljenja s TMZ in RT naj bolnik jemlje TMZ do 6 ciklov monoterapije. V 1. ciklu (monoterapije) je odmerek zdravila 150 mg/m2 enkrat na dan 5 dni, temu pa naj sledi 23 dni brez terapije. Na začetku 2. cikla odmerek povečajte na 200 mg/m2, če je SMT za nehematološko toksičnost za 1. cikel stopnje ≤ 2 (z izjemo alopecije, slabosti in bruhanja), absolutno število nevtrofilcev (ANC) ≥ 1,5 x 109/l in število trombocitov ≥ 100 x 109/l. Če odmerka niste povečali v 2. ciklu, ga v naslednjih ciklih ne smete povečevati. Ko pa odmerek enkrat povečate, naj ostane na ravni 200 mg/m2 na dan v prvih 5 dneh vsakega naslednjega cikla, razen če nastopi toksičnost. Zmanjšanje odmerka in ukinitev zdravila med fazo monoterapije opravite, kot je opisano v preglednicah 2 in 3. Med zdravljenjem morate 22. dan pregledati celotno krvno sliko (21 dni po prvem odmerku TMZ). **Odrasli in pediatrični bolniki, stari 3 leta ali več, s ponavljajočim se ali napredujočim malignim gliomom:**Posamezen cikel zdravljenja traja 28 dni. Bolniki, ki še niso bili zdravljeni s kemoterapijo, naj jemljejo TMZ v odmerku 200 mg/m2 enkrat na dan prvih 5 dni, temu pa naj sledi 23-dnevni premor (skupaj 28 dni). Pri bolnikih, ki so že bili zdravljeni s kemoterapijo, je začetni odmerek 150 mg/m2 enkrat na dan, v drugem ciklu pa se poveča na 200 mg/m2 enkrat na dan 5 dni, če ni bilo hematoloških toksičnih učinkov. **Kontraindikacije:** Preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov. Preobčutljivost za dakarbazin (DTIC). **Posebna opozorila in previdnostni ukrepi: Pljučnica, ki jo povzroča Pneumocystis carinii** Pilotno preskušanje podaljšane 42-dnevne sheme zdravljenja je pokazalo, da pri bolnikih, ki so sočasno prejemali TMZ in RT, obstaja še posebej veliko tveganje za nastanek pljučnice zaradi okužbe s Pneumocystis carinii (PCP). **Malignosti** Zelo redko so poročali tudi o primerih mielodisplastičnega sindroma in sekundarnih malignostih, vključno z mieloidno levkemijo. Antiemetično zdravljenje Navzea in bruhanje sta pogosto povezana z zdravljenjem s TMZ. **Antiemetično zdravljenje** se lahko da pred uporabo TMZ ali po njej. **Odrasli bolniki z novo diagnosticiranim multiformnim glioblastomom** Antiemetična profilaksa je priporočljiva pred začetnim odmerkom sočasne faze in je močno priporočljiva med fazo monoterapije. **Ponavljajoči se ali napredujoči maligni gliom** Pri bolnikih, ki so močno bruhali (stopnja 3 ali 4) v prejšnjih ciklih zdravljenja, je potrebno antiemetično zdravljenje. **Laboratorijske vrednosti** Pred jemanjem zdravila morata biti izpolnjena naslednja pogoja za laboratorijske izvide: ANC ≥ 1,5 x 109/l in število trombocitov ≥ 100 x 109/l. Na 22. dan (21 dni po prvem odmerku) ali v roku 48 ur od navedenega dne, morate pregledati celotno krvno sliko in jo nato spremljati vsak teden, dokler ni ANC > 1,5 x 109/l in število trombocitov > 100 x 109/l. Če med katerimkoli ciklom ANC pade na < 1,0 x 109/l ali število trombocitov na < 50 x 109/l, morate odmerek zdravila v naslednjem ciklu zmanjšati za eno stopnjo (glejte poglavje 4.2). Stopnje odmerka so 100 mg/m2, 150 mg/m2 in 200 mg/m2. Najmanjši priporočeni odmerek je 100 mg/m2. **Pediatrična uporaba** Kliničnih izkušenj z uporabo TMZ pri otrocih, mlajših od 3 let, ni. Izkušnje z uporabo tega zdravila pri starejših otrocih in mladostnikih so zelo omejene. **Starejši bolniki (stari > 70 let)** Videti je, da je pri starejših bolnikih tveganje za nevtropenijo ali trombocitopenijo večje, kot pri mlajših. Zato je pri uporabi zdravila TMZ pri starejših bolnikih potrebna posebna previdnost. **Moški bolniki** Moškim, ki se zdravijo s TMZ je treba svetovati, naj ne zaplodijo otroka še šest mesecev po prejetem zadnjem odmerku in naj se pred zdravljenjem posvetujejo o možnostih za shranitev zmrznjene sperme. **Natrij** To zdravilo vsebuje 2,4 mmol natrija na vialo. To je treba upoštevati pri bolnikih na nadzorovani dieti z malo natrija. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Študije medsebojnega delovanja so izvedli le pri odraslih. V ločeni študiji 1. faze, sočasna uporaba TMZ in ranitidina ni povzročila spremembe obsega absorpcije temozolomida ali izpostavljenosti njegovem aktivnem presnovku monometiltriazenoimidazol karboksamidu (MTIK). Analiza populacijske farmakokinetike v preskušanjih 2. faze je pokazala, da sočasna uporaba deksametazona, proklorperazina, fenitoina, karbamazepina, ondansetrona, antagonistov receptorjev H2 ali fenobarbitala ne spremeni očistka TMZ. Sočasno jemanje z valprojsko kislino je bilo povezano z majhnim, a statistično pomembnim zmanjšanjem očistka TMZ. Študij za določitev učinka TMZ na presnovo ali izločanje drugih zdravil niso izvedli. Ker pa se TMZ ne presnavlja v jetrih in se na beljakovine veže le v majhni meri, je malo verjetno, da bi vplival na farmakokinetiko drugih zdravil. Uporaba TMZ v kombinaciji z drugimi mielosupresivnimi učinkovinami lahko poveča verjetnost mielosupresije. **Neželeni učinki:** Pri bolnikih, ki se zdravijo s TMZ v kombinaciji z RT ali monoterapijo po RT zaradi novo diagnosticiranega multiformnega glioblastoma ali z monoterapijo pri bolnikih s ponavljajočim se ali napredujočim gliomom, so bili zelo pogosti neželeni učinki podobni; slabost, bruhanje, zaprtje, neješčnost, glavobol in utrujenost. Pri bolnikih z novo diagnosticiranim glioblastomom multiforme na monoterapiji so zelo pogosto poročali o konvulzijah, medtem ko je bil izpuščaj opisan zelo pogosto pri bolnikih z novo diagnosticiranim multiformnim glioblastomom, ki so prejemali TMZ sočasno z RT, ter pri tistih, ki so zdravilo prejemali v obliki monoterapije, pogosto pa pri tistih s ponavljajočim se gliomom. Pri obeh indikacijah so o večini hematoloških neželenih reakcij poročali pogosto ali zelo pogosto. **Imetnik dovoljenja za promet:** Schering-Plough Europe, Rue de Stalle 73, Bruselj Belgija **Način in režim izdaje zdravila: Zdravilo Temodal 20 mg, 100 mg, 140mg, 180 mg, 250 mg** se izdaja na recept (Rp/Spec), Temodal 2,5 mg/ml prašek za raztopino za infundiranje pa je namenjeno uporabi samo v bolnišnicah (H). **Datum priprave informacije:** februar 2010

Literatura: 1 Povzetek temeljnih značilnosti zdravila Temodal 2 Stupp R, et. al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised III study: 5-year analysis of the EORTC-NCIC trial

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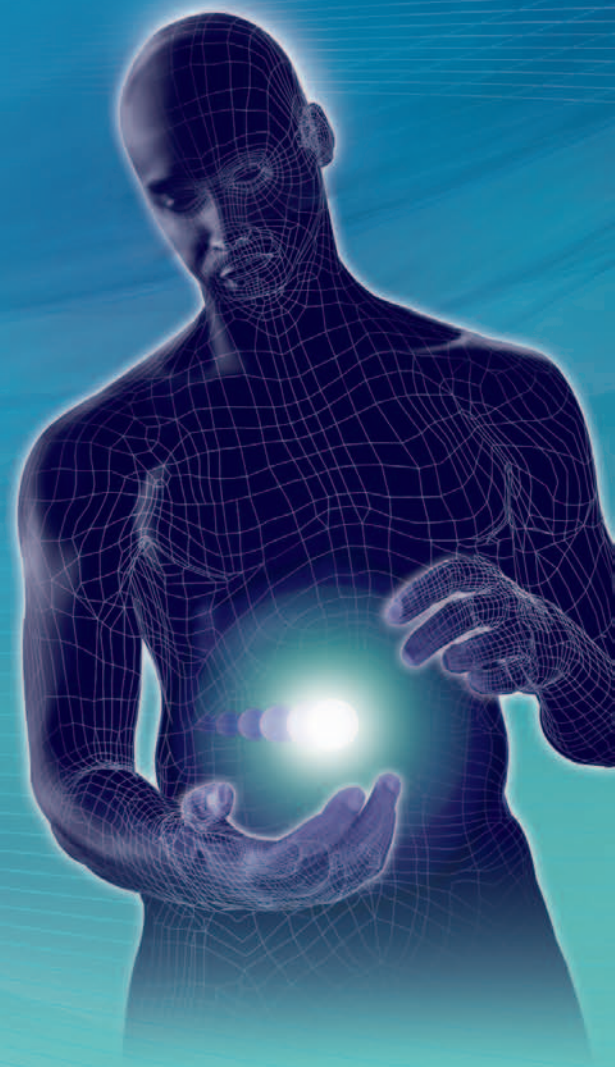
Nova, intravenska oblika zdravila Temodal, za zdravljenje bolnikov z novo diagnosticiranim glioblastomom multiforme in bolnikov s ponavljajočim se ali napredujočim malignim gliomom.[1]



Resnični napredek

Pomembno izboljšanje preživetja potrjeno tudi ob daljšem spremljanju bolnikov.[2]

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SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Samo za strokovno javnost.

Ime zdravila: Tarceva 25 mg/100 mg/150 mg filmsko obložene tablete
Kakovostna in količinska sestava: Ena filmsko obložena tableta vsebuje 25 mg, 100 mg ali 150 mg erlotiniba (v obliki erlotinibijevega klorida).
Terapevtske indikacije: Nedrobnocelični rak pljuč: Zdravilo Tarceva je indicirano za zdravljenje bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč po neuspehu vsaj ene predhodne kemoterapije. Pri predpisovanju zdravila Tarceva je treba upoštevati dejavnike, povezane s podaljšanim preživetjem. Koristnega vpliva na podaljšanje preživetja ali drugih klinično pomembnih učinkov zdravljenja niso dokazali pri bolnikih z EGFR-negativnimi tumorji. Rak trebušne slinavke: Zdravilo Tarceva je v kombinaciji z gemcitabinom indicirano za zdravljenje bolnikov z metastatskim rakom trebušne slinavke. Pri predpisovanju zdravila Tarceva je treba upoštevati dejavnike, povezane s podaljšanim preživetjem. Koristnega vpliva na podaljšanje preživetja niso dokazali za bolnike z lokalno napredovalo boleznijo.
Odmerjanje in način uporabe: Zdravljenje z zdravilom Tarceva mora nadzorovati zdravnik z izkušnjami pri zdravljenju raka. Zdravilo Tarceva vzamemo najmanj eno uro pred zaužitjem hrane ali dve uri po tem. Kadar je potrebno odmerek prilagoditi, ga zmanjšujemo v korakih po 50 mg. Pri sočasnem jemanju substratov in modulatorjev CYP3A4 bo morda potrebna prilagoditev odmerka. Pri dajanju zdravila Tarceva bolnikom z jetrno okvaro je potrebna previdnost. Če se pojavijo hudi neželeni učinki pride v poštev zmanjšanje odmerka ali prekinitev zdravljenja z zdravilom Tarceva. Uporaba zdravila Tarceva pri bolnikih s hudo jetrno ali ledvično okvaro ter pri otrocih ni priporočljiva. Bolnikom kadilcem je treba svetovati, naj prenehajo kaditi, saj so plazemske koncentracije erlotiniba pri kadilcih manjše kot pri nekadilcih. Nedrobnocelični rak pljuč: Priporočeni dnevni odmerek zdravila Tarceva je 150 mg. Rak trebušne slinavke: Priporočeni dnevni odmerek zdravila Tarceva je 100 mg, v kombinaciji z gemcitabinom. Pri bolnikih, pri katerih se kožni izpuščaj v prvih 4 do 8 tednih zdravljenja ne pojavi, je treba ponovno pretehtati nadaljnje zdravljenje z zdravilom Tarceva.
Kontraindikacije: Huda preobčutljivost za erlotinib ali katero koli pomožno snov.
Posebna opozorila in previdnostni ukrepi: Močni induktorji CYP3A4 lahko zmanjšajo učinkovitost erlotiniba, močni zaviralci CYP3A4 pa lahko povečajo toksičnost. Sočasnemu zdravljenju s temi zdravili se je treba izogibati. Bolnikom, ki kadijo, je treba svetovati, naj prenehajo kaditi, saj so plazemske koncentracije erlotiniba pri kadilcih zmanjšane v primerjavi s plazemskimi koncentracijami pri nekadilcih. Verjetno je, da je velikost zmanjšanja klinično pomembna. Pri bolnikih, pri katerih se akutno pojavijo novi in/ali poslabšajo nepojasnjeni pljučni simptomi, kot so dispneja, kašelj in vročina, je zdravljenje z zdravilom Tarceva treba prekiniti, dokler ni znana diagnoza. Bolnike, ki se sočasno zdravijo z erlotinibom in gemcitabinom, je treba skrbno spremljati zaradi možnosti pojava toksičnosti, podobni intersticijski pljučni bolezni. Če je ugotovljena intersticijska pljučna bolezen, zdravilo Tarceva ukinemo in uvedemo ustrezno zdravljenje. Pri približno polovici bolnikov, ki so se zdravili z zdravilom Tarceva, se je pojavila driska. Zmerno do hudo drisko zdravimo z loperamidom. V nekaterih primerih bo morda potrebno zmanjšanje odmerka. V primeru hude ali dolgotrajne driske, navzeje, anoreksije ali bruhanja, povezanih z dehidracijo, je zdravljenje z zdravilom Tarceva treba prekiniti in dehidracijo ustrezno zdraviti. O hipokaliemiji in ledvični odpovedi so poročali redko. Posebno pri bolnikih z dejavniki tveganja (sočasno jemanje drugih zdravil, simptomi, bolezni ali drugi dejavniki, vključno z visoko starostjo) moramo, če je driska huda ali dolgotrajna oziroma vodi v dehidracijo, zdravljenje z zdravilom Tarceva prekiniti in bolnikom zagotoviti intenzivno intravensko rehidracijo. Dodatno je treba pri bolnikih s prisotnim tveganjem za razvoj dehidracije spremljati ledvično delovanje in serumske elektrolite, vključno s kalijem. Pri uporabi zdravila Tarceva so poročali o redkih primerih jetrne odpovedi. K njenemu nastanku je lahko pripomogla predhodno obstoječa jetrna bolezen ali sočasno jemanje hepatotoksičnih zdravil. Pri teh bolnikih je treba zato premisliti o rednem spremljanju jetrnega delovanja. Dajanje zdravila Tarceva je treba prekiniti, če so spremembe jetrnega delovanja hude. Bolniki, ki prejemajo zdravilo Tarceva, imajo večje tveganje za razvoj perforacij v prebavilih, ki so jih opazili občasno. Pri bolnikih, ki sočasno prejemajo zdravila, ki zavirajo angiogenezo, kortikosteroide, nesteroidna protivnetna zdravila (NSAID) in/ali kemoterapijo na osnovi taksanov, ali so v preteklosti imeli peptični ulkus ali bolezen divertiklov, je tveganje večje. Če pride do tega, je treba zdravljenje z zdravilom Tarceva dokončno ukiniti. Poročali so o primerih kožnih bolezni z mehurji in luščenjem kože, vključno z zelo redkimi primeri, ki so nakazovali na Stevens-Johnsonov sindrom/ toksično epidermalno nekrolizo in so bili v nekaterih primerih smrtni. Zdravljenje z zdravilom Tarceva je treba prekiniti ali ukiniti, če se pri bolniku pojavijo hude oblike mehurjev ali luščenja kože. Zelo redko so poročali o primerih perforacije ali ulceracije roženice; opazili so tudi druge očesne bolezni. Zdravljenje z zdravilom Tarceva je treba prekiniti ali ukiniti, če se pri bolnikih pojavijo akutne očesne bolezni, kot je bolečina v očeh, ali se le-te poslabšajo. Tablete vsebujejo laktozo in jih ne smemo dajati bolnikom z redkimi dednimi stanji: intoleranco za galaktozo, laponsko obliko zmanjšane aktivnosti laktaze ali malabsorpcijo glukoze/galaktoze.
Medsebojno delovanje z drugimi zdravili in druge oblike interakcij: Erlotinib se pri ljudeh presnavlja v jetrih z jetrnimi citokromi, primarno s CYP3A4 in v manjši meri s CYP1A2. Presnova erlotiniba zunaj jeter poteka s CYP3A4 v črevesju, CYP1A1 v pljučih in CYP1B1 v tumorskih tkivih. Z zdravilnimi učinkovinami, ki se presnavljajo s temi encimi, jih zavirajo ali pa so njihovi induktorji, lahko pride do interakcij. Erlotinib je srednje močan zaviralec CYP3A4 in CYP2C8, kot tudi močan zaviralec glukuronidacije z UGT1A1 *in vitro*. Pri kombinaciji ciprofloksacina ali močnega *zaviralca CYP1A2* (npr. fluvoksamina) z erlotinibom je potrebna previdnost. V primeru pojava neželenih dogodkov, povezanih z erlotinibom, lahko odmerek erlotiniba zmanjšamo. Predhodno ali sočasno zdravljenje z zdravilom Tarceva ni spremenilo očistka prototipov *substratov CYP3A4*, midazolama in eritromicina. Inhibicija glukoronidacije lahko povzroči interakcije z zdravili, ki so *substrati UGT1A1* in se izločajo samo po tej poti. Močni *zaviralci aktivnosti CYP3A4* zmanjšajo presnovo erlotiniba in zvečajo koncentracije erlotiniba v plazmi. Pri sočasnem jemanju erlotiniba in močnih zaviralcev CYP3A4 je zato potrebna previdnost. Če je treba, odmerek erlotiniba zmanjšamo, še posebno pri pojavu toksičnosti. Močni *spodbujevalci aktivnosti CYP3A4* zvečajo presnovo erlotiniba in pomembno zmanjšajo plazemske koncentracije erlotiniba. Sočasnemu dajanju zdravila Tarceva in induktorjev CYP3A4 se je treba izogibati. Pri bolnikih, ki potrebujejo sočasno zdravljenje z zdravilom Tarceva in močnim induktorjem CYP3A4 je treba premisliti o povečanju odmerka do 300 mg ob skrbnem spremljanju njihove varnosti. Zmanjšana izpostavljenost se lahko pojavi tudi z drugimi induktorji, kot so fenitoin, karbamazepin, barbiturati ali šentjanževka. Če te zdravilne učinkovine kombiniramo z erlotinibom, je potrebna previdnost. Kadar je mogoče, je treba razmisliti o drugih načinih zdravljenja, ki ne vključujejo močnega spodbujanja aktivnosti CYP3A4. Bolnikom, ki jemljejo varfarin ali druge *kumarinske antikoagulante*, je treba redno kontrolirati protrombinski čas ali INR. Sočasna uporaba *zaviralcev P-glikoproteina*, kot sta ciklosporin in verapamil, lahko vodi v spremenjeno porazdelitev in/ali spremenjeno izločanje erlotiniba. Za erlotinib je značilno zmanjšanje topnosti pri pH nad 5. *Zdravila, ki spremenijo pH v zgornjem delu prebavil*, lahko spremenijo topnost erlotiniba in posledično njegovo biološko uporabnost. Učinka antacidov na absorpcijo erlotiniba niso proučevali, vendar je ta lahko zmanjšana, kar vodi v nižje plazemske koncentracije. Kombinaciji erlotiniba in zaviralca protonske črpalke se je treba izogibati. Če menimo, da je uporaba antacidov med zdravljenjem z zdravilom Tarceva potrebna, jih je treba jemati najmanj 4 ure pred ali 2 uri po dnevnem odmerku zdravila Tarceva. Če razmišljamo o uporabi ranitidina, moramo zdravili jemati ločeno: zdravilo Tarceva je treba vzeti najmanj 2 uri pred ali 10 ur po odmerku ranitidina. V študiji faze Ib ni bilo pomembnih učinkov *gemcitabina* na farmakokinetiko erlotiniba, prav tako ni bilo pomembnih učinkov erlotiniba na farmakokinetiko gemcitabina. Erlotinib poveča koncentracijo platine. Pomembnih učinkov *karboplatina* ali paklitaksela na farmakokinetiko erlotiniba ni bilo. *Kapecitabin* lahko poveča koncentracijo erlotiniba. Pomembnih učinkov erlotiniba na farmakokinetiko kapecitabina ni bilo.
Neželeni učinki: *Zelo pogosti neželeni učinki* so kožni izpuščaj in driska, kot tudi utrujenost, anoreksija, dispneja, kašelj, okužba, navzeja, bruhanje, stomatitis, bolečina v trebuhu, pruritus, suha koža, suhi keratokonjunktivitis, konjunktivitis, zmanjšanje telesne mase, depresija, glavobol, nevropatija, dispepsija, flatulenca, alopecija, okorelost, pireksija. *Pogosti neželeni učinki* so gastrointestinalne krvavitve, krvavitev iz nosu, nenormalnosti testov jetrne funkcije, keratitis, zanohtnica. *Redko* so poročali o jetrni odpovedi. *Občasno* pa o perforacijah v prebavilih, poraščenosti moškega tipa pri ženskah, spremembah obrvi, krhkih nohtih, odstopanju nohtov od kože, blagih reakcijah na koži (npr. hiperpigmentacija), spremembah trepalnic, resni intersticijski pljučni bolezni, vključno s smrtnimi primeri. *Zelo redko* so poročali o primerih, ki so nakazovali na Stevens-Johnsonov sindrom/ toksično epidermalno nekrolizo in so bili v nekaterih primerih smrtni, ter o ulceracijah in perforacijah roženice.
Režim izdaje zdravila: H/Rp. **Imetnik dovoljenja za promet:** Roche Registration Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, AL7 1TW, Velika Britanija. **Verzija:** 2.0/09. **Informacija pripravljena**: januar 2010.

002-10-TAR

DODATNE INFORMACIJE SO NA VOLJO PRI:
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ČAS ZA ŽIVLJENJE.

DOKAZANO PODALJŠA PREŽIVETJE PRI BOLNIKIH:

- z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč[1]
- z metastatskim rakom trebušne slinavke[1]

[1] Povzetek glavnih značilnosti zdravila TARCEVA, www.emea.europa.eu

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Zamenjava opioidnega zdravljenja: pri zamenjavi peroralnih ali parenteralnih opioidov s fentanilom je treba začetni odmerek izračunati na osnovi količine analgetika, ki je bila potrebna v zadnjih 24 urah, jo pretvoriti v odgovarjajoči odmerek morfina s pomočjo razpredelnice in nato preračunati ustrezen odmerek fentanila, spet s pomočjo razpredelnice (glejte SmPC). Prvih 12 ur po prehodu na transdermalni obliž Epufen bolnik še vedno dobiva predhodni analgetik v enakem odmerku kot prej; v naslednjih 12 urah se ta analgetik daje po potrebi. Titracija odmerka in vzdrževalno zdravljenje: obliž je treba zamenjati vsakih 72 ur. Odmerek je treba titrirati individualno, dokler ni dosežen analgetični učinek. Odmerek 12,5 mikrogramov/uro je primeren za titriranje odmerka v manjšem odmernem območju. Če analgezija na koncu začetnega obdobja nošenja obliža ni zadostna, se lahko odmerek po 3 dneh zveča. Možno je, da bodo bolniki potrebovali občasne dodatne odmerke kratko delujočih analgetikov (npr. morfina) za prekinitev bolečine. Sprememba ali prekinitev zdravljenja: vsaka zamenjava z drugim opioidom mora potekati postopoma, z majhnim začetnim odmerkom in počasnim zvečevanjem. Splošno veljavno pravilo je postopna ustavitev opioidne analgezije, da bi preprečili odtegnitvene simptome, kot so navzeja, bruhanje, diareja, anksioznost in mišični tremor. Uporaba pri starejših bolnikih: starejše in oslabljene bolnike je treba skrbno opazovati zaradi simptomov prevelikega odmerjanja ter odmerek po potrebi zmanjšati. Uporaba pri otrocih: transdermalni obliži Epufen se lahko uporabljajo le pri pediatričnih bolnikih (starih od 2 do 16 let), ki tolerirajo opioide in peroralno že dobivajo opioide v odmerku, enakovrednemu najmanj 30 mg morfina na dan. Bolnik mora prvih 12 ur po prehodu na Epufen še vedno dobivati predhodni analgetik v enakem odmerku kot prej. V naslednjih 12 urah je treba ta analgetik dajati odvisno od kliničnih potreb. Titracija odmerka in vzdrževalno zdravljenje: če je analgetični učinek Epufena prešibak, je treba bolniku dodati morfin ali drugi opioid s kratkim delovanjem. Odvisno od dodatnih potreb po analgeziji in jakosti bolečine pri otroku se lahko uporabi več obližev. Odmerek je treba prilagajati korakoma, po 12,5 mikrogramov/uro. Uporaba pri bolnikih z jetrno ali ledvično okvaro: Zaradi možnosti pojava simptomov prevelikega odmerjanja je treba te bolnike skrbno spremljati in odmerek ustrezno zmanjšati. Uporaba pri bolnikih s povečano telesno temperaturo: Pri teh bolnikih bo morda treba prilagoditi odmerek. **Način uporabe:** transdermalni obliž Epufen je treba takoj po odprtju vrečke nalepiti na nerazdraženo, neobsevano kožo, na ravno površino prsnega koša, zgornjega dela hrbta ali nadlakti. Po odstranitvi zaščitne plasti je treba obliž trdno pritrditi na izbrano mesto in z dlanjo pritiskati približno 30 sekund, da se obliž popolnoma nalepi, še zlasti na robovih. Uporaba pri otrocih: pri mlajših otrocih je obliž priporočljivo nalepiti na zgornji del hrbta, ker je manjša verjetnost, da bi otrok odstranil obliž. Transdermalnega obliža se ne sme deliti, ker podatkov o tem ni na voljo. **KONTRAINDIKACIJE:** Preobčutljivost za zdravilno učinkovino, hidrogenirano kolofonijo, sojo, arašide ali katerokoli pomožno snov. Akutna ali pooperativna bolečina, ko v kratkem časovnem obdobju ni možno titriranje odmerka in obstaja verjetnost za življenjsko ogrožajočo respiratorno depresijo. Huda okvara osrednjega živčnega sistema. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Zaradi razpolovne dobe fentanila je treba bolnika v primeru pojava neželenega učinka opazovati še 24 ur po odstranitvi obliža. Pri nekaterih bolnikih, ki uporabljajo transdermalni obliž Epufen, se lahko pojavi respiratorna depresija. Epufen je treba previdno dajati: bolnikom s kronično pljučno boleznijo, zvišanim intrakranialnim tlakom, možganskim tumorjem, boleznimi srca, jeter in ledvic, tistim z zvišano telesno temperaturo, pri starejših bolnikih in otrocih, bolnikih z miastenijo gravis. Odvisnost od zdravila: kot posledica ponavljajoče se uporabe se lahko razvijeta toleranca na učinkovino ter psihična in/ali fizična odvisnost od nje. Ostali: lahko se pojavijo neepileptične (mio)klonične reakcije. **MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI IN DRUGE OBLIKE INTERAKCIJ:** Derivati barbiturne kisline, opioidi, anksiolitiki in pomirjevala, hipnotiki, splošni anestetiki, fenotiazini, mišični relaksanti, sedativni antihistaminiki in alkoholne pijače, zaviralci MAO, itrakonazol, ritonavir, ketokonazol, nekateri makrolidni antibiotiki, pentazocin, buprenorfin. **VPLIV NA SPOSOBNOST VOŽNJE IN UPRAVLJANJA S STROJI:** Zdravilo ima močan vpliv na sposobnost vožnje in upravljanja s stroji. **NEŽELENI UČINKI:** Najbolj resen neželen učinek fentanila je respiratorna depresija. Zelo pogosti (≥ 1/10): dremavost, glavobol, navzeja, bruhanje, zaprtje, znojenje, srbenje, somnolenca. Pogosti (≥ 1/100 do < 1/10): kserostomija, dispepsija, reakcije na koži na mestu aplikacije, sedacija, zmedenost, depresija, tesnoba, živčna napetost, halucinacije, zmanjšan apetit. Občasni (≥ 1/1000 do < 1/100): tahikardija, bradikardija, tremor, parestezija, motnje govora, dispneja, hipoventilacija, diareja, zastajanje urina, izpuščaj, rdečina, hipertenzija, hipotenzija, evforija, amnezija, nespečnost, vznemirljivost. Nekateri od naštetih neželenih učinkov so lahko posledica osnovne bolezni ali drugih zdravljenj. Drugi neželeni učinki: odpornost, fizična in psihična odvisnost se lahko razvijejo med dolgotrajno uporabo fentanila. Pri nekaterih bolnikih se lahko pojavijo odtegnitveni simptomi, ko zamenjajo prejšnje opiodne analgetike s transdermalnim obližem s fentanilom ali po nenadni prekinitvi zdravljenja. **NAČIN IZDAJE:** Samo na zdravniški recept. **OPREMA:** Škatle s 5 transdermalnimi obliži. **IMETNIK DOVOLJENJA ZA PROMET:** Lek farmacevtska družba, d.d., Verovškova 57, Ljubljana, Slovenija **INFORMACIJA PRIPRAVLJENA:** avgust 2009

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POMEMBEN JE **VSAK** KORAK!

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- izboljša splošno počutje bolnikov [3,4]

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA: MEGACE 40 mg/ml peroralna suspenzija

Sestava: 1 ml peroralne suspenzije vsebuje 40 mg megestrolacetata. **TERAPEVTSKE INDIKACIJE:** Zdravljenje anoreksije-kaheksije ali nepojasnjene, pomembne izgube telesne mase pri bolnikih z AIDS-om. Zdravljenje anorektično-kahektičnega sindroma pri napredovalem raku. **ODMERJANJE IN NAČIN UPORABE:** Pri aidsu je priporočeni začetni odmerek Megace za odrasle 800 mg (20 ml peroralne suspenzije) enkrat na dan eno uro pred jedjo ali dve uri po jedi in se lahko med zdravljenjem prilagodi glede na bolnikov odziv. V raziskavah bolnikov z aidsom so bili klinično učinkoviti dnevni odmerki od 400 do 800 mg/dan (10 do 20 ml), uporabljani štiri mesece. Pri anorektično-kahektičnem sindromu zaradi napredovalega raka je priporočljiv začetni odmerek 200 mg (5 ml) na dan; glede na bolnikov odziv ga je mogoče povečati do 800 mg na dan (20 ml). Običajni odmerek je med 400 in 800 mg na dan (10–20 ml). V raziskavah bolnikov z napredovalim rakom so bili klinično učinkoviti dnevni odmerki od 200 do 800 mg/dan (5 do 20 ml), uporabljani najmanj osem tednov. Pred uporabo je potrebno plastenko s suspenzijo dobro pretresti. Uporaba pri otrocih: Varnosti in učinkovitosti pri otrocih niso dokazali. Uporaba pri starostnikih: Zaradi pogostejših okvar jeter, ledvic in srčne funkcije, pogostejših sočasnih obolenj ali sočasnega zdravljenja z drugimi zdravili je odmerek za starejšega bolnika treba določiti previdno in običajno začeti z najnižjim odmerkom znotraj odmernega intervala. **KONTRAINDIKACIJE:** Preobčutljivost za megestrolacetat ali katerokoli pomožno snov. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Uporaba gestagenov med prvimi štirimi meseci nosečnosti ni priporočljiva. Pri bolnikih s tromboflebitisom v anamnezi je treba zdravilo Megace uporabljati previdno. Zdravljenje z zdravilom Megace se lahko začne šele, ko so bili vzroki hujšanja, ki jih je mogoče zdraviti, ugotovljeni in obravnavani. Megestrolacetat ni namenjen za profilaktično uporabo za preprečitev hujšanja. Učinki na razmnoževanje virusa HIV niso ugotovljeni. Med zdravljenjem z megestrolacetatom in po prekinitvi kroničnega zdravljenja je treba upoštevati možnost pojava zavore nadledvične žleze. Morda bo potrebno nadomestno zdravljenje s stresnimi odmerki glukokortikoidov. Megestrolacetat se v veliki meri izloči prek ledvic. Ker je verjetnost zmanjšanega delovanja ledvic pri starostnikih večja, je pri določitvi odmerka potrebna previdnost, prav tako je koristno spremljanje ledvične funkcije. Peroralna suspenzija vsebuje saharozo. Bolniki z redko dedno intoleranco za fruktozo, malabsorpcijo glukoze/galaktoze ali pomanjkanjem saharoza-izomaltaze ne smejo jemati tega zdravila. Peroralna suspenzija vsebuje tudi majhne količine etanola (alkohola), in sicer manj kot 100 mg na odmerek. **INTERAKCIJE:** Aminoglutetimid: poročali so o zmanjšanju koncentracije progestogena v plazmi z možno izgubo terapevtskega delovanja zaradi inducirane presnove. Sočasno jemanje megestrolacetata (v obliki peroralne suspenzije) in zidovudina ali rifabutina ne povzroča sprememb farmakokinetičnih parametrov. **NEŽELENI UČINKI:** Pogosti (≥ 1/100, < 1/10): navzea, bruhanje, driska, flatulenca, izpuščaj, metroragija, impotenca, astenija, bolečina, edem. Neznana pogostnost (pogostnosti ni mogoče oceniti iz razpoložljivih podatkov): poslabšanje osnovne bolezni (širjenje tumorja), adrenalna insuficienca, kušingoidni izgled, Cushingov sindrom, diabetes mellitus, motena toleranca za glukozo, hiperglikemija, spremembe razpoloženja, sindrom karpalnega kanala, letargija, srčno popuščanje, tromboflebitis, pljučna embolija (v nekaterih primerih usodna), hipertenzija, navali vročine, dispneja, zaprtje, alopecija, pogosto uriniranje. **Vrsta ovojnine in vsebina:** Plastenka z 240 ml suspenzije. **Režim izdaje:** Rp/Spec. **Imetnik dovoljenja za promet:** Bristol-Myers Squibb spol. s r.o., Olivova 4, Praga 1, Češka; **Odgovoren za trženje v Sloveniji:** PharmaSwiss d.o.o., Ljubljana, tel: 01 236 4 700, faks: 01 236 4 705; MGS-120609. **Pred predpisovanjem preberite celoten povzetek glavnih značilnosti zdravila!**

Reference: 1. Povzetek glavnih značilnosti zdravila Megace – 12. junij 2009; **2.** Register zdravil Republike Slovenije XI – leto 2008; **3.** Beller, E., 1997. Ann Oncol 8: 277-283; **4.** Čufer, T, 2002. Onkologija 9(2): 73-75; **5.** Yavuzsen, T., 2005. J Clin Oncol 23(33): 8500-8511; **6.** Bilten Recept 7(1), 22.5.2009

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	Fresubin 2 kcal DRINK	+++ 2,0	+++ 10	–	vanilija, gozdni sadeži	povečane potrebe po energiji in beljakovinah ter omejen vnos tekočine
	Fresubin 2 kcal fibre DRINK	+++ 2,0	+++ 10	+	čokolada	povečane potrebe po energiji in beljakovinah ter omejen vnos tekočine in potreba po vlakninah
	Fresubin protein energy DRINK	++ 1,5	+++ 10	–	vanilija, lešnik, tropski sadeži	povečane potrebe po beljakovinah, okrevanje po bolezni ali operaciji, težka kronična obolenja
Sadni napitki						
Napitki na osnovi sadnih sokov	ProvideXtra DRINK	++ 1,5	+ 4,0	–	češnja, črni ribez, pomaranča-ananas	občutljivost na mlečne beljakovine; pred in po operativnem posegu
Prehrana za posebne namene						
Posebni napitki za odrasle	Fresubin hepa DRINK	++ 1,3	+ 4,0	+	kapučino	jetrna odpoved, podhranjenost
	Diben DRINK	++ 1,5	+++ 7,5	+	vanilija, gozdni sadeži	sladkorni bolniki, intoleranca na glukozo
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Podrobnejše informacije so na voljo pri: **Grünenthal d.o.o.**,
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The *title page* should include a concise and informative title, followed by the full name(s) of the author(s); the institutional affiliation of each author; the name and address of the corresponding author (including telephone, fax and E-mail), and an abbreviated title. This should be followed by the *abstract page*, summarizing in less than 250 words the reasons for the study, experimental approach, the major findings (with specific data if possible), and the principal conclusions, and providing 3-6 key words for indexing purposes. Structured abstracts are preferred. The text of the report should then proceed as follows:

Introduction should state the purpose of the article and summarize the rationale for the study or observation, citing only the essential references and stating the aim of the study.

Materials and methods should provide enough information to enable experiments to be repeated. New methods should be described in detail. Reports on human and animal subjects should include a statement that ethical approval of the study was obtained.

Results should be presented clearly and concisely without repeating the data in the figures and tables. Emphasis should be on clear and precise presentation of results and their significance in relation to the aim of the investigation.

Discussion should explain the results rather than simply repeating them and interpret their significance and draw conclusions. It should review the results of the study in the light of previously published work.

Illustrations and tables must be numbered and referred to in the text, with the appropriate location indicated. Graphs and photographs, provided electronically, should be of appropriate quality for good reproduction. Colour graphs and photographs are encouraged. In photographs, mask the identities of the patients. Tables should be typed double-spaced, with a descriptive title and, if appropriate, units of numerical measurements included in the column heading.

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Dent RAG, Cole P. *In vitro* maturation of monocytes in squamous carcinoma of the lung. *Br J Cancer* 1981; **43:** 486-95.

Chapman S, Nakielny R. *A guide to radiological procedures.* London: Bailliere Tindall; 1986.

Evans R, Alexander P. Mechanisms of extracellular killing of nucleated mammalian cells by macrophages. In: Nelson DS, editor. *Immunobiology of macrophage.* New York: Academic Press; 1976. p. 45-74.

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Pred predpisovanjem se seznanite s celotnim povzetkom glavnih značilnosti zdravila.

SUT-01-10

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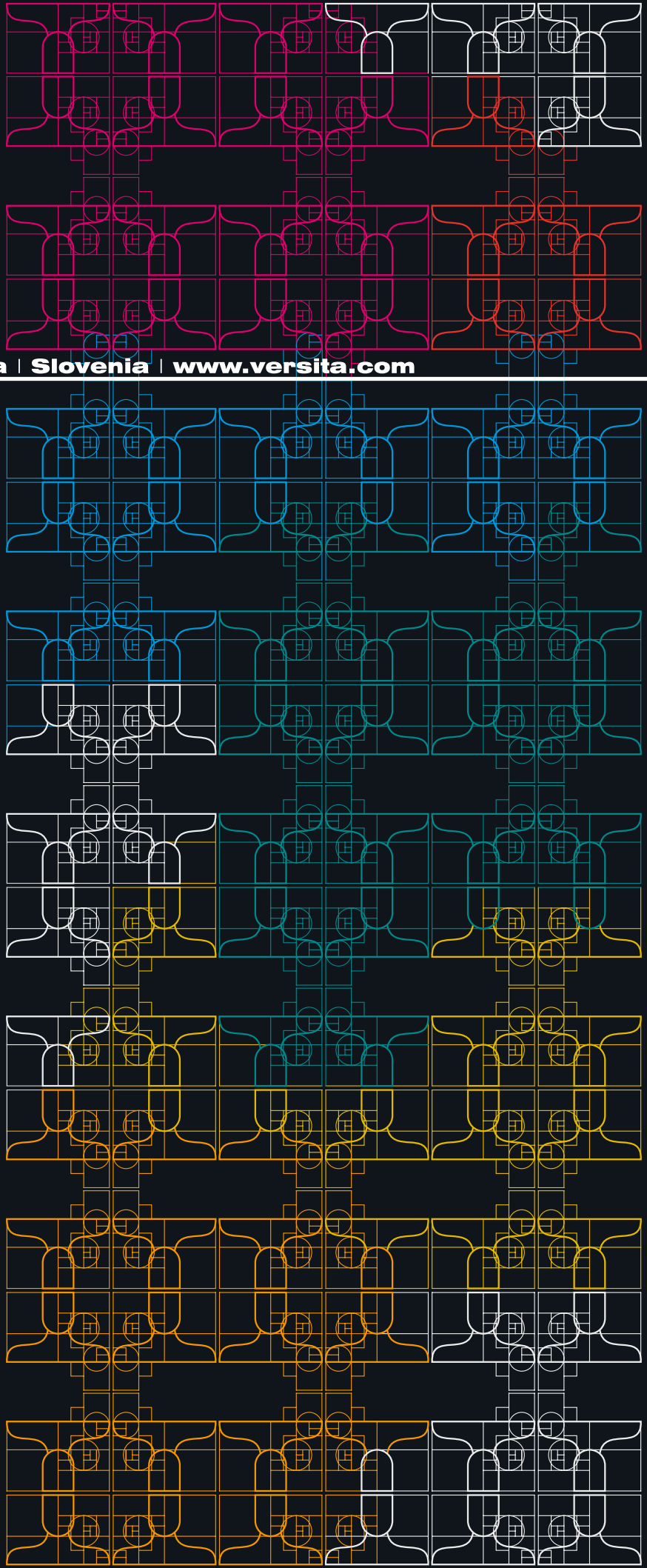

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