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ALIMTA®
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Edina histološko usmerjena citostatična terapija

Pravi **Izbor**
Pravi **Bolnik**
Pravi **Čas**

ALIMTA/Cisplatin:

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

Edina kombinirana terapija z signifikantno izboljšanim preživetjem:
12,6 meseca pri bolnikih z adenokarcinomom pljuč[1]

*vs. Gemcitabin/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). ALIMTA 500 mg: vsaka viala vsebuje 500 mg pemetrekseda (v obliki dinatrijevega pemetrekseda). **Pomožne snovi:** 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 histologije. ALIMTA je indicirana kot monoterapija za zdravljenje bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim karcinomom, ki nima pretežno luskaste 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 monoterapija: 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 pemetrekseda. **Kontraindikacije:** Preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov, Med zdravljenjem s pemetreksedom je treba dojenje prekiniti. 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 proti 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. **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 peroralnimi antikoagulacijskimi učinkovinami ter kemoterapijo proti raku zahtevata povečano pogostnost spremljanja INR. Kontraindicirana sočasna uporaba: Cepivo proti rumeni mrzlici: teganje za smrtno generaliziranо bolezen po cepljenju, Odsvetovana sočasna uporaba: Živa oslabljena cepiva (razen proti rumeni mrzlici): teganje za sistemsko, potencialno smrtno bolezen, **Neželeni učinki** Klinične študije malignega plevralnega mezotelioma Zelo pogosto: znižani nevtrofilci/granulociti, znižani levkociti, znižan hemoglobin, znižani trombociti, slabost, bruhanje, stomatitis/faringitis, anoreksija, diareja, zaprtje, utrujenost, nevropatija- senzorična, povišan kreatinin, znižan očistek kreatinina, izpuščaj, alopecija. Pogosti: konjuktivitis, dispepsija, dehidracija, motnje okusa, Klinične študije nedrobnoceličnega pljučnega karcinoma - ALIMTA monoterapija: Zelo pogosti: znižan hemoglobin, znižani levkociti, znižani nevtrofilci/granulociti, slabost, bruhanje, anoreksija, stomatitis/faringitis, diareja, utrujenost, izpuščaj/luščenje, Pogosti: znižani trombociti, zaprtje, povišana telesna temperatura, povišanje SGPT (ALT), povišanje SGOT (AST), srbenje, alopecija, Klinične študije nedrobnoceličnega pljučnega karcinoma - ALIMTA v kombinaciji s cisplatinom Zelo pogosti: znižan hemoglobin, znižani levkociti, znižani nevtrofilci/granulociti, znižani trombociti, slabost, bruhanje, anoreksija, zaprtje, stomatitis/faringitis, diareja brez kolostomije, utrujenost, povišan kreatinin, alopecija, izpuščaj/luščenje, Pogosti: dispepsija/zgaga, nevropatija-senzorična, motnje okusa. 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 insuficienco in primerih edema. 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** 06.01.2009
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CONTENTS

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review

Cancer cachexia-anorexia syndrome and skeletal muscle wasting

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Background. *Cachexia-anorexia syndrome is a common and important indicator of cancer. It occurs in 30% to 80% of cancer patients. Cachexia means "bad condition" and may be present in the early stages of tumor growth, before any signs of malignancy. Cancer cachexia is a syndrome of progressive body wasting, characterized by loss of adipose tissue and skeletal muscle mass. In most cancer patients, cachexia is characterized by anorexia, which implies a failure of food intake, regulated through a complex system of hormones and neuropeptides. A decline in food intake relative to energy expenditure is a fundamental physiologic derangement leading to cancer associated weight loss. The weight loss in patients with cachexia-anorexia syndrome differs from that in caloric starvation or anorexia nervosa. The pathophysiology of cancer cachexia is not fully understood; however, studies have shown that cytokines are important in the alteration of the carbohydrate, lipid and protein metabolism. Cancer, prolonged bed rest, HIV infection and aging are conditions in which muscle wasting is a common feature. An intervention that may potentially attenuate the progression of muscle wasting in cancer patients is resistance exercise training, defined as multiple repetitions of static or dynamic muscular contractions that increase muscle mass.*

Conclusions. *The main components of the pathological state of cachexia are anorexia and metabolic abnormalities such as fat depletion and muscle protein catabolism. Future developments may concentrate on the molecular abnormalities of cachexia and on examination of the functional benefit of resistance exercise training for cancer related muscle wasting.*

Key words: cancer cachexia; muscle wasting; cytokines; muscle

Introduction

Many patients with chronic or end-stage diseases, such as infection, cancer, acquired immunodeficiency syndrome (AIDS), congestive heart failure, cystic fibrosis, tuberculosis, rheumatoid arthritis, and Crohn's disease, develop cachexia.[1]

The word "cachexia" is derived from Greek "*kakos*" meaning "bad" and "*hexis*" meaning "condition". Cachexia is characterized by weight loss involving depletion of host adipose tissue and skeletal muscle mass. Weight loss in cancer patients differs from that found in caloric starvation, where body fat is lost preferentially (Table 1). Cachexia is an important cause of mortality in cancer patients, between 10-22% of all cancer

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Table 1. Characteristics of starvation and cancer cachexia. Cancer associated weight loss arises from the loss of equal amounts of muscle and fat and is characterized by increased catabolism of skeletal muscle and decreased protein synthesis.

Starvation	Cancer cachexia
Mobilization of fat, sparing of skeletal muscle	Equal mobilization of fat and skeletal muscle
Decreased basal metabolic rate	Normal or increased basal metabolic rate
Liver atrophy	Increased liver size
Normal lipoprotein lipase	Reduced lipoprotein lipase
Decreased protein breakdown	Increased protein breakadown Increased synthesis of acute- phase protein
Reduced glucose turnover	Normal or increased glucose turnover

deaths, as well as death from other causes such as infection.[2] An effective therapy for cachexia should improve the quality of life of cancer patient and it may also be expected to extend the survival time. In general, patients with solid tumors have a higher frequency of cachexia.[3] About 80% of patients with upper gastrointestinal cancer and 60% of patients with lung cancer have had substantial weight loss.[3] Cachexia is more common in children and elderly patients and becomes more pronounced as the disease progresses.

Tumor growth is associated with profound metabolic and neurochemical alterations. About half of all cancer patients show a syndrome of cachexia, causing the clinical manifestations of anorexia, one of the most common symptoms in advanced cancer.[4] Anorexia is defined as the loss of desire to eat, which frequently leads to reduced food intake. Based on various diagnostic tools, anorexia has been detected at the point of cancer diagnosis in 13-55% of patients.[5] In cancer patients, the development of anorexia is associated with the presence of cachexia, resulting in cancer anorexia-cachexia syndrome.[6] Symptoms which are usually identified as part of cachexia-anorexia syndrome include weight loss, anorexia, early satiety, muscular weakness and anemia.[7] Anorexia alone is unlikely to be responsible for the wasting seen in cancer patients, although it may be a contributing factor because the degree of weight loss cannot be ascribed completely to reduced food intake. Anorexia and cachexia can co-exist in cancer patients. The muscle wasting observed in cancer patients occurs even in the presence of normal food intake, and increased muscle proteolysis is detectable even before weight loss occurs.[8] The pathogenesis of anorexia is multifactorial and is related to disturbance of the central physiological mechanisms controlling food intake. The presence of anorexia can be characterized by identification of objective symptoms, including early satiety, taste and smell alterations, meat aversion and nausea/vomiting.[9]

The presence of anorexia is an extremely distressing syndrome, because appetite and the ability to eat have been reported to be the most important factors in the physical and psychological aspects of a patient's quality of life.[10] In anorexic cancer patients, early satiety together with a reduced appetite has been postulated to be caused by the production of factors by the tumor that exert their effects by acting on the hypothalamic sensory cells.[11]

Loss of appetite can arise from decreased taste and smell of food, as well as from cytokine production.[4] An alteration in the regulation of feeding, controlled by a complex of hormones and neuropeptides in the ventral hypothalamus, are also involved in cachexia-anorexia. For instance, neuropep-

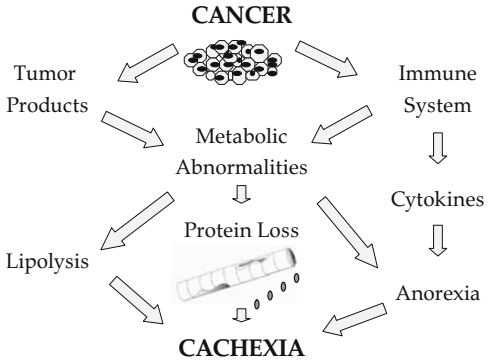

Figure 1. Metabolic abnormalities caused by cancer, resulting in losses of fat and skeletal muscle. Tumors produce factors inducing breakdown of adipose tissue into fatty acids, and protein degradation (amino acids) in skeletal muscle. Cytokines synthesised by tumor or host induced anorexia, the most common symptoms in advanced cancer. Progressive weight loss and skeletal muscle protein loss, profound anorexia characterized Cachexia-Anorexia Syndrome.

tide Y (NPY) is considered to be among the most potent of the feeding stimulatory peptides.[12,13] Increased levels of leptin, a hormone secreted by adipocytes, can block NPY and induce satiety.[14]

Cytokines, in particular, appear to play a key role in satiety disturbances, and an emerging view is that anorexia-cachexia syndrome is caused predominantly by cytokines, either produced by cancer cells or released by the immune system of the host as a response to the presence of cancer.[14]

The wasting observed in cancer cachexia can arise from a decreased energy intake and increased energy expenditure (EE - the amount of calories required for a 24-hour period by the body during an active period) or a combination of both. Hypermetabolism and weight loss are significant predictors of decreased survival and they also indicate that weight loss cannot be explained solely by diminished dietary intake.[15]

Patients with cancer have a highly variable change in energy expenditure. Cancer associated weight loss arises from the loss of equal amounts of muscle and fat and is characterized by increased catabolism of skeletal muscle and decreased protein synthesis.[16] Weight loss can occur during any phase of the disease, and some tumors seem more capable than others of inducing the metabolic changes apparent in cancer cachexia syndrome. In comparison with control groups, patients with malignant disease have been reported to have reduced, normal or elevated energy expenditure.[17-19] The metabolic abnormalities caused by cancer are shown in Figure 1.

Patients with lung and pancreatic cancer have an increased resting energy expenditure (REE - the amount of calories required for a 24-hour period by the body during a non active period) compared with healthy control subjects.[20,21] However, patients with gastric and colorectal cancer were reported to have no elevation of REE.[20] Cachexia can occur even with a normal EE, which suggests that tumor and host factors play an important role in depletion of body lipids (FAT) and protein during the process of cachexia.

The metabolic profile of cachexia is not the same as that of caloric starvation.[7] During the first few days of starvation, glucose utilization by the brain and erythrocytes necessitates depletion of liver and muscle glycogen and increased glucose production by the liver, using gluconeogenic amino acids derived from catabolism of muscle. This phase is replaced in long-term starvation by the use of fat as a fuel, in which free fatty acids released from adipose tissue are converted into ketone bodies, which are utilized for energy by peripheral tissues and, eventually, to some extent by the brain. This leads to prolonged conservation of muscle mass.[6] In contrast, in cancer cachexia, there is equal loss of both fat and muscle, so that for a given degree of weight loss there is more loss of muscle in a patient with cancer cachexia than in caloric starvation.[22] In starving persons, the body

quickly adapts to the lack of nutrients by decreasing its EE and by oxidizing store lipids for energy. This metabolic adaptation to a decreased food intake is critical for maintaining a functional muscle mass. Cancer-bearing hosts, however, fail to develop such metabolic adaptation and thus continue to deplete their skeletal muscle proteins.[6] Metabolic abnormalities in cancer cachexia are described in Figure 1.

The role of cytokines

Cytokines are polypeptides synthesised and released primarily by activated monocytes and macrophages. They are also produced by some types of tumor cells.[23]

Skeletal muscle plays an important role in the immune system as one of the producers of cytokines. Cytokines are primarily responsible for cell-to-cell communication and stimulate the arrival of lymphocytes, neutrophils, monocytes and other healer cells to the injury site to repair the injured tissue.[24] Three important cytokines, tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1) and interleukin 6 (IL-6), are responsible for protein breakdown in skeletal muscle and increased production of prostaglandins. Cytokines are also involved in muscle hypertrophy and in the muscle regeneration process.[25]

Numerous cytokines, including (TNF-α), (IL-1), (IL-6), interferon γ (IFN- γ) and leukemia-inhibitory factor (LIF), play an important role in the etiology of cancer cachexia. Such cytokines may be produced by tumor or host tissue and are characterized by the induction of anorexia and a decrease in the clearing enzyme lipoprotein lipase.[6]

Cytokines are implicated in changes in sensory function in the chorda tympani (involved in the transduction of taste), leading to alterations in taste and in specific food preferences.[4] Patients with end stage cancer have altered taste thresholds with respect to the bitter modality and these changes are most apparent in patients with higher concentrations of C-reactive protein, ILβ-1, IL-6 and TNF-α. The odor threshold was also lower in these patients than in healthy subjects.[26] The described cytokines also sensitize the vagal afferent nerve fibers, resulting in increased activation of the mechanisms that mediate sensations of fullness and thus contribute to the process of satiety.[26] Cytokines may inhibit feeding by causing nausea and vomiting and also by decreasing gastric motility and gastric emptying, intestinal motility or by modifying gastric acid secretion. Their effects may result from direct action on the gastrointestinal system or indirect effects mediated by cytokines (ILβ-1, IL-2, IFN-γ and TNF- α) on the central nervous system.[12,27-33] Cytokines can also interact with prostaglandins (members of a group of lipid compounds that are derived enzymatically from fatty acids and have important functions in the body) and corticotrophin-releasing hormone (CRH), a polypeptide hormone and neurotransmitter involved in the stress response to inhibit gastric emptying.[29,30]

Cytokine interaction with neuropeptides may induce anorexia and can also modify serotonin and catecholamine pathways in the central and peripheral nervous system. Cytokines; TNF-α, IL-1, IL-6 and IFN-γ have been implicated in the induction of cancer related muscle wasting.[34]

Interleukin-1 (IL-1)

IL-1 was observed to induce anorexia, weight loss and hypoalbuminemia in mice[35] and satiety in rats as a result of activation of gluco-sensitive neurons in the ventromedial nucleus of the hypothalamus.[36,37] Otterness *et al.*[38] and Mrosovsky *et al.*[39] observed cachexia-causing effects of IL-1 when it is administrated to animals. Transfection of a cachectic tumor cell line

(colon-26) with the gene for IL-1 receptor antagonist failed to abolish the capacity of the tumor to produce cachexia.[40] These results confirmed a role of this cytokine in the induction of tissue wasting in cancer.

Tumor necrosis factor alpha (TNF- α)

Many of the metabolic disturbances associated with IL-1 are similar to those of TNF-α, a possible cachectic factor. Administration of TNF-α to laboratory animals induces a state of cachexia, with anorexia and depletion of adipose tissue and lean body mass. Its effects are mediated centrally and in the gastrointestinal tract. TNF-α infusions directly reduce gastric emptying and peristalsis,[41] they can induce lipolysis and inhibit lipid synthesis,[42-46] and increase proteolysis in peripheral muscle.[47] TNF-α has been shown in several studies to activate muscle protein degradation directly and induces IL-6 release.[48] It has been demonstrated that IL-6 can impair TNF-α expression in cardiac muscle; one potential role of IL-6 expression in contracting skeletal muscle is therefore to downregulate TNF-α expression.[49]

Llovera *et al.*[50] demonstrated that TNF-α administration to healthy, cancer free rats brought about an enhanced rate of degradation of skeletal muscle protein, even though body weight loss was not apparent in the animals.

Hyperexpression of TNF-α has been identified as one of the key cytokine responses involved in cachexia.[51] TNF-α appears to influence several other abnormalities present during cancer cachexia: adipose and muscle wasting, insulin resistance, increased thermogenesis, and alteration in lipid and protein metabolism.[52,53]

Interleukine - 6 (IL-6)

The role of IL-6 in the development of cancer cachexia has mainly been provided from animal studies involving the use of murine colon-26 adenocarcinoma model. Evidence of a causative role of IL-6 in the pathogenesis of anorexia and cachexia comes from experiments reporting that treatment with anti-mouse IL-6 antibody was successful in reversing the key parameters of anorexia in mice bearing adenocarcinoma.[54,55]

Elevated serum concentration of IL-6 has been reported in cancer patients. IL-6 increased in lung cancer patients and has been identified as a mediator of cachexia by the growth of a uterine cervical carcinoma (Yomoto) in nude mice.[56] An elevated level of serum IL-6 has been reported in patients with colon cancer and in acute–phase response,[57] however, since all patients had lost weight, it is difficult to associate this elevation with the induction of cachexia.

Muscle atrophy has been observed in IL-6 transgenic mice, and another study reported that administration of IL-6 to rats acutely activated both total and myofibrillar protein degradation in skeletal muscle.[58] It has been demonstrated that contracting human skeletal muscle released IL-6 but not TNF-α, because it negatively affects glucose uptake in skeletal muscle.[59]

Glucocorticoids can prevent the stimulatory effects of proinflammatory factors on IL-6 secretion, which in general stimulate proliferation at the earliest, myoblast stage of muscle formation. Prelovsek *et al.*[48] reported that a high dexametasone concentration prevents the stimulatory effects of TNF-α and LPS on IL-6 secretion from the precursors of human muscle regeneration. It results in prevention of myoblast proliferation, leading to a reduced final mass of the regenerated muscle.

The results of human and animal studies strongly implicate IL-6 in the cachectic process. IL-6 probably does not act alone but may either induce or act in synergy with other cachectic factors.

Interferon gamma (IFN-γ)

In mice bearing Lewis lung tumors, the development of tumors is associated with IFN-γ production and with progressive weight loss. IFN-γ antibodies counteract the wasting syndrome observed in cancer cachexia.[60] In rats that had received transplants of MCG 101 sarcoma, anti-IFN-γ antibody reduced weight loss and anorexia but the treatment was partial and short-lived, suggesting that IFN-γ may not be the sole mediator.[61] Such a result should not be interpreted to mean that IFN-γ by itself can induce cachexia, since both IFN-γ release and the presence of tumor cells were found to be required.

Leukemia- inhibitory factor (LIF)

LIF is proposed to be a mediator of cachexia through its ability to decrease lipoprotein lipase activity. LIF plays an important role in the development of cancer cachexia syndrome observed in melanoma-bearing nude mice. The expression of LIF mRNA was examined in four melanoma xenografts, SEKI, G361, A375 and MEWO, in nude mice. SEKI- and G361-bearing nude mice developed cancer cachexia syndrome, and their body weights decreased. A375- and MEWO-bearing nude mice, however, did not develop the syndrome. Northern blot analysis revealed that G361 as well as SEKI expressed a large amount of LIF mRNA, but A375 and MEWO did not, suggesting a close relationship between the expression of LIF mRNA and the development of the syndrome.[62]

Proteolysis-inducing factor (PIF)

Proteolysis-Inducing Factor, which induces muscle wasting, was purified from cachexia-inducing MAC-16, a murine adenocarcinoma. The exogenous administration of PIF to healthy mice resulted in a 50% decrease in muscle protein synthesis and a 50% increase in muscle protein degradation.[63]

A PIF of identical characteristics and molecular weight was detected in the urine of persons losing weight due to pancreatic or gastrointestinal cancers but not in the urine of weight-stable patients with cancer or weight-losing non-cancer patients.[64]

PIF appears to induce muscle wasting via activation of the ubiquitin proteosome pathway.[65] It seems unlikely that any of the cytokines alone are able to explain the complex mechanism of wasting seen in cancer cachexia, and other factors must be involved.

Effects of cachexia on skeletal muscle

Cancer cachexia is associated with perturbations in protein metabolism, leading to significant wasting of tissue proteins. Muscle wasting results from an imbalance between the rates of muscle protein synthesis and degradation. Body composition analysis shows that skeletal muscle is the major site of protein loss in patients with solid (non hematological) tumors.[66] There are also changes in the concentration of plasma amino acids, and most studies report a decrease in gluconeogenic amino acids, in contrast with severe malnutrition, in which the concentration of branched-chain amino acids in plasma is normal or even increased. Protein degradation of amino acid results in the release of amino acids, particularly alanine and glutamine. The former is channeled to the liver for gluconeogenesis and APP (acute phase protein) synthesis, whereas glutamine is taken up by the tumor to sustain energy and nitrogen demands.[1]

Intracellular protein breakdown can be mediated by three pathways; lysosomal, Ca^{2+}-dependent and ATP-ubiquitin dependent proteolytic pathways. All three

pathways may be involved in cachexia, although the ubiquitin-dependent system is considered to be the most important and has been most studied.[67] Recent evidence suggests that PIF and TNF-α, but not other cytokines, can induce expression of the key regulatory components of this pathway.[65,68]

TNF-α induces muscle wasting via inhibition of pathways involved in muscle cell differentiation and regeneration.[69] Exposure of myocites to TNF-α activates the transcription factor-nuclear factor kappa B (NF-κB), which in turn inhibits muscle cell differentiation by suppressing the synthesis of MyoD, a transcriptional factor that is essential for muscle cell differentiation and for the repair of damaged muscle tissue.[69] The activation of NF-κB is also involved in upregulation of cytokine synthesis, which can contribute to paracrine effects of cytokines on skeletal muscle tissue, as previously described. Cytokine-induced skeletal muscle wasting is probably a multifactorial process, involving increased protein degradation and reduced myocite regeneration and repair.[70]

The consequence of skeletal muscle wasting is fatigue. Atrophy of skeletal muscle leads to asthenia and muscle weakness, which causes a reduction of physical activity level in affected persons. This leads to more muscle deconditioning and atrophy, which in turn aggravates the feeling of fatigue.

Physical exercise training may potentially attenuate muscle wasting and/or reduce fatigue in cancer patients. Data obtained from healthy humans and from experimental animals demonstrate that regular endurance exercise training of submaximal intensity (below maximal heartbeat) increases muscle endurance and resistance to fatigue.[71] Resistance exercise training also increases the mass of healthy muscles and attenuates muscle wasting associated with some catabolic conditions. Resistance exercise training in cancer patients increases muscle mass by accelerating the rate of protein synthesis and by attenuating muscle protein breakdown and/or muscle wasting. The effect of resistance exercise training on increased muscle mass in cancer has not been adequately studied.

A mouse model of cancer cachexia (mice bearing the colon-26 adenocarcinoma) was used to test the hypothesis that resistance training, performed by electrical stimulation of the motor nerve would attenuate cancer-related wasting in the contracting muscles.[72] The results of the study demonstrate that resistance training attenuates wasting of the *extensor digitorum longus* muscle (EDL) in tumor-bearing mice. This attenuation of wasting was paralleled by an increase in muscle weight, which was due to an increase in the actual mass of muscle and not merely due to edema. The findings of this study also suggested that the dose of training that attenuates wasting of the EDL muscle in tumor-bearing mice is not sufficient to induce hypertrophy in the EDL muscle in the control non-tumor-bearing mice. These data show that wasted muscles respond differently than non-wasted muscles to exercise training. Attenuation of muscle wasting may be easier to achieve than the induction of hypertrophy of healthy non-wasted muscles, which suggests that persons with wasted muscle may not need to exercise as vigorously in order to attenuate wasting of their muscles.

The loss of skeletal muscle mass that accompanies cancer cachexia is associated with changes in muscle functional properties, with a shift in muscle fiber type distribution or a shift in myosin isoform expression. Myosin heavy chain (MHC) and myosin light chain (MLC) expression are characterized by a decrease in the phenotypic expression of "slow" myosine isoforms (type I MHC and slow forms of MLC) and an increase in the phenotypic

expression of "fast" myosine isoforms (type II MHC and fast MLC isoforms) in the soleus muscle.[68] These changes are likely to have a significant impact on the functional properties of muscles during cachexia. The exact mechanism of this effect on myosin isoform expression is unknown at present. Evidence suggests that the problem is a multifactorial process mediated by host-released and tumor-released factors. The activation of pro-inflammatory cytokines and various proteolytic pathways, particularly the ubiquitin proteasome pathway within skeletal muscle may be responsible for the problem.[69] These factors apparently disturb the balance between the rates of skeletal muscle protein synthesis and muscle protein degradation, leading to muscle protein depletion and muscle wasting. Wasting of skeletal muscle contributes to muscle weakness, fatigue and to the morbidity and mortality of cancer.

Conclusions

There is a high incidence of cancer cachexia-anorexia syndrome in cancer patients. This syndrome is observed in 80% of patients in advanced stage cancer. Cachexia is characterized by wasting of skeletal muscle and depletion of host adipose tissue, while anorexia is associated with persistent loss of appetite and decrease in food intake. Both induce weight loss that differs from caloric starvation.

The cachectic state is particularly problematic in cancer. It typifies a poor prognosis and often lowers responses to chemotherapy and radiation treatment.[74] Radiation damages mitotically active muscle satellite cells, prevents compensatory hypertrophy of skeletal muscle, and prevents small fiber formation.[75]

Skeletal muscle wasting during cancer cachexia is well documented, and resistance exercises should be examined as a potential intervention for attenuating cancer-induced muscle wasting. Exercise-induced changes of skeletal muscle mass should be pursued in terms of changes in muscle protein synthesis and muscle protein degradation pathways in the exercised versus the unexercised muscles, both of which have been implicated in cancer induced muscle wasting.

Exercise that induces attenuation of muscle wasting is a result of an increase in muscle protein synthesis or a decrease in muscle protein breakdown. Its mechanisms need to be explored in future research. It would be important to examine the functional benefit of training-induced attenuation of cancer-related muscle wasting.

The intervention of preserving muscle mass has an important clinical implication, since it improves the prognosis and the quality of life of cachetic cancer patients.

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research article

Xanthogranulomatous cholecystitis remains a challenge in medical practice: experience in 24 cases

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Background. *Xanthogranulomatous cholecystitis (XGC) is a rare, benign, chronic inflammatory disease of the gallbladder. Its importance lies in the fact that imaging studies and intraoperative appearance that may be confused with tumour of the gallbladder. This study aimed to evaluate pre-and intraoperative findings of XGC and to remind it in difficult cholecystectomy patients.*

Patients and methods. *The clinical data of 24 patients with XGC over a period of 7 years were analyzed retrospectively (mean age, 53 years (32-68) M/F ratio 1:1.4).*

Results. *The clinical symptoms were abdominal pain, nausea and jaundice in 79%, 62% and 12% of the patients. Preoperative ultrasonography for 24 patients revealed gallstone (95.8%) and bile sludge (8%). Pericholecystic fluid, polyp and tumour of the gallbladder was present in 20%, 4% and 4% of the patients. The gallbladder was thickened (>3mm) in 10 patients. On computed tomography, all patients showed abnormal findings. The intraoperative findings were as follows: gallstones (100%), chronic cholecystitis (54%), hydropic gallbladder, emphysematous gallbladder, adhesions of the gallbladder to adjacent organs and tumoural mass of gallbladder.*

Conclusions*. XGC is difficult to diagnose pre-or intraoperatively and remains a challenge in medical practice. The definitive diagnosis depends on the histopathologic examination.*

Key words: xanthogranulomatous; cholecystitis; gallbladder

Introduction

Xanthogranulomatous cholecystitis (XGC) is an uncommon inflammatory disease of the gallbladder characterized by the infiltration of plasma cells, lipid-laden histiocytes, and the proliferation of fibroblasts in the gallbladder wall.[1]

The term of xanthogranulomatous cholecystitis was initially proposed by Goodman and Ishak in 1981.[2] The pathogenesis of XGC is the rupture of Rokitansky-Aschoff sinuses and extravasation of bile into the muscular layer. The rupture of the serosa results in adhesion to the adjacent liver, duodenum, and transverse colon. Gallstones

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may have an important role in the pathogenesis, since they appear to be present in most patients.[3]

The clinical and laboratory findings of cases with XGC are similar to those of acute or chronic cholecystitis.[4] The major intraoperative findings are frequently characterized by thickening of the gallbladder wall, tumour-like mass and adherence of the gallbladder to adjacent organs.[5] Patients with XGC are frequently misdiagnosed with imaging studies and even during the operation as having carcinoma of the gallbladder.

The aim of this study is to evaluate pre- and intraoperative findings of XGC and to remind it in difficult cholecystectomy patients.

Patients and methods

Twenty four histologically confirmed cases of XGC were identified from the retrospective analysis of the patient records of 749 cholecystectomy operations over a period of 7 years (January 2000-April 2007). The study included 14 female and 10 male (male/female ratio 1:1.4) having a mean age of 53 years (range, 32- 68 years**).**

The clinical presentation, laboratory and radiological findings, surgical findings, histopathological characteristics, morbidity and mortality were investigated from the surgeon's registry.

All patients underwent preoparative ultrasound examination with 3 MHz transabdominal probe (General Electric Logic5 Pro, Milwaukee, Wisconsin, USA). CT examination was performed for 6 patients with Toshiba spiral CT Asteion. A standard abdominal MRI protocol was employed to one patient. All images were acquired using on 1.5 Tesla MR units. MR studies were performed on a 1.5 T unit (Achieva; Philips Medical Systems, Eindhoven, The Netherlands).

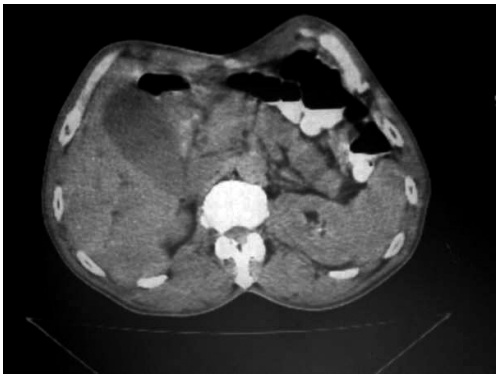

Figure 1. 59-year-old woman with xanthogranulomatous cholecystitis. Contrast-enhanced CT scan shows hydropic gallbladder.

The US findings used for the diagnosis were presence of gallstone and bile sludge, pericholecystic fluid and thickness of the gallbladder wall. Thickening of the gallbladder wall was considered abnormal if it exceeded 3 mm to Kim *et al*.[6] The CT feature used for the diagnosis were presence of gallstones; increased thickness of the gallbladder wall (>3mm), loss of interface between the gallbladder and the liver; pericholecystic fluid; and choledocholithiasis. In one patient, a MRI study was made because of suspected gallbladder carcinoma in CT appearance.

The gallbladder was fixed with 10% formalin and specimens were stained with H and E. Descriptive statistics were used to describe the features of the data in our study.

Results

The clinical symptoms were right hypochondrial pain in 19 (79%) patients, nausea in 15(62%) icteric sclera in three (12%), and fever in two (8%). Main signs included positive Murphy's sign, palpable mass in the right upper quadrant and yellow skin were found in 17 (70%), 24 (100%) and three (12%) patients.

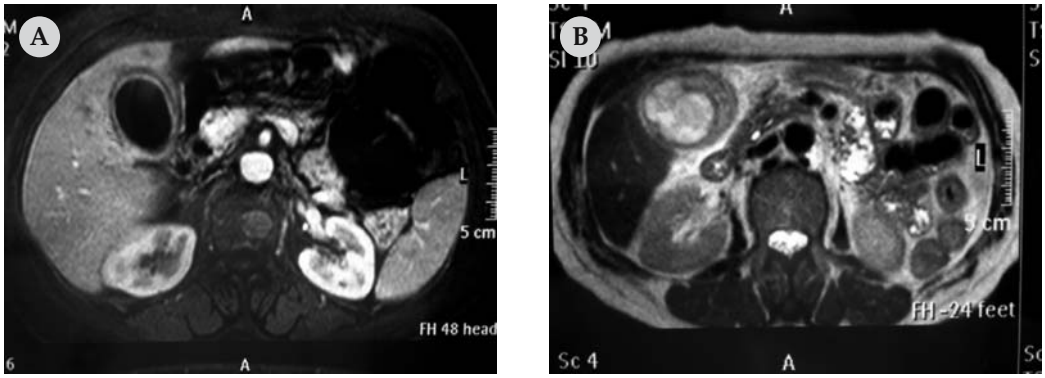

Figure 2. Abdominal MR imaging demonstrating suspected gallbladder mass involving the liver in T1 weighted series(A), and a soft tissue mass in T2 weighted series (B).

Laboratory tests were within normal ranges except leucocytosis in five patients (>12.000/μl), elevated ALT-AST and biluribin levels were found in three patients in each group.

Preoperative US for 24 patients revealed gallstone in 23 (96%) patients, pericholecystic fluid in five (20%), bile sludge in two (8%), polyp of gallbladder in one (4%), and tumour of gallbladder in one (4%) patient. The gallbladder was thickened (>3mm) in ten (41.6%) patients. Upper abdominal CT was performed in six (25%) patients. CT revealed hydrops of gallbladder in two (33.3%) patients (Figure 1), carcinoma of the pancreas also in two (33.3%) patients, pericholecystic abscess in one and one carcinoma of gallbladder. Thickening of the gallbladder wall was seen, more than 3 mm thick, in six patients. MRI showed suspected gallbladder cancer involving the liver in one patient (Figure 2A-B).

The intraoperative findings were as follows: chronic cholecystitis in 13 (54%), adhesions of the gallbladder to adjacent organs in four (adhesions to the transverse colon in two patients, the duodenohepatic ligament in one, and fundus of the gallbladder to abdominal wall in one), emphysematous gallbladder in three (perforation of the gallbladder in two patient and carcinoma of the pancreas in one), hydrops of the gallbladder in two (carcinoma of the pancreas in one patient and enlarged gallbladder with a stone impacted in Hartmann's pouch in one), and tumoural mass of the gallbladder in two patients (Figure 3). The gallbladder stones were found in all patients. Furthermore, the frozen section was performed in one patient and malignancy was not found. The surgical treatment was elective open cholecystectomy in 23 (95.8%) patients and laparoscopic cholecystectomy (LC) in one (4.2%) patient. In one patient, who could not rule out the possibility of

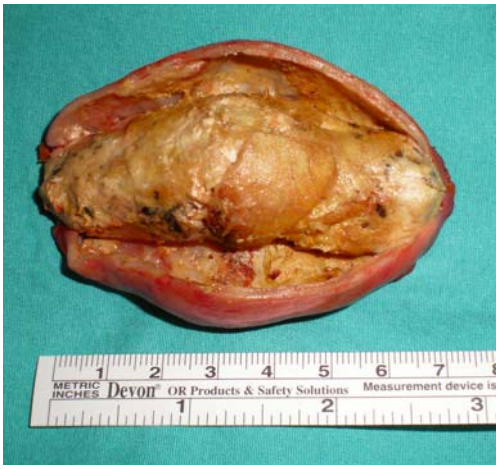

Figure 3. Tumoular like deposits of xanthogranulomatous cholecystitis in surgical specimen.

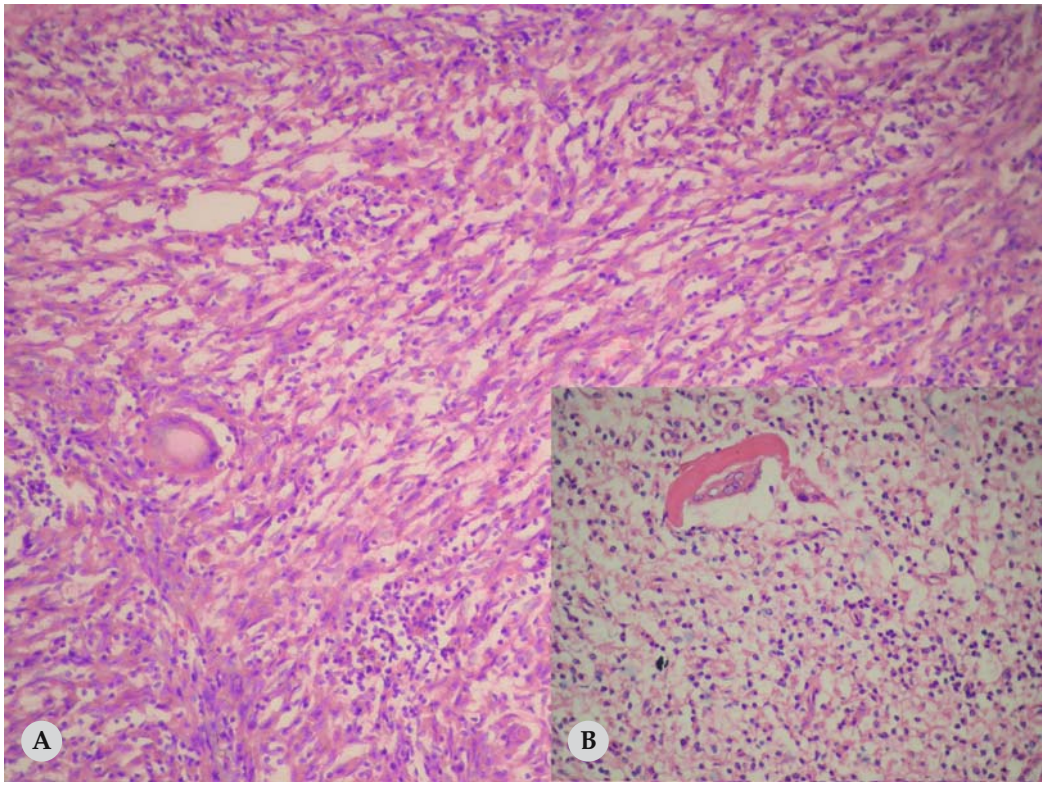

Figure 4. Histiocytes, lymphocytes and giant cells present in a granulomatous focus of xanthogranulomatous inflammation. A; (HE X20) B; (HE X40)

carcinoma, cholecystectomy and wedge resection of adjacent liver was performed.

The postoperative complications were found in 7(29%) patients who included wound infection and pleural effusion in four and two patients, respectively. Acute renal failure developed in one patient with pancreas cancer. The average hospital stay time was 8 days (range, 3- 22 days). No mortality was seen.

Histopathologically there were a focal or diffuse inflammatory process with xanthogranulomatous changes, histiocytosis and giant cells of foreign body in all patients (Figure 4-5). The examination of specimen showed gallstones in 24 (100%) patients, presence of sludge in 13 (54.4%), thickening gallbladder wall (>5 mm) in 15 (62%), dysplasia in four (16%) and mucosal ulcers in three (12%). In addition four (16%) patients had lymphadenopathy which showed reactive lymphadenitis.

Discussion

XGC was previously described as an uncommon form of chronic cholecystitis.[7] Christensen AH and Ishak KG initially described it as a pseudotumour with destructive type of gallbladder inflammation, pericholecystic infiltration, hepatic involvement and lymphadenopathy. In 1981 the term xanthogranulomatous cholecystitis was proposed in a review of 40 cases collected over a 10-year period.[2]

Even though the number of published cases is not large, XGC not as rare as gen-

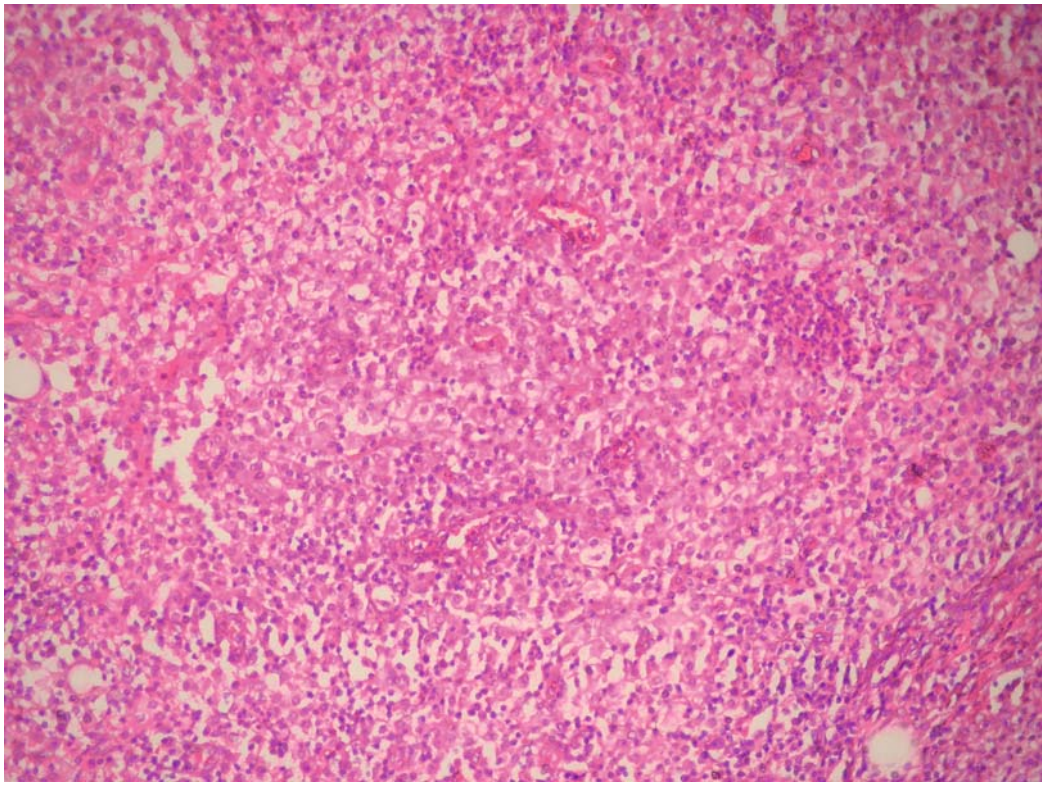

Figure 5. Xanthogranulomatous inflammation of the gallbladder wall, characterized by histiocytes (HEx20).

erally believed.[7] In the literature, the incidence of XGC is reported to be 0.7% to 13.2%.[8] Higher incidence was reported from the Eastern countries.[9-11] In our series, the incidence of XGC was 4% among 749 patients who were operated on gallstone. XGC mostly affects middle-aged women and old persons between 60-70 years. Similar to the previous reports the mean age was 63 years in our study.[12,13] This suggests that age must be one of the significant factors in the development of the XGC. The male to female ratio range is from 2:1 to 1:2 in other series.[14] A study from India[12] reported a 1:9 male to female ratio while in our report male to female ratio was found 1:1.4. The different incidence of XGC may be due to misdiagnosis by clinicians.

Clinically, XGC does not have a typical presentation. Our patients were presented with right upper quadrant pain, nausea, fever, icterus and palpable mass; they are similar to acute or chronic cholecystitis. These clinical features are not specific for XGC and there was no difference between the patients with cholecystitis and gallbladder carcinoma.[11,15] We noted that all of the patients with these symptoms required an elective surgical procedure at first presentation. The association of XGC with a perforated gallbladder, abscess formation, enterobiliary fistula and Mirizzi syndrome were supported by increasing series.[4,16] In our series, these lesions were found in 16% patients. In one case, the adhesion of fundus to abdominal wall was considered as the potential of XGC for fistula formation.

The reported series support the existence of this comorbid factors seen in nearly 23% of patients.[4]

Preoperative biochemical tests or imaging studies are not suggestive of XGC.[12] Neither the liver function abnormalities nor the tumour markers (CEA, CA-19.9) are suggestive of XGC, although Adachi *et al*.[17] argued that serum level of CA-19.9 may be elevated in both carcinoma and XGC patients. It has been reported that thickening of the gallbladder, adhesion to neighbouring tissues or organs were specific findings to XGC, although there are other series that it is difficult to differentiate XGC from other lesions.[18,19] All these findings can be seen in acute cholecystitis; however, the presence of intramural low-attenuation nodules, preservation of mucosal lining and degree of enhancement of the gallbladder wall are suggestive of the XGC.[18] In our study none of the patients was diagnosed by radiologist as XGC with imaging findings. US misdiagnosed one case of XGC as carcinoma of the gallbladder (misdiagnose rate 4.3%), while CT misdiagnosed two cases of XGC as carcinoma (misdiagnose rate 33.3%). In this study, high misdiagnosis rate may be related to our insufficient experience for imaging findings of XGC. Chun *et al*.[18] concluded that a definitive diagnosis of gallbladder carcinoma is not possible with only imaging findings. In our study, thickening of the gallbladder wall was found in 24% and 46% patients with US and CT respectively. While thickening of the gallbladder wall was found in 62% of patients pathologically. This leads to the conclusion that thickening of the gallbladder wall in imaging findings itself is not a predictive factor in the diagnosis of XGC. Hatakenaka et al.[20] have demonstrated that MR imaging may play an important role in differentiating XGC from carcinoma in patients with a thickened gallbladder wall. In our series, MR imaging did not differentiate XGC from gallbladder carcinoma.

In our series as in others reported in the literature, all patients had gallstones (100%), frequently sludge of bile (54%) or biliary obstruction (12%).[4] Its importance lies in the fact that gallstones can have an important role in the pathogenesis of XGC via extravasation of bile into the gallbladder wall. In contrary, in a series noted the presence of gallstone in only 85% of the XGC, which leads to the conclusion that the presence of gallstone is only an associated condition and not the cause of the inflammatory process.[21] The small ulcerations in the mucosa reported to be a precipitating factor in other studies.[7,8] The extravasation of bile causes that fibrous reaction and scarring healing within the gallbladder wall, response due to thickening of the gallbladder wall. The ulcerations in the mucosa were found pathologically in 12% of our patients.

Open cholecystectomy is the first choice for XGC, either complete or partial.[14] In our series, single cholecystectomy was performed on 23 patients and cholecystectomy with partial hepatic wedge resection on one patient. LC may be contraindicated in XGC because of a high incidence of complications. According to the study carried by Guzmán-Valdivia G, LC was not completed due to difficulty in dissecting the gallbladder and converted into the open procedure in 80% of the patients diagnosed as XGC.[21] The necessity of radical surgery is not cleared with extra-gallbladder involvement.[4] The intraoperative frozen section or fine needle aspiration has been suggested to confirm the diagnosis of XGC.[9] The frozen section is valuable when there is no invasion of pericholecystic organs. In our series, the frozen section was used (negative result) in a case with an extensive invasion of liver. We believe it was not change the approach of surgeon. Nevertheless, in the patients with negative results, the radical

surgery can be performed due to the coexistence of XGC and carcinoma of the gallbladder. On the other hand, studies report that the radical surgery may be associated with a high perioperative morbidity.[22]

Although XGC is a benign disease, patients usually have a longer hospital stay with a postoperative complications.[4] In our study, complications occurred in 7(29%) patients including wound infection, pleural effusion and renal failure related to hepatorenal failure. Complications were reported including leakage of bile and bile peritonitis which largely related to the difficulty in cholecystectomy.[9] The complications are thus related more to the technical difficulty in stripping the gallbladder, the mode of the operation and the clinical condition of the patient than to the disease itself.

Conclusions

The pre- or intraoperative differential diagnosis of XGC from other gallbladder diseases remains a challenge in medical practice. The presence of firm adhesions of the gallbladder to neighbouring organs and tissues, thickened gallbladder wall, together with gallstone in a patient with chronic disease, is highly suggestive of XGC. The definitive diagnosis depends on the histopathologic examination. XGC can be treated successfully with an accurate diagnosis and proper operation.

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case report

Spinal subdural haematoma in von Willebrand disease

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Background. *Von Wilebrand disease (vWD) is the most common inherited disorder of hemostasis. Bleeding in patients with von Wilebrand disease is a frequently reported complaint. Patients with inherited bleeding disorders are also in a large number of cases infected with hepatitis C virus (HCV). Studies showed an increased risk factor for intracerebral hemorrhage (ICH) in patients infected with HCV receiving the combination treatment.*

Case report. *A 44-year old man reported to our Emergency Department with low back pain, headaches, neck pain, leg weakness and urinary retention. The neurological examination showed nuchal rigidity and spastic paraparesis. Thoracic spine MRI revealed a subacute subdural haematoma at T8-T11 level.*

Conclusions. *To the best of our knowledge, this is the first report of a subdural haematoma of the thoracic spine in a patient with vWD and chronic HCV infection. The presented patient was receiving a combination treatment, a fact that has also to be taken in consideration as a possible risk factor for a bleeding episode.*

Key words: von Willebrand disease; subdural haematoma; hepatitis C; complications of treatment; interferon; ribavirin

Introduction

Von Wilebrand disease (vWD) is an inherited bleeding disorder that results from quantitative and qualitative deficiencies of von Wilebrand factor (vWF), a glycoprotein with an essential role in primary and secondary hemostasis.[1] vWD is the most common inherited bleeding disorder, with a prevalence from 0.6% to 1.2%.[2] Patients with vWD are prone to complain of different bleeding episodes like mucocutaneus bleeding, menorrhagia, gastrointestinal bleeding with angiodysplasia or excessive postsurgical bleeding.[1] The infection with hepatitis C virus (HCV) is a major comorbidity in patients affected by bleeding disorders, with a prevalence of 39% in vWD patients.[3] In patients with chronic HCV infection the combination therapy of pegylated interferon alpha-2b and ribavirin is becoming a standard treatment.[4] Recent studies suggested that patients with chronic HCV infection receiving combination therapy were more prone to develop an intracerebral haemorrhage (ICH), indicating

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a possible association between HCV, HCV treatment and ICH.[5, 6]

Spinal subdural hematoma (SSDH) is a rare condition that can lead to spinal cord or cauda equina compression.[7] A variety of causes, like bleeding disorders, anticoagulant therapy, arteriovenous malformations or underlying neoplasm have been described as possible pathogenic factors that could promote the formation of an acute SSDH.[8] Therefore, SSDH is a neurological and neurosurgical emergency that has to be promptly diagnosed and treated in order to provide the best possible recovery.

We report on a 44-year old man with vWD and chronic HCV receiving the combination therapy that developed sudden leg weakness and low back pain as the initial presentation of spontaneous acute SSDH. The diagnosis was confirmed by MRI and the patient was treated with a conservative approach.

Here we discuss the clinical features, imaging findings, treatment decision and outcome.

Case report

A 44-year old man reported to our Emergency Department (ED) with acute low back pain, headaches, neck pain, leg weakness and urinary retention. During the last two days before he reported to our ED, the patient had high body temperature. Since childhood, he was diagnosed vWD. He was serologically diagnosed as infected with HCV in 1999, and had been treated with the combination therapy of pegylated interferon alpha-2b and ribavirin for a period of five months before he reported to our ED. A month and a half before this episode, he was admitted to our Gastroenterology Department because of a bleeding duodenal ulcer. There was no history of trauma.

The physical examination showed blood pressure 100/60 mm Hg, pulse 88/min and body temperature of 37.7 °C. The neurological examination showed nuchal rigidity and spastic paraparesis of the legs. The blood test revealed a platelet count of 89.000/mm^3, a prothrombin time of 65% and a partial thromboplastin time of 54 s. Laboratory studies for vWD showed a factor VIII activity of 43% and a vWF activity (ristocetin factor) of 17%. A MRI of the thoracic spine revealed a high intensity signal in T1- and T2-weighted images (WI) extending from T8 to T11 that belonged to a dorsal subdural haematoma. There was no abnormal signal visible in the spinal cord (Figure 1). The treatment was conservative and cryoprecipitates were administered according to the level of factor VIII and vWF activity. The antiviral combination therapy was withdrawn. The patient responded well to our therapy and physiotherapy treatment followed after the initial mobilization.

After a month, on discharge from our centre, the patient was able to sit independently and his neurological improvement was satisfactory. On a follow-up MRI scan done 3 months after he reported to our ED there was a complete regression of the subdural hematoma and the neurological examination was normal.

Discussion

Acute SSDH is a rare condition that may result from iatrogenic factors, but also from coagulopathies, anticoagulant therapy, severe liver failure, underlying neoplasm, arteriovenous malformations or after poisoning with rodenticides of the coumarin group.[8-10] It usually presents with acute back pain and signs of spinal cord and cauda equina compression. Bleeding episodes often accompany vWD, but they rarely lead to the formation of a hematoma.[1] In two

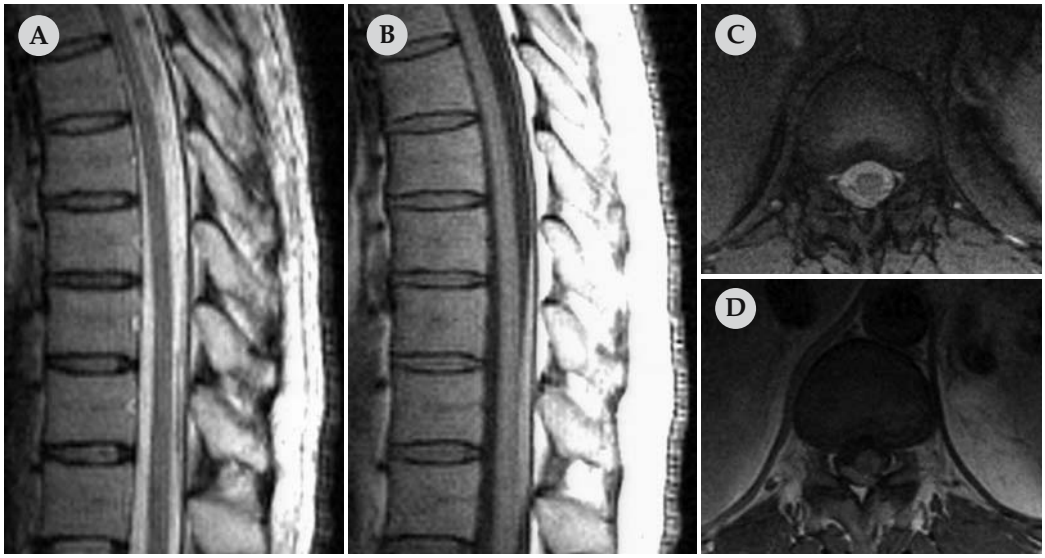

Figure 1A, B, C, D. Sagital T2-WI (A), sagital T1-WI (B), axial T2-WI (C) and axial T1-WI (D) showing a T1 and T2 high intensity signal dorsal spinal subdural haematoma.

cases, patients with vWD reported with a subgaleal hematoma and an encapsulated hematoma of the thigh, both of them occurring after a trauma.[11,12]

Our patient with SSDH and vWD did not have any recent history of trauma, but he was on combination therapy for chronic HCV infection. Recent studies and case reports suggested that HCV infection alone or with combination therapy raised the possibility to develop an ICH.[5,6,13] Taking this in consideration, we could hypothesize that chronic HCV infection and combination therapy could be a risk factor to develop SSDH. Therefore, we decided to withdraw the antiviral combination therapy from his treatment.

MRI is the imaging method of choice to determine the location, evolution, extent and shape of the hematoma, as well as the follow-up of the patient like at the patients with the others causes of spine cord compresion.[9,10,14,15] Our patient had a dorsal hematoma, with a crescent shape, extending from T8 to T11 and a high intensity signal in T1- and T2-weighted images indicating a hematoma in the subacute phase. The epidural fat was well delineated, a fact that confirms the subdural location of the hematoma. Follow-up MRI scans were done in order to asses the evolution of the hematoma, that in our case in a 3 months period showed a complete regression, and to look for possible long term complications represented by arachnoidal fibrosis or spinal cord atrophy.[10]

The current literature suggests three treatment options in case of a subdural hematoma: surgical decompression, percutaneous clot drainage and conservative treatment.[9,10,14] In a previous case of a subdural hematoma in a patient with idiopathic thrombocytopenic purpura a conservative approach was preferred, because of a significant risk of bleeding.[16] Because our patient was diagnosed with vWD, a factor that could promote bleeding complications during a surgical procedure, we opted for a conservative treatment.

Reviewing the literature regarding spinal hematoma and vWD, Kakazu *et al.*[17] reported of a spontaneous spinal epidural haem-

orrhage associated with vWD. Therefore, to the best of our knowledge, this is the first report of a subdural hematoma of the thoracic spine in a patient with vWD and chronic HCV infection under the combination therapy. Our patient had an indicative clinical presentation, the diagnosis was confirmed by MRI and we decide to pursue a conservative treatment approach. We withdrew the antiviral combination therapy because of the possibility that it could be a risk factor for spontaneous bleeding. After three months, our patient showed a complete recovery.

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research article

Increased late urinary toxicity with whole pelvic radiotherapy after prostatectomy

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Background. *Radiotherapy aimed at prostatic bed (PBRT) can prevent recurrence or reestablish remission in prostate cancer patients primarily treated with prostatectomy. In selected patients results may be improved with the additional irradiation of pelvic nodes (WPRT).*

Patients and methods. *The objective of the study was to evaluate late toxicity of postoperative radiotherapy in 43 patients – 21/43 treated with WPRT. Dysuria, haematuria, nocturia, continence and obstructive urination problems as well as urgency, continence, frequency, pain and bleeding of defecations were prospectively registered and converted to a modified Radiation Therapy Oncology Group (RTOG) – late effects normal tissue (LENT) scoring system. Median tumour dose (TD) for PBRT was 64.8 (59.4-70.0) Gy and for WPRT 50.4 (48.0-56.0) Gy.*

Results*. More important than the deterioration of intestinal function (worsening for 1 grade in 54% and ≥2 grades in 5% of patients) was the deterioration of urinary function (worsening for 1 grade in 33% and ≥2 grades in 26% of patients). This appeared to be more frequent in patients with WPRT than PBRT (67% vs. 50% of patients) especially in conjunction with WPRT TD >52 Gy (deterioration in 71% of patients).*

Conclusions. *Although several factors may influence increased urinary toxicity after WPRT, it seems reasonable to lower the urinary bladder dose as it possible with novel radiation techniques.*

Key words: prostate cancer; postoperative radiotherapy; whole pelvic radiotherapy; late toxicity

Introduction

Radiotherapy has a well established role in the treatment of patients after radical prostatectomy. Immediately after the operation it can prevent recurrence in patients with high risk features[1-3] or reestablish the remission when applied as a salvage treatment for biochemical or local recurrence.[4] It can be used as a sole treatment or in combination with hormonal therapy.[5]

Regardless whether it is used immediately postoperatively, or as a salvage treatment, radiation fields are focused on a believable position of prostate and seminal vesicles as it was before the resection[1-3,6,7] and/or areas of the most probable local recurrence.[8]

It is suggested that with broadening of the treatment fields with the inclusion of pelvic nodes in selected patients considered at high risk of lymph node involvement, the

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improved biochemical relapse-free survival can be acquired.[9]

The objective of the study was to evaluate late toxicity of this treatment.

Patients and methods

Documents of 49 patients with prostate cancer that received the radiation treatment after prostatectomy between June 2001 and December 2005 at Institute of Oncology Ljubljana were analysed to evaluate the consequences of the radiation treatment. The treatment and the follow-up with the attentive evaluation of side effects were conducted by the author.

Radiation started 1.8-88.5 (median 6.1) months after prostatectomy. Forty-three % (21/49) of patients were treated a few months after prostatectomy because of a high risk of recurrence and with preradiation PSA below 0.2 ng/ml; the others (28/49 (57%) patients) were submitted to radiotherapy because of the biochemical or local recurrence.

Treatment fields were limited to prostatic bed (PBRT) in 24/49 (49%) of patients – in 25/49 (51%) patients pelvic nodes up to the lumbosacral (S1/L5) interspace were also included (WPRT). The median dose defined by 95% isodose, encompassing target volume applied to the prostatic bed, was 64.8 Gy (59.0-70.0 Gy) and 50.4 Gy (48.0-56.0 Gy) to the pelvic region in fractions of 1.8-2.2 Gy (median 2.0 Gy). Bioequivalent doses for a fraction of 2 Gy (BED_2) were computed using $\alpha/\beta = 3$. Median BED_2 to the prostatic bed was 62.2 Gy (range 57.3-73.0 Gy) and to the whole pelvis 50 Gy (dose range 48.0-57.3 Gy). Treatments were delivered on the linear accelerator using four-field technique and standard fractionation. A dose calculation with 2D planning was performed in 20/49 (41%) patients and 3D in 29/49 (59%) patients. The posterior rectal wall was excluded on lateral fields otherwise no dose constraints were used to limit the dose to organs at risk. Regular portal images were used to limit set up errors.

Long term side effects were assessed in 43/49 (88%) patients still alive and with no sign of recurrence at the time of evaluation.

Side effects rising as a consequence of urinary damage were prospectively registered at each visit with regard to dysuria, haematuria, nocturia, incontinence and obstructive problems. Similarly, the consequences of intestinal toxicity were registered with regard to urgency, continence, frequency of defecations, as well as pain and bleeding problems.

Side effects were converted to late lower gastrointestinal and urinary toxicity scores according to Storey's modification of the Radiation Therapy Oncology Group (RTOG) – late effects normal tissue (LENT) late toxicity scoring system at the time of evaluation.[10] Still some additional modification was performed regarding urinary frequency and obstruction primarily aimed to evaluate toxicity already present after prostatectomy. A minor modification was made also with regard to intestinal toxicity with the inclusion of urgency of defecations (Table 1). Erectile problems were not prospectively evaluated and therefore, they were not included and analysed.

Urination characteristics of the initial (before radiotherapy) and the final (at the last follow-up visit) evaluation were compared also in the sense of the possible improvement. On the other hand, worsening of urination characteristics for a grade was considered as a minor deterioration, while worsening of two or more grades was considered as a major deterioration. The same principle was applied also in the evaluation of late intestinal toxicity.

The Kaplan Meier survival method was used to assess the time of the appearance of toxicity. The first appearance of the most

Table 1. Modifications of delayed radiation toxicity grading using Radiation Therapy Oncology Group (RTOG) and Late Effects Normal Tissue Task Force (LENT) criteria with regard to urinary and intestinal toxicity

Criteria	Grade1	Grade 2	Grade 3	Grade 4
Nocturnal urinary frequency	2-3	3-4	hourly or less	dysfunction requiring cystectomy/urinary diversion/nephrostomy
Obstructive urinary symptoms	occasional	regular	operative treatment / urethrotomy	dysfunction requiring cystectomy/urinary diversion/nephrostomy
Urinary incontinence	occasional/drops	regular us of up to 1 sanitary pad	regular use of 2 sanitary pads or more	dysfunction requiring cystectomy/urinary diversion/nephrostomy
Urgency of defecations	present/ without incontinence	intermittent use of sanitary pads	regular use of sanitary pads	dysfunction requiring surgery

pronounced change in urination and defecation characteristics in each patient was considered as the observed event. 1-cummulative survival curve was used to present the results graphically. Chi square test was used for the estimation of differences in late toxicity between patients with prostate only and additional pelvic radiation as well as between patients with different dose regimens.[11] P-value ≤0.05 was considered significant in all statistical tests. The SPSS 15.0 for Windows was used as a tool for the analysis.

Results

Considerable urination difficulties already existed at the start of the radiation treatment. These were mostly related to the increased nocturnal urination frequency and difficulties with continence. Problems with increased nocturnal urinations experienced 21/43 (49%) patients – difficulties were considerable (grade 3) in 5/43 (12%). Even more pronounced were efforts to remain continent. More or less pronounced dripping of urine was evident in 24/43 (56%) patients. Urinary incontinence without the need for sanitary pads was evident in 14/43 (33%) patients, up to one sanitary pad per day was used by the next 6/43 (14%) patients while pronounced incontinence with 2 or more pads per day was evidenced in 4/43 (9%) patients.

Late toxicity was evaluated after a median follow up of 52 months. During follow up visits some of urination difficulties have actually resolved or became less pronounced (Table 2). The improvement in the sense of less frequent nocturnal urinations was noted in 9/21 (43%) patients with this problem after the surgery and the improvement in the sense of less pronounced incontinence was noted in 11/24 (46%) pa-

Table 2. Change in urination characteristics after postoperative radiotherapy comparing the condition after prostatectomy and at the last follow-up

Change in urination characteristics	Dysuria	Haematuria	Nocturia	Incontinence	Obstruction
Improvement	0	0	9	11	0
No change	40	37	18	20	40
Minor deterioration	2	3	12	7	1
Major deterioration	1	3	4	5	2

tients who had continence difficulties after the surgery.

The comparison of all urination characteristics between the situation at the start of radiation and at the last follow up visit showed that some improvement, or no change, was evident in 18/43 (42%) patients, and minor deterioration was noticed in 14/43 (33%) patients. However, the major deterioration of urination characteristics was evident in 11/43 (26%) patients. These eleven patients included two patients with severe haemorrhagic cystis and 6 patients with G3 urinary toxicity. In both patients with haemorrhagic cystitis cystectomy was obligatory. However, in 2 of 6 patients with G3 toxicity symptoms have resolved after the endoscopic incision for the bladder neck obstruction.

As expected, in comparison to crude incidence rates, results of the survival analysis of toxicity events were even more unpromising showing the deterioration of urinary function in 73% of patients at 5 years (Figure 1).

The comparison of the incidence of deteriorations between patients that received PBRT and WPRT, as well as the comparison of BED_2 for WPRT (lower or greater than 52 Gy) and of BED_2 for prostate bed (lower or greater than 65 Gy) showed no statistical significance. However, differences were seen in favour of PBRT and also in favour of lower radiation doses to either prostate bed or pelvic nodes (Table 3).

Compared to urinary deterioration, the deterioration of the intestinal function was markedly less pronounced. It appeared as more frequent defecations (in 23/43 (54%) patients), urgency to defecate usually as a consequence to some dietary offence (in 15/43 (35%), occasional bleeding (7/43 (16%) or occasional uncontrolled mucous discharge (in 4/43 (9%) patients). None of the patients experienced G3/G4 toxicity. Altogether defecation characteristics remained unchanged in 13/43 (30%) patients, the minor deterioration as G1 toxicity was evident in 28/43 (65%) and major as G2 toxicity in 2/43 (5%) patients.

Actuarial rates of intestinal radiation late effects were slightly lower than for the urinary tract (63% at 5 years) and with decreasing appearance of new cases after 40 months after the start of radiotherapy.

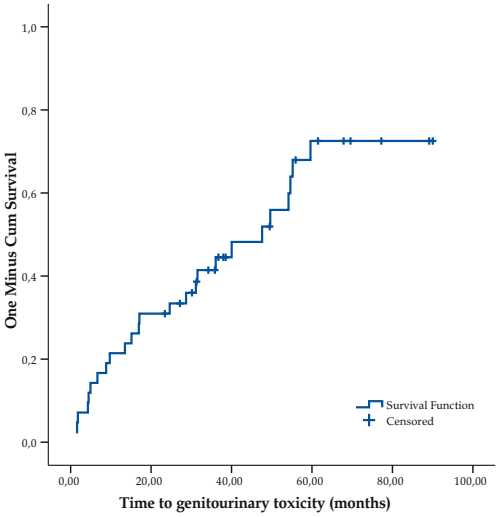

Figure 1. Survival without deterioration of urination characteristics.

Table 3. Incidence of deterioration in urinary function regarding treatment fields and radiation doses

Characteristics	Incidence of deteriorations	P
Prostate bed RT	11/22 (50%)	0.226
Whole pelvic RT	14/21 (67%)	
Whole pelvic dose <52 Gy	8/13 (62%)	0.243
Whole pelvic dose ≥52 Gy	6/8 (75%)	
Prostate bed dose <64 Gy	15/29 (52%)	0.259
Prostate bed dose ≥64 Gy	10/14 (71%)	

No difference in the rate of side effects was found between pelvic and prostatic only radiation and also not between different dosages

Discussion

Postoperative radiotherapy is generally reported as non toxic with a few severe late side effects. With treatment fields limited to prostatic bed and doses of 60-62 Gy, rates of serious side effects are lower than 5%,[2,12] and seems to remain within this range also with the dose escalation to 70 Gy.[13,14]

However, low grade toxicity seems to be quite common. In the survey of 75 patients, Pearce reported the incidence of any toxicity registered at any point during the median follow up of 45 months, after the irradiation of 60-66 Gy to prostatic bed, to be 51% for intestinal and 78% for urinary tract.[15] Similarly in the report of EORTC trial 22911 5-year cumulative incidence rate of complications (of any grade) based on the competive risk analysis in the radiotherapy arm is about 70%.[2] Doses applied in the trial were 60 Gy while treatment fields were limited more or less to prostatic areas. Initial 50 Gy were applied to somewhat larger area including the region of seminal vesicles and larger margins around prostatic bed. Compared to these reports our results are disappointing. They do not differ so much in overall toxicity as in the rate of severe complications. A straightforward explanation for these events is hard to give. Especially if we consider that both patients that required cystectomy were treated with conventional doses of 60 and 64 Gy and fractionation of 2 Gy and with treatment fields limited to the initial position of prostate and seminal vesicles.

Long term intestinal toxicity, although common in our study, does not turn out to be an important problem. In all patients it was of a low grade. In the majority it was manifested with slightly increased number of defecations. They also needed some kind of diet, and in the case of an offence, urgency that may in some patients be aggravated to minor continence problems occurred. No difference was noted whether treatment fields were limited to a prostate region or they encompass also pelvic nodes.

Prevalent problems in our patients were related to postirradiation damage to urinary bladder. However, our results suggest that considerable problems with urination were already present after the prostatectomy. Furthermore, results of already mentioned EORTC trial suggest that problems arise with time also when prostatectomy was the sole treatment. So, we can not look at the consequences of the postoperative radiation neglecting the impact of the surgical treatment. At least in a part, long term side effects of this combined treatment are due to the prostatectomy. The improvement of urination characteristics in 24% of patients after radiotherapy suggests a prolonged healing after the prostatectomy at least in some patients. For these patients often used interval of 2-3 months to start adjuvant radiation after the surgery may be too short. According to this, lower toxicity could be expected in patients irradiated after an interval that would suggest a complete healing after the prostatectomy. However, there was no difference in the rates of major deterioration of urination characteristics between patients irradiated with an interval to surgery shorter or longer than 2 years. It appeared in 5/15 (33%) and 6/28 (21%) patients respectively. Nevertheless, an uncompleted healing after the surgery may be an additional factor contributing to long term toxicity. It seems reasonable to wait with irradiation until patients report no further improvement, perhaps jointly with the introduction of the hormonal treatment.

Further reasons for urination problems may ground in the properties of the postoperative irradiation. Limited data are available as a predictor of late urinary toxicity in the postoperative radiotherapy.[12] Less obscured is a situation with the radical radiotherapy treatment of prostate cancer and some of these data may be valid also in the context of postoperative radiotherapy. Pinkawa stated in a study of 80 patients with the use of Expanded Prostate Cancer Index Composite questionnaire, that the patients' ability to fill the bladder has a major impact on the dose-volume histogram parameters and on both, acute and late urinary toxicity.[16] Similar was the conclusion of Harsolia stating the importance of urinary bladder dose-volume parameters on 331 patients using National Cancer Institute Common Toxicity Criteria 2.0 for the evaluation of chronic urinary toxiciy.[17] A further similarity of both studies is a finding of the importance of the exposure of urinary bladder to relatively low doses of radiation in the range of 30-40 Gy. In our study, due to the inclusion of pelvic nodes and the use of box technique, large parts of bladder were exposed to irradiation. Even when treatment fields were restricted to prostatic bed regularly about 80% of the bladder volume received dose equal or greater than 60 Gy (dose that is similar to target doses). The interdependence of late urinary toxicity and volume and dose of irradiation is also suggested by our results. The increase in urinary problems, although not statistically significant, was seen in patients with larger fields by the comparison of pelvic and prostate only irradiation. The increase in toxicity was also evident with larger doses – greater than 52 Gy to pelvic field and 64 Gy to prostatic bed. To reduce the radiation damage to urinary bladder it seems important not only to limit the target dose but also, and for most, to exclude as much as possible of the bladder out of the irradiated volume and to limit the dose to the rest of the bladder wall. New radiation techniques such as intensity modulated radiotherapy (IMRT) can produce concave dose distribution and, as shown by planning studies, can reduce the volume of the bladder exposed to high doses for 20-50%.[18,19] Less effective is IMRT in the low dose range. How effective will be this and other techniques that make it possible to reduce the dose to the urinary bladder, in the reduction of late urination toxicity with pelvic irradiation is still unclear.

Perhaps the easiest and most reliable way to solve the marked urinary toxicity is to reduce the target volume. EORTC giudelines[8] for the target volume definition in postoperative radiotherapy for prostate cancer, with the limitation of target volume to the sites of most probable local recurrence, enable a considerable reduction in dose-volume parameters of the urinary bladder. However, certain assurance is needed to exclude pelvic nodes. Reliable information is offered by extended, and perhaps also, but with less trustworthiness, classic lymphadenectomy.[20] In our study lymphadenctomy was performed in 77% of patients with a median 6 resected nodes. If, and how much, the omitment of pelvic radiotherapy in our patients would compromise the results of the treatment, could be anticipated from the initial results that suggest the significantly improved progression free survival (86% and 71% at 5 years) in patients treated with whole pelvic radiotherapy (WPRT) for the relapsed or progressive disease after the prostatectomy. They support the effectiveness and superiority of WPRT to prostatic bed radiation for patients with the risk of nodal invasion greater than 15% according to Roach equation[21] and with no reliable information on the lymph node status through lymphadenectomy. More secure is to make some compromise in the target volume. One possibility is the less strict

inclusion of the lymphatic region around a distal part of external iliacal vessels,[22] and the other is to adapt target volume to the extensiveness of lymphadenectomy with the exclusions of regions that were already submitted to lymphadenectomy.

The hormonal therapy can be a component of postoperative treatment.[23] In the majority of our patients, goserelin 10.8 mg every 3 months was used for one year. The treatment started at least one month before the commencement of radiotherapy, so castrate testosterone levels were achieved at the time of radiotherapy. Considering the study of Taussky in which a relationship between chronic toxicity and testosterone level at the time of radiotherapy was stated, hormonal therapy should also be considered as one of the possible factors that influence our results.[24] What will be the risks of the exclusion of the immediate hormonal therapy will be eventually demonstrated by RTOG 96-01 trial results.

Nevertheless, some caution is needed with the interpretation of the results on late urinary toxicity. The fibrosis of the bladder wall and the loss of muscle function as a result of late radiation damage, were in the majority of our patients demonstrated with continence problems and increased urination frequency with the need to wake up at night. Common criteria used to evaluate and grade this problem, are toxic criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer.[25] However, in RTOG scales these problems are almost completely ignored, as is the case with incontinence, or inexact (and subjective) for correct grading of the problem, such is the case with the increased urination frequency. Modifications of this scoring system as proposed by Storey[10] also leave some uncertainties in the evaluation of mild, initial continence problems not necessitating the use of pads, and also severe problems with the use of multiple pads per day. It also remains too broad to register changes in the urination frequency – especially in connection with the postoperative radiation of prostatic carcinoma with distinctive problems present already after the prostatectomy. Not so rare scenario of 2-3 wakening per night after the prostatectomy with the increase to 4-5 wakening can be scored as no toxicity (not twice baseline), G1 toxicity (twice baseline) and also as G2 or G3 (moderate or severe) toxicity depending on the impression of the inquirer. One can speculate that exaggerated urinary toxicity in our study can be the consequence of more rigorous grading – even with the regard to, generally more exact, grading of serious toxicity.

As a conclusion we can say that pelvic radiotherapy in the postoperative treatment of prostatic carcinoma may result in the increased and perhaps unacceptable urinary morbidity at least in connection with a classic box technique of irradiation and concurrent testosterone deprivation. Like in other irradiated areas, toxicity is strongly correlated with the irradiated volume in conjunction with the dose.[26] The quality of life became more and more important in the case of a good prognosis of patients' outcomes regarding a disease control and survival, therefore, we have to carefully look for late toxicity assessing restrictions in the combined modality treatment.[27] However, there are several possibilities to make pelvic radiotherapy after the radical prostatectomy more acceptable. Nevertheless, the individual response to radiotherapy is important, even with regard to high grade morbidity.

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research article

Usage of the standard and modified comet assay in assessment of DNA damage in human lymphocytes after exposure to ionizing radiation

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Background. *Human organisms are extremely sensitive to ionizing radiation, which has the strong genotoxic effect on the DNA molecule. The aim of the present study was to detect the type of DNA damage and cell death caused by ionizing radiation as well as the sensitivity of the standard and modified comet assay.*
Methods. *The effect of gamma radiation (0.1 Gy and 4 Gy) on human lymphocytes was observed using the standard alkaline and Fpg-modified comet assay with ability to detect oxidized purines as well as with the DNA diffusion test.*
Results. *Parameters of the standard comet assay showed significantly higher values in samples exposed to 4 Gy than in samples exposed to radiation dose of 0.1 Gy and control sample. The Fpg-modified comet assay showed significantly higher values already at dose of 0.1 Gy as the result of oxidative DNA damage. The DNA diffusion test showed that gamma rays lead to apoptosis more often than to necrosis.*
Conclusions. *This observation suggests that the standard alkaline and Fpg-modified comet assays as well as the DNA diffusion test are reliable techniques for estimation of DNA damage and form of the cell death caused by gamma radiation in vitro. In addition Fpg-modified comet assay prove to be more sensitive for detection of gamma irradiation induced DNA damage than the standard assay.*

Key words: human peripheral blood lymphocytes; ROS; gamma radiation; alkaline comet assay; Fpg-modified comet assay; diffusion test

Introduction

Ionizing radiation is nowadays omnipresent in human lives because of the increasing development of technology, industry and medicine. People live and work near nuclear facilities and places of testing nuclear weapon and also use nuclear (gamma) radiation in medical purposes. In each case, human organism bears the strong genotoxic effect making the structure of the DNA molecule unstable and causing the genesis of many changes in it.[1] The damage of the genetic material caused by ionizing radiation is one of the best precondition indicator for development of malign diseases such as breast, gall-bladder or thyroid can-

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cer.[2,3] Gamma radiation affects the DNA structure directly, causing strand breaks, or indirectly causing cleavage of the water molecules and damaging the DNA molecule by reactive oxygen species (ROS).[4] Strand breaks and oxidative damage in the DNA causes further cell death in the form of apoptosis or necrosis.[5] These changes can be examined at human lymphocytes using different cytogenetic techniques. The most frequently used cytogenetic techniques are the comet assay, micronucleus test, chromatid exchange test, chromosomal aberrations test and fluorescence *in situ* hybridization (FISH) test.[6-10]

The comet assay (SCGE; single cell gel electrophoresis) is a rapid, simple, visual and sensitive technique for measuring and analyzing DNA damage at the single cell level.[6,11-17] This technique can be performed at the level of individual cells and requires a small number of cells per sample. Single cells can be used in *in vivo* and *in vitro* as well as in biomonitoring of population exposed to radiation or chemical mutagens.[6,18,19] The comet assay detects single and double stranded breaks at the level of DNA molecule, sites of incomplete repair, alkali labile sites, DNA-DNA and DNA-protein cross-links. Besides, the comet assay can be used for detection of the level of the DNA fragmentation in apoptosis.[17, 20-22] In addition; particular enzymes such as formamidopyrimidine glycosilase can be used for detection of oxidative damage at the level of the DNA molecule caused by ROS.[23-25]

The DNA diffusion assay, a simple, sensitive, and reliable cytogenetic method is often used for quantification of apoptosis, is based on the principle that nuclear DNA of apoptotic cells have abundant alkali-labile sites and under alkaline conditions small pieces of DNA thus generates diffuse in agarose, giving the appearance of a halo if stained with a sensitive fluorescent dye. Apoptotic cells show a circular gradient of granular DNA with a dense central zone and a lighter and hazy outer zone, giving the overall appearance of a halo.[26]

Peripheral blood lymphocytes were exposed to gamma radiation doses of 0.1 Gy and 4 Gy *in vitro.* In that manner, the aim of this study was to detect the type of DNA damage caused by ionizing radiation. Considering that, two forms of the comet assay, standard alkaline and Fpg-modified were used. In addition, sensitivity of both techniques toward different doses of gamma radiation was measured. The DNA diffusion test was also used to detect the form of the cell death caused by gamma radiation.

Materials and methods

Blood sampling

The study was performed on peripheral blood samples obtained from a healthy female non-smoking donor (age 24 years). The donor was not exposed to ionizing radiation, vaccinated or used medicals for a year before blood sampling. Whole venous blood was collected under sterile conditions in heparinised vacutainer tubes (Becton Dickinson, NJ, USA) containing lithium heparin as anticoagulant. After collection, blood was divided into a large number of samples. All experiments were conducted on peripheral blood lymphocytes cultivated at 37 °C in an atmosphere of 5% CO_2 in air.

Exposure conditions

The whole blood samples were irradiated with gamma radiation on the ice. As a source of radiation Gammacell 220 (Institute "Ruđer Bošković", Zagreb, Croatia) was used. Vacutainers (volume 5 cm^3) containing blood samples were exposed to radiation doses defined as doses in the water, but irradiation was performed

in the air. Samples were irradiated with radiation doses of 0.1 Gy and 4 Gy that is equal to radiation periods of 32.3 s and 23 min and 9 s at temperature of 21 °C. Significance of the absorbed dose was 3%.[27] To get homogenate samples, they were stirred after irradiation, cooled to 4 °C, transported to the laboratory on ice and processed as quickly as possible.

Determination of cell viability

The indices of cell viability and necrosis were obtained from differential staining with acridine orange and ethidium bromide, using fluorescence microscopy.[28] Lymphocytes were isolated using a modification of the Ficoll-Histopaque centrifugation method.[29] The slides were prepared using 200 μl of human peripheral blood lymphocytes and 2 μl of stain (acridine orange and ethidium bromide, both diluted in PBS). A total of 100 cells were analyzed to determine the percentage of viable cells using an Olympus AX-70 microscope with 60× magnification and 515–560 nm fluorescence filters. The cells were classified according the following description: live cells with a functional membrane, with uniform green staining of the nucleus and necrotic cells with uniform red staining of the nucleus.

The alkaline comet assay

To evaluate DNA damage after irradiation and to test sensitivity of the technique towards gamma radiation the comet assay was carried out under alkaline conditions, basically as described by Singh *et al.*[14] Fully frosted slides were covered with agarose (Sigma) containing the whole blood sample. The slides were then immersed for 1 h in lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris–HCl, 1% sodium sarcosinate (Sigma), pH 10) with 1% Triton X-100 (Sigma) and 10% dimethyl sulfoxide (Kemika, Zagreb, Croatia). The slides were then placed on a horizontal gel electrophoresis tank. The unit was filled with electrophoresis buffer (0.3 M NaOH, 1 mM Na_2EDTA; pH 13) and the slides were placed in this alkaline buffer for 20 min. Denaturation and electrophoresis were performed at 4 °C under dim light. Electrophoresis was carried out for 20 min at 25 V (300 mA). After electrophoresis the slides were rinsed with neutralization buffer (0.4 M Tris–HCl, pH 7.5). Each slide was stained with ethidium bromide (20 μg/ml) and covered with a coverslip. The slides were then stored in sealed boxes at 4 °C until analysis.

Fpg-modified comet assay

For evaluation of possible oxidative DNA-damaging effect of gamma radiation and to test the sensitivity of the technique, modified version of the comet assay was performed using an Fpg FLARE™ assay kit (Trevigen Inc, Gaithersburg, USA) with some modification.[30] Within the kit the manufacturer provided all the reagents used. Fully-frosted microscopic slides were prepared. Each slide was covered with 1% normal melting point (NMP) agarose (Sigma). After solidification, the gel was scraped off the slide. The slides were then coated with 0.6% NMP agarose. A low melting point (LMP) agarose was melted and stabilized in a water bath at 37 °C. For each sample and control, 5 μl of cell homogenate was mixed with 100 μl of LMP agarose and placed on the slides. After 10 min of solidification on ice, the slides were covered with 0.5% LMP agarose. The slides were then immersed in a pre-chilled lysis solution and kept in a refrigerator at 2 °C for 60 min. Followed the immersion in the buffer, three times for 15 min. After lysis, the slides were treated with 100 μl of Fpg enzyme (1:500 in REC dilution buffer). The enzyme was di-

luted right before use. Control slides were treated with 100 μl of REC dilution buffer only. The slides were placed horizontally in a humidity chamber at 37 °C for 30 min. All slides were then immersed in an alkali solution (0.3 M NaOH, 1 mM Na_2EDTA; pH 12.1) for 40 min. Followed electrophoresis in a pre-chilled alkali solution (0.3 M NaOH, 1 mM Na_2EDTA; pH 12.1) at 1 V/cm for 20 min. After electrophoresis, the slides were rinsed gently three times with neutralization buffer (0.4 M Tris–HCl, pH 7.5) to remove excess alkali and detergents. Each slide was stained with ethidium bromide (20 μg/ml) and covered with a coverslip. Slides were stored at 4 °C in sealed boxes until analysis.

Comet capture and analysis

A total of one hundred randomly captured comets from each slide were examined at 250x magnification using an epifluorescence microscope (Zeiss, Germany) connected through a black and white camera to an image analysis system (Comet Assay II; Perceptive Instruments Ltd., UK). The analysis did not include the edges and damaged parts of the gel as well as debris, superimposed comets, and comets without distinct head ("clouds", "hedgehogs", or "ghost cells").

Differences in the tail length, tail intensity and tail moment between samples obtained with standard alkaline comet assay (basic DNA damage) and Fpg-modified comet assay (total DNA damage) were considered as oxidative DNA damage in a single cell.

DNA diffusion test

For evaluation of the type of the cell death DNA diffusion assay was performed following the protocol described by Singh.[12,31] The chemicals needed for the DNA diffusion assay were provided by the Sigma Chemical Company. Agarose precoated slides were made by spreading 50 ml of 0.7% normal melting agarose on each slide and drying them at room temperature. Microgels were made on agarose-precoated slides by mixing 5 μl of whole blood culture with 50 μl of 0.7% high-resolution agarose and pipetting it onto the slide. The gel was immediately covered with a cover glass. The slides were coded and cooled on ice for 1 min. The cover glasses were removed, and 200 ml of 2% agarose solution was layered. After keeping the slides for 1 min on ice, the cover glasses were removed and the slides were immersed in a freshly made lysing solution (1.25 M NaCl, 1 mM tetra sodium EDTA, 5 mM Tris–HCl pH 10, 0.01% sodium lauroyl sarcosine, 0.2% DMSO freshly added, 0.3 M NaOH freshly added) for 10 min at room temperature. After lysis, the slides were twice immersed in a neutralising solution (50% ethanol, 1 mg/ml spermine, 20 mM Tris–HCl pH7.4) for 30 min at room temperature. The slides were air-dried and stored at room temperature. The slides were stained with ethidium bromide (20 μg/ml) and covered with a coverslip for 10 min. 1000 lymphocytes per slide were analysed. Lymphocytes undergoing apoptosis or necrosis were distinguished from normal cells in accordance with the figures and instructions given by Singh.[31] Apoptotic cell nuclei have a hazy or undefined outline without any clear boundary due to nucleosomal-sized DNA diffusing into the agarose. Necrotic cell nuclei are bigger and are poorly defined. They have a clear, defined outer boundary of the DNA halo and a relatively homogeneous halo appearance.

Statistical analysis

Each experimental set contained duplicated slides. The various parameters measured in

the exposed and control groups were evaluated using Statistica 5.0 package (StaSoft, Tulsa, USA). Each sample was characterized for the extent of DNA damage by considering the mean ± SE (standard error of the mean), median and range of the comet parameters. Multiple comparisons between groups were done by means of ANOVA on log-transformed data. Post-hoc analysis of differences was done by Scheffé test. As for the DNA diffusion test, values were analyzed using the chi-square test. The level of statistical significance was set at $P<0.05$.

Results

Cell viability test

The viability of the cells as determined by acridine orange and ethidium bromide, using fluorescence microscopy, was consistently above 89% in all the exposed samples and 100% in control samples. This is considered to be in acceptable range for conducting the comet assay.[32,33]

Comet assay and Fpg-modified comet assay

Results of the standard alkaline comet assay are presented in the Table 1. These results showed statistically significant increase of the mean values for all three parameters of the standard comet assay ($P<0.05$) in the sample irradiated with the dose of 4 Gy in contrast to control sample and sample irradiated with the dose of 0.1 Gy. Sample irradiated with the dose of 0.1 Gy showed slightly increased values for all parameters measured but there were no significant differences in compare to the corresponding control (Figure 1).

Results of the Fpg-modified comet assay are presented in the Table 2. These results showed statistically significant increase in all three parameters of modified comet assay ($P<0.05$) for both irradiation doses in compare to the control sample (Figure 2).

Generally, the mean values of all three Fpg-modified comet assay parameters were significantly higher than of the standard comet assay. These findings suggest that the Fpg-modified comet assay is more sensitive to gamma irradiation than the standard comet assay.

DNA diffusion test

Percentage of apoptotic and necrotic cells gained for the diffusion test is presented in Table 3 whereas Figure 3 presents microphotographs of viable, apoptotic, and necrotic cells from the un-exposed sample and samples irradiated with gamma radiation. These results showed statistically significant increase ($P<0.05$) of apoptotic cells in samples irradiated with the dose of 0.1 Gy and 4 Gy in comparison to the control sample. Significant increase in apoptotic cells was also found in sample irradiated with 4 Gy in comparison to the sample irradiated with 0.1 Gy. Number of necrotic cells significantly increased only in sample irradiated with 4 Gy in comparison to the control sample, while statistically significant increase in comparison to the sample irradiated with the dose of 0.1 Gy was not present.

Discussion

Our goal was to test the sensitivity of different protocols for comet assay, and to evaluate the type of cell death caused by different doses of gamma radiation. This type of radiation is a potent carcinogen mainly due to it potential as oxidative-induced damage agent. It produces variety of primary lesions in DNA such as single and double strand breaks, DNA-DNA

Table 1. Results of the alkaline comet assay and DNA diffusion test in human peripheral blood lymphocytes after exposure to 0.1 Gy and 4 Gy gamma irradiation

Sample	Alkaline comet assay											
	Tail length (μm)				Tail intensity				Tail moment			
	Min	Max	Mean±SE	Median	Min	Max	Mean±SE	Median	Min	Max	Mean±SE	Median
4 Gy	25.00	92.31	55.78±1.90*,#	54.49	5.74	49.21	22.51±1.02*,#	20.53	1.09	11.99	5.06±0.27*,#	4.52
0.1 Gy	13.46	25.00	17.09±0.31	16.03	0.00	19.21	3.14±0.38	1.91	0.00	2.46	0.43±0.05	0.25
Control	12.18	21.79	16.19±0.27	15.70	0.00	9.74	2.64±0.26	1.57	0.00	1.28	0.36±0.03	0.21

* statistically significant increase in compare to the corresponding control (P<0.05)
# statistically significant increase in compare to the 0.1 Gy irradiated sample(P<0.05)

Table 2. Results of the Fpg-modified comet assay in human peripheral blood lymphocytes after exposure to 0.1 Gy and 4 Gy gamma irradiation

Sample	Fpg-comet assay											
	Tail length (μm)				Tail intensity				Tail moment			
	Min	Max	Mean±SE	Median	Min	Max	Mean±SE	Median	Min	Max	Mean±SE	Median
4 Gy	31.41	90.38	60.87±1.47*,#,+	60.90	8.76	66.86	32.14±1.38*,#,+	29.92	1.57	22.29	8.15±0.48*,#,+	7.15
0.1 Gy	14.74	28.20	18.61±0.32*,+	17.95	0.00	17.98	4.81±0.40*,+	4.28	0.00	2.70	0.70±0.06*,+	0.57
Control	12.82	20.51	16.18±0.18	16.03	0.00	8.29	2.42±0.21	2.13	0.00	1.12	0.33±0.03	0.29

* statistically significant increase in compare to the corresponding control (P<0.05)
# statistically significant increase in compare to the 0.1 Gy irradiated sample(P<0.05)
+ statistically significant increase in compare to the standard comet assay (P<0.05)

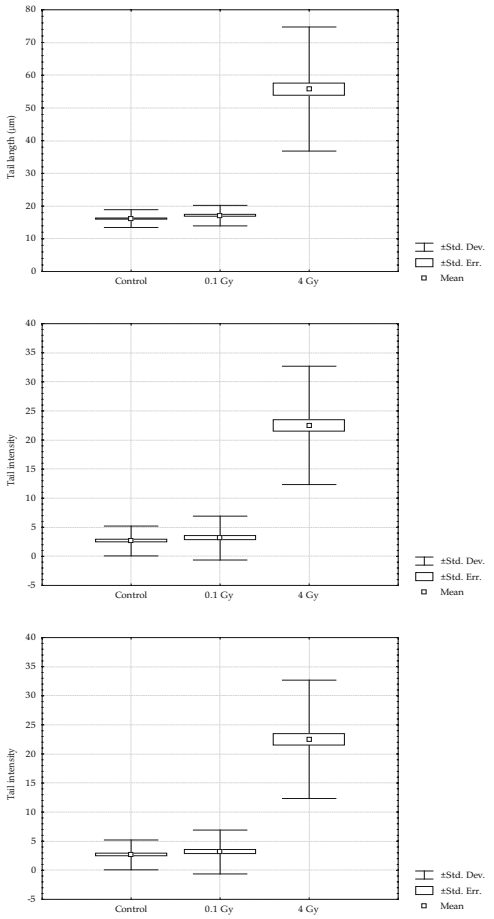

Figure 1. Parameters of the alkaline comet assay (tail length, tail intensity and tail moment), for human peripheral blood lymphocytes exposed to 0.1 Gy and 4 Gy gamma irradiation.

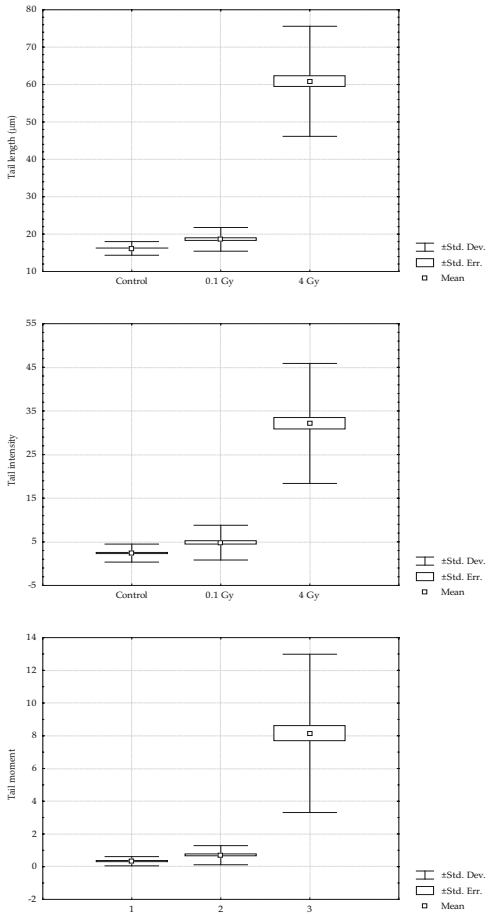

Figure 2. Parameters of the Fpg-modified comet assay (tail length, tail intensity and tail moment), for human peripheral blood lymphocytes exposed to 0.1 Gy and 4 Gy gamma irradiation.

Table 3. Results of the DNA diffusion test in human peripheral blood lymphocytes after exposure to 0.1 Gy and 4 Gy gamma irradiation.

DNA diffusion test		
Sample	Apoptosis	Necrosis
	%	%
4 Gy	4.90*,#	4.10*
0.1 Gy	3.00*	2.50
Control	1.10	1.40

* statistically significant increase in compare to the corresponding control (P<0.05)

statistically significant increase in compare to the 0.1 Gy irradiated sample(P<0.05)

and DNA-protein cross-links, alkali-labile sites and damage to purine and pyrimidine bases as well as oxidize bases and abasic sites.[34,35,36,37]

In that manner, standard alkaline and Fpg-modified version of the comet assay were used as well as the DNA diffusion test to access whether this type of radiation induces apoptotic or necrotic cell death. Techniques used in this study showed genotoxic effect of gamma rays on DNA molecule of peripheral blood human lym-

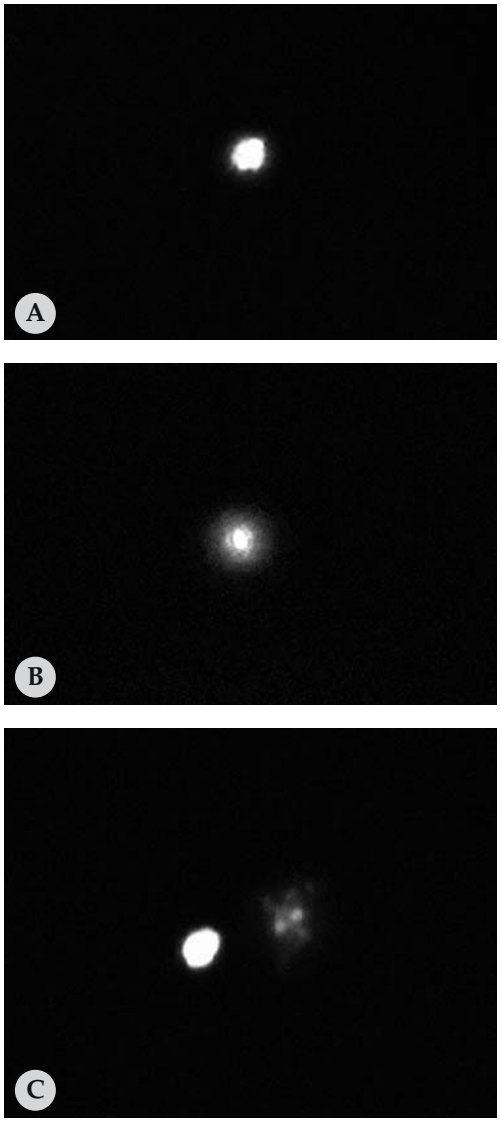

Figure 3. DNA diffusion microphotographs of viable lymphocytes from the un-exposed sample (A), and apoptotic (B) and necrotic (C) cells from samples irradiated with gamma radiation.

phocytes *in vitro*. With the standard alkaline comet assay increase in DNA damage was noticed in both exposure doses but it was significant only at higher dose of 4 Gy whereas at lower dose there were no statistically significant increase in neither of the standard comet assay parameters. Usage of the Fpg-modified protocol showed significant increase in all the parameters measured at both exposure doses indicating that the modified version of this assay is capable to detect wider scale of DNA damage induced by gamma irradiation. In addition, with modified protocol it is possible to detect ROS mediated DNA damage, thus significant increase in modified comet parameters in comparison to the standard one suggests that gamma radiation did induce oxidative damage in DNA molecule *in vitro*.

Some of the previous studies also showed that the Fpg-modified version of the comet assay is more sensitive for detection of DNA damage than the standard alkaline one.[18,22, 24, 38-41] According to that fact, scientists revealed that using the standard alkaline comet assay it is possible to detect damages at radiation doses from 5 cGy to 10 cGy [42,43] and in some adapted experimental conditions (*e.g.* addition of the Fpg enzyme) it is possible to detect damages even at radiation doses of 0.6 cGy.[44,45] The additional reason why the Fpg-modified comet assay is more sensitive than the standard alkaline one is that the Fpg enzyme helps to detect oxidative damage of the DNA molecule by cleavage of 8-oxodG, FaPyGua, FaPyAde and other ring-opened purines.[46,47,48]

In one of our previous research done on atorvastatin toxicity towards peripheral blood lymphocytes modified comet protocol also showed greater sensitivity in the manner of DNA damage. In that study all parameters obtained with the standard comet assay and Fpg-modified comet assay were significantly higher in the treated than in control lymphocytes, but the Fpg-modified protocol showed a significantly greater tail length, tail intensity, and tail moment in all treated lymphocytes than did the standard comet assay.[18]

Another comparison of the standard alkaline and Fpg-modified comet assay was made on leukocytes collected from Wistar

rats after exposure to 915 MHz microwave radiation. Both the standard and the Fpg-modified comet assay detected increased DNA damage in blood leukocytes of the exposed rats. The significant increase in Fpg-detected DNA damage in the exposed rats suggests that oxidative stress is likely to be responsible and that Fpg-modified protocol is much more sensitive than the standard one. These results are in compliance with our results because of the higher values obtained with Fpg-modified comet assay than with standard alkaline indicating oxidative DNA damage.[39]Strand breaks and oxidative damage at the level of DNA molecule have further consequences for the cells. Depending on the kind and the level of the damage, these effects often lead to the cell death which appears in the form of apoptosis or necrosis.[49] In our study this was evaluated by using the DNA diffusion test. Observing results, it was noticed that higher radiation dose increased the number of apoptotic cells in compare to the number of necrotic cells. That was because; gamma radiation damages the DNA molecule by producing reactive oxygen species more often than hitting the DNA molecule directly and causing strand breaks. These reactive oxygen species initiate cascade reactions in the cells witch leads to apoptosis, what makes apoptosis more frequent form of cell death than necrosis.[50]

Conclusion

In this study correlation between different protocols of the comet assay was made suggesting that Fpg-modified version is more sensitive to gamma radiation by virtue of measuring oxidative DNA damage in addition to basal DNA strand breaks. Moreover, evaluation of the type of cell death was made using DNA diffusion test indicating that gamma rays more often leads to apoptosis. Results obtained lead to the same conclusion that gamma radiation affects the DNA molecule by ROS that are most frequent product of the gamma radiation effect. Human peripheral blood lymphocytes proved to be sensitive to ionizing radiation depending on the radiation dose and are suitable biomarkers for this type of research.

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research article

Cell size dynamics and viability of cells exposed to hypotonic treatment and electroporation for electrofusion optimization

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Background. *Various electrofusion parameters have to be adjusted to obtain the optimal electrofusion efficiency. Based on published data, good electrofusion conditions can be achieved with the hypotonic treatment. However, the duration of the hypotonic treatment before electroporation and buffer hypoosmolarity have to be adjusted in order to cause cell swelling, to avoid regulatory volume decrease and to preserve cell viability. The aims of our study were to determine cell size dynamics and viability of four different cell lines in hypotonic buffer and to study the influence of the electroporation on the selected cell line in hypotonic buffer.*

Materials and methods. *Cell size dynamics of different cell lines exposed to hypotonic buffer and electroporation were analyzed by time-resolved cell size measurements. The viability of hypotonically treated or/and electroporated cells was determined 24 h after the experiment by a modified crystal violet (CV) viability assay.*

Results. *In our experimental conditions the hypotonic treatment at 100 mOsm was efficient for CHO, V79 and B16-F1 cell lines. The optimal duration of the treatment was between two and five minutes. On the other hand the same hypotonic treatment did not cause cell swelling of NS1 cells. Cell swelling was also observed after electroporation of B16-F1 in isotonic buffer and it was amplified when hypotonic buffer was used. In addition, the regulatory volume decrease was successfully inhibited with electroporation.*

Conclusions. *Cell size dynamics in hypotonic conditions should be studied for each cell line since they differ in their sensitivity to the hypotonic treatment. The inhibition of cell regulatory volume decrease by electroporation may be beneficial in achieving higher electrofusion efficiency. The hypotonic treatment in itself did not significantly affect the cell viability; however, electric field parameters for electroporation should be carefully selected taking into account the hypotonically induced volume increase of cells.*

Key words: hypotonic treatment; cell swelling; regulatory volume decrease; cell size measurements; viability; electrofusion; electroporation

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Introduction

Electrofusion of two different types of cells generates a third polynuclear type, which displays hybrid characteristics of the two parental cells. Different methods

are used to achieve cell fusion; however, electrofusion is gaining on its importance because it is easy to use, potentially highly efficient, reproducible and controllable.[1-3] Electrofusion has great potential for clinical applications, with respect to viral and chemical methods, because it is a safe method that does not introduce any foreign substances into the body.

Cell fusion is a two-condition process: (I) cell membrane has to be brought into fusogenic state and (II) a close physical contact of two fusogenic membranes has to be established. The fusogenic state of cell membrane is achieved by electroporation.[4-6] Electroporation is a method, widely used in medicine[7-12], where a dramatic increase in membrane permeability is caused by cell exposure to short and intense electric pulses.[13-15] With the appropriate selection of electrical parameters and media, taking into account biological characteristics of the treated cells, the reversible electroporation can be obtained. The reversible electroporation does not affect cell viability, because cell membranes reseal after the treatment.[16-21]

As mentioned before, the second condition required for cell fusion is a close physical contact between cells which has to be established during the fusogenic state of the membrane. A physical contact between cells can be achieved by the application of alternating electric filed which causes dielectrophoretic forces that result in cell migration and pearl chain formation.[4,22]

Even though electrofusion of biological cells is potentially a useful method, achieving the sufficient efficiency still requires extensive trial-and-error studies.[23-26] One of the earliest approaches to improve electrofusion efficiency was the use of hypotonic electrofusion buffer that resulted in the considerable fusion efficiency increase.[27-34] To ensure the improvement of fusion efficiency in hypotonic buffer the duration and the osmolarity of the hypotonic treatment has to be selected and used properly. Rapid cell swelling in the hypotonic environment due to influx of water namely triggers regulatory the volume decrease. If it is triggered before the induction of cell fusion, it can inhibit the positive effect of the hypotonic treatment on electrofusion by reducing cell size, restoring microvilli and excessive leaking of cytosolic electrolytes.[33,35] The prolonged treatment thus leads to poor fusion efficiency and also decreases the cell viability.[30,32-34]

The aim of our study was first to determine cell size dynamics and the viability of four different cell lines in hypotonic buffer. The volume regulation of different cell lines exposed to strongly hypotonic buffers was analyzed by means of time-resolved cell size measurements. The second aim of our study was to determine the influence of the electroporation on the selected cell line in hypotonic buffer. B16-F1 cell line was selected because electroporation parameters and swelling induced by electroporation in isotonic buffer were previously well described for this cell line.[21,36]

Materials and methods

Chemicals, cell culture media

Eagle's minimal essential medium (EMEM), Ham's Nutrient Mixtures (F-12 HAM), Dulbecco's Modified Eagle's Medium (DMEM), trypsin, fetal bovine serum (FBS), L-glutamine, sucrose, phosphate (K_2HPO_4/KH_2PO_4), $MgCl_2$, crystal violet, trypsin and EDTA were purchased from Sigma (Sigma-Aldrich Chemie GmbH, Germany). Antibiotics (crystacillin and gentamicin) were purchased from Lek (Ljubljana, Slovenia).

Cells

All cell lines were cultured in an incubator (Kambič, Slovenia) in the humidified

Table 1. Chemical composition, conductivity and osmolarity of isotonic and hypotonic buffers used in our experiments.

Ingredients	Isotonic	Hypotonic
Phosphate buffer	10 mM	10 mM
$MgCl_2$	1 mM	1 mM
Sucrose	250 mM	75 mM
pH	7,2	7,2
Conductivity	1.62 mS/cm	1.62 mS/cm
Osmolarity	260 mOsm	93 mOsm

atmosphere at 37°C and 5% CO_2 in the following culture media: murine melanoma (B16-F1) and Chinese hamster lung fibroblast (V79) in EMEM supplemented with 10% fetal bovine serum (FBS), antibiotics (gentamicin, crystacillin) and L-glutamine; Chinese hamster ovary cells (CHO) in F-12 HAM supplemented with 10% FBS, antibiotics and L-glutamine; mouse myeloma NS1 in DMEM supplemented with 13% FBS, antibiotics and L-glutamine. Cell lines were grown in 25 cm^2 culture flask (TPP, Switzerland) until they reached 80-90% confluence. Adherent cells were exposed to 0.25% trypsin/EDTA solution for 1 minute. Trypsin solution was then removed and 5 ml of culture media was added. Cells were gently rinsed from the bottom with a plastic pipette and the homogenous cell suspension was prepared. For cell size dynamics and viability measurements in hypotonic buffer 5 x 10^5 cells were placed on Petri dish (see *Cell size measurement and analysis* section). For viability tests after the electroporation, cell suspension was centrifuged (290 x g, 5 min, 4°C) and then resuspended in the electroporation buffer (details explained in *Cell viability* section).

Isotonic and hypotonic buffers

Iso- and hypo-tonic buffers (phosphate buffer saline – PBS) of osmolarities 260 and 93 mOsm (mOsmol/kg) and conductivity 1.62 mS/cm were used (Table 1). The osmolarity of solutions was determined with Knauer vapor pressure osmometer K-7000 (Knauer, Wissenschaftliche Gerätebau, Germany). Buffers pH was 7.2.

Cell size measurement and analysis

Cell volume changes were measured by protocol which allowed a rapid exchange of media. For that purpose we used 9.2 cm^2 tissue culture Petri dishes (TPP, Switzerland). Before the microscopic measurement, cells in suspension were counted by haemocytometer and 5 x 10^5 cells were placed on Petri dish. Cells were incubated at 37°C for 20-40 min (except for NS1, which were incubated overnight) in culture medium allowing cells to slightly adhere but still preserving a round shape. Culture medium was removed and cells were washed with 1 ml isotonic buffer leaving 300 μl to avoid drying of the sample during the acquisition of the first image (represents time t = 0 min). Cells were observed under the Axiovert 200 microscope (Zeiss, Germany) with 40 x objective in transmitted light. Phase contrast images were acquired with cooled CCD video camera VisiCam 1280 (Visitron, Germany) and PC software MetaMorph 7.0 (Molecular Devices, USA). After the first image was acquired 3.3 ml hypotonic buffer was added to the cells (in the control samples isotonic buffer was used). The resulting osmolarity of hypotonic buffer was calculated to 100 mOsm. Images of cells were then taken at various time intervals up to 30 min after the buffer exchange (every 15 s until 2 min and in minute steps after that). One to three sequences were recorded of each repetition of every experiment (cell line).

Cell diameters of 5-10 cells per image were determined at each time interval (37 images in every sequence). Cell size dynamic was defined as the relative change of cell diameter $= d/d_0$, where d is an actual

cell diameter and d_0 is an initial one (at t = 0 min). The mean values (± STD) for a given experiment (cell line) were calculated from at least three independent repetitions of the experiments and plotted against time. The script was written in Matlab (Matlab 2008a, Math Works, USA) for all calculations to enhance calculation speed and graph performance. Observed differences between cell lines were statistically tested using ANOVA test or One-Sample T test (SPSS Statistics).

Electroporation

In the second part of our study, where we studied the effect of electroporation on B16-F1 cells swelling in isotonic and hypotonic buffers, the protocol was slightly altered from the one described previously. One minute after the buffer exchange from isotonic to hypotonic (or to isotonic again for control) electric pulses were delivered using two parallel electrodes (Pl/Ir = 90/10) and an electric pulse generator Cliniporator (IGEA, Italy). We used electrical parameters optimal for transient permeabilization of B16-F1 cells in the isotonic buffer (1000 V/cm, 8 x 100 μs, 1 Hz) described in our previous studies.[21,36] Cell sizes were measured and analyzed as described previously.

Cell viability

The viability of hypotonically treated cells as well as electroporated cells was analyzed 24 h after experiments by means of a modified crystal violet (CV) viability assay.[37] From prepared cell suspension 10^5 cells per well were plated to 24 – well microplates (TPP, Switzerland) and incubated at 37°C for 20-40 min in culture medium to settle and to slightly adhere. After the incubation culture medium was carefully removed and cells were washed with 1 ml isotonic buffer. One ml of hypotonic buffer was added to the cells in each well. In control 1 ml of culture medium was added. After a hypotonic treatment (30 min at room temperature) all media (buffer and culture medium in control) was carefully removed and 1 ml of fresh culture medium was added. Cells were then cultured in the incubator for 24 h. In described protocol washing, removing and adding medium were the same for treated cells as well as for the control ones.

After 24 h culture medium was removed and cells were washed with the isotonic buffer (1 ml per well) 0.1% CV solution prepared in the isotonic buffer was then added (200 μl per well). After 30 min of incubation at room temperature dye was carefully removed and cells were washed three times with the isotonic buffer per well (200 μl, 500 μl, 1000 μl). After the washing procedure cells were lysed by 10% acetic acid (1 ml per well). The same lysis procedure was also applied to wells without cells and their absorption values were used as the background. The absorption of lysate was measured with a microplate reader Infinite M200 (Tecan, Switzerland) at 595 nm wavelength controlled with PC software i-Control (Tecan, Switzerland) at maximum 1 h after the dying procedure. The viability of treated cells was defined as

$$Vc = \frac{(A_{TC} - A_{Bg})}{(A_{CC} - A_{Bg})} \times 100,$$

where

V_c ... viability [%]

A_{TC} ... absorption value of treated cells

A_{CC} ... absorption value of control cells (100 % viability)

A_{Bg} ... absorption value of background

The mean V_c values (± STD) for a given cell line were calculated from three independent experiments.

In the second part of our study, where we studied the effect of electroporation on

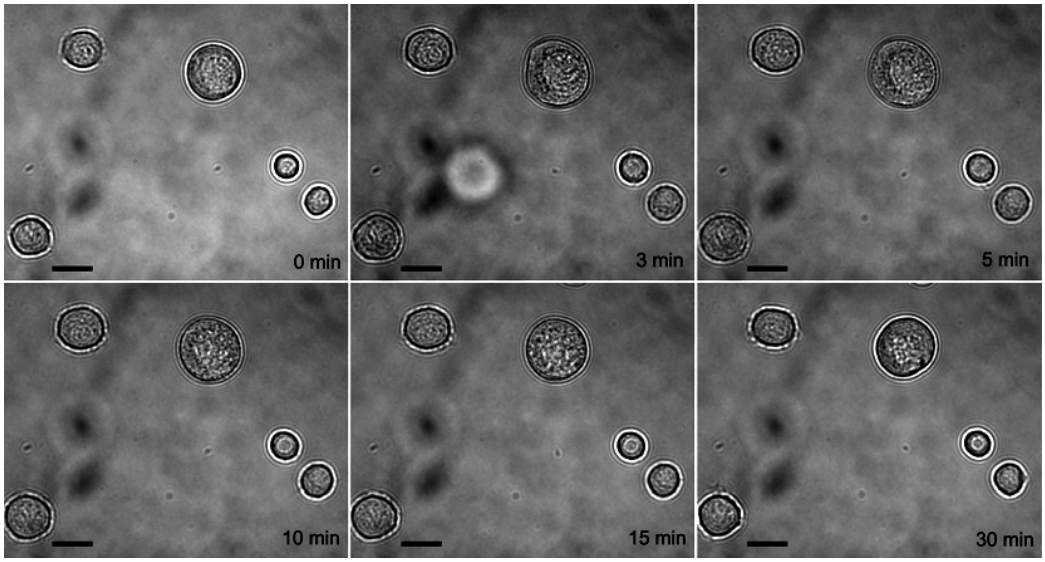

Figure 1. Swelling and regulatory volume decrease of B16-F1 cells in 100 mOsm buffer containing sucrose as the major osmolyte. The images show the same cells before (0 min) and during the hypotonic treatment at the indicated time intervals. After the rapid change of isotonic to hypotonic buffer at zero time, the cells swelled within 5 min and then shrank gradually within the observed time (5-30 min). Scale bar corresponds to 30 μm.

B16-F1 cells swelling, the viability protocol was slightly altered from the one described above. In order to expose cells to an electric field, electroporation cuvettes with 4 mm gap (Eppendorf, Germany) were used. The cell suspension prepared in cell culture media was counted and aliquots of 5×10^5 cells were prepared and centrifuged (290 x g, 5 min, 4 °C). Supernatant was carefully removed and cells were resuspended in 1 ml of hypotonic buffer (or culture media for control). One minute after the hypotonic buffer was added, 800 μl of cell suspension was electroporated. In the control treatment no pulses were delivered. After the electroporation cells were kept at room temperature for 10 min and on 37 °C for another 10 min to allow membrane resealing. From each cuvette 600 μl of cell suspension was plated in three microplate wells (24 – well microplates, TPP, Switzerland). Finally 1 ml of culture medium was added to each well and cells were incubated for 24 h. The cell viability was then determined with the crystal violet assay as described above. Differences between electroporated and non-electroporated (control) cells were tested by the Paired samples T – test (SPSS Statistics).

Results

Cell size dynamics due to exposure to hypotonic buffer

We monitored cell size (diameter) changes in B16-F1, CHO, V79 and NS1 cells after the rapid change of isotonic to hypotonic buffer. From the microphotographs such as shown in Figure 1, the diameters of individual cells were evaluated and normalized to the original isotonic diameter ($\nu = d/d_0$, Figure 2). The hypotonic treatment caused CHO, V79, B16-F1 cells to swell rapidly within first minutes from their initial isotonic diameters (d_0) to their maximum diameters (d_{max}) due to a fast water uptake driven by the imposed osmotic gradient.

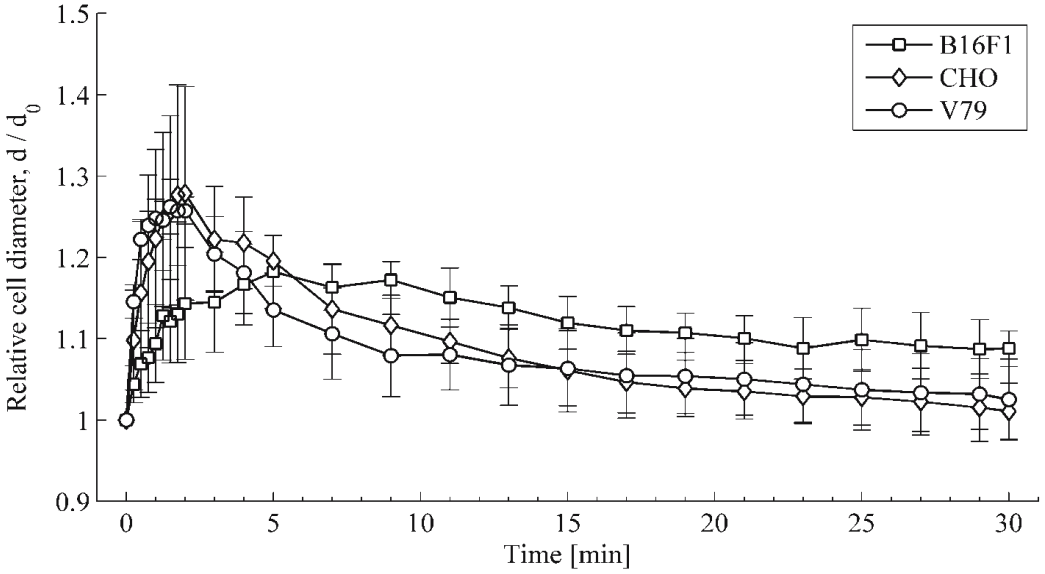

Figure 2. Cell dynamics (d/d_0) in hypotonic buffer for three different cell lines. Cell dynamics was obtained by directly measuring the size of the cells. Each data point represents the mean ± STD of at least three repetitions. One-Sample T test showed that after 30 min in the hypotonic buffer cell size of V79 and CHO did not differ significantly from their initial isotonic size ($P > 0,1$) while B16-F1 cells remained significantly enlarged $d_{end}/d_0=1.09$ ($P = 0,004$).

We estimate that B16-F1 cells reached their maximal size 5 min after exposure to hypotonic buffer, while V79 and CHO cells reached their maximal size at approximately 2 min. The size increase was the smallest for B16-F1 cells ($d_{max}/d_0 = 1.18$) and larger for V79 and CHO cells ($d_{max}/ d_0 = 1.26$ and 1.29 respectively). However, described differences among cell lines were not statistically significant. Cells in the isotonic buffer did not change their size (data not shown).

After the initial swelling all cell lines underwent regulatory volume decrease and cells shrank gradually to diameters near original ones, despite the persisting hypotonic treatment, but with some differences between cell lines. During our observation, V79 and CHO cells shrank to their original size, while B16-F1 cells remained significantly enlarged $d_{end}/d_0=1.09$ ($P = 0,004$). Consequently, a regulatory volume decrease was faster with CHO and V79 cells and slower with B16-F1 cells.

A completely different behaviour was found in NS1 cell line under the same hypotonic treatment. The hypotonic treatment led NS1 cells to blebbing and expressing non-spherical shapes (Figure 3). Therefore, no cell swelling and consequent regulatory volume decrease was observed or measured for this cell line.

Cell size response to electroporation in isotonic and hypotonic buffer

We monitored cell size (diameter) changes in B16-F1 cells after the electroporation. Cells were exposed to the hypotonic buffer for 1 min before delivery of the pulses. In this study we used electrical pulse parameters that are optimal for **the reversible electroporation** of cells in the isotonic buffer as described in Materials and methods.

From the microphotographs the diameter of an individual cell was measured and normalized to the original diameter in the

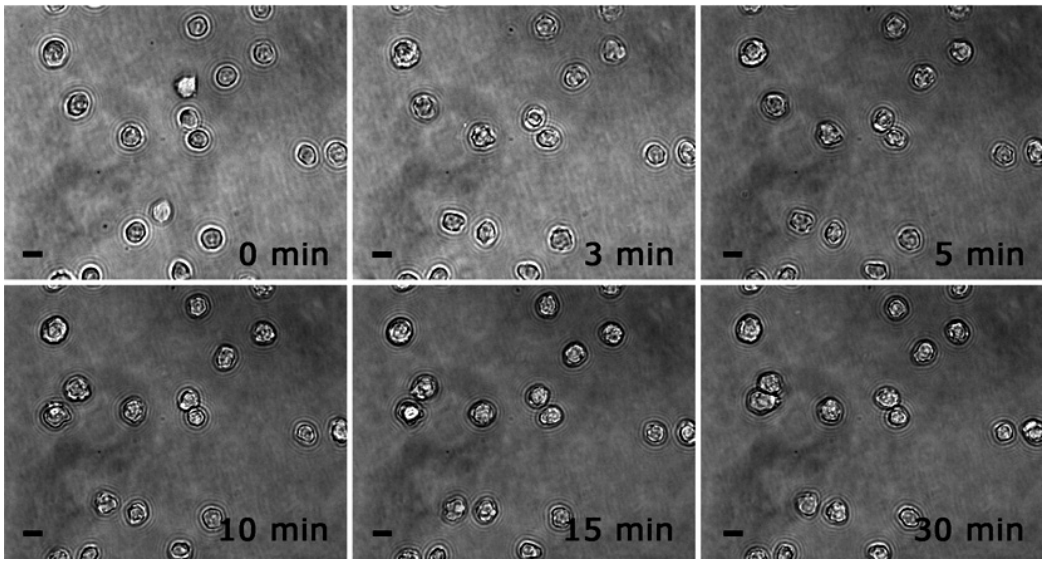

Figure 3. Mouse myeloma cells NS1 in 100 mOsm buffer containing sucrose as the major osmolyte. The image shows the behaviour of the cells during the hypotonic treatment. These cells did not exhibit any regular size alterations. Instead, NS1 cells started to bleb and express non-spherical shapes. Scale bar corresponds to 10 μm.

isotonic buffer ($\nu = d/d_0$, Figure 4). Within 1 min after the buffer change from isotonic to hypotonic, cells started to swell as in previous experiments without electroporation. We observed that the electroporation inhibited the effect of regulatory volume decrease completely during the 30 min of our observation. Cell swelling dynamics was also observed in B16-F1 cells electroporated in the isotonic buffer where a lower magnitude of swelling was measured (Figure 4).

Cell viability after hypotonic treatment

The cell viability of fusion partners needs to be preserved in order to produce viable hybrid cells. We, thus, analyzed the cell viability for all cell lines with a crystal violet (CV) viability assay. Results of the viability assay for hypotonically treated cells are shown in Figure 5. We observed no decrease in viability indicating that most of the cells survive the hypotonic treatment.

In the second part of our study, where we studied the effect of electroporation on B16-F1 cells swelling in hypotonic buffer, cell the viability was also analyzed (Figure 6). The viability of electroporated cells significantly decreased in the hypotonic buffer to 63% ($P = 0.002$) while in the isotonic buffer the viability was not affected ($P > 0.1$).

Discussion

Electrofusion in the hypotonic buffer is a promising approach for improving the cell fusion efficiency. Improved electrofusion conditions can be achieved with the hypotonic treatment. However, the duration and the strength (the osmolarity) of the hypotonic treatment should be optimized for the specific cell type. In the first part of our study, we determined cell size dynamics and survival of different cell lines in the hypotonic buffer. In the second part, we determined the influence of the electroporation on B16-F1 cell size dynamics and the survival in hypotonic buffers.

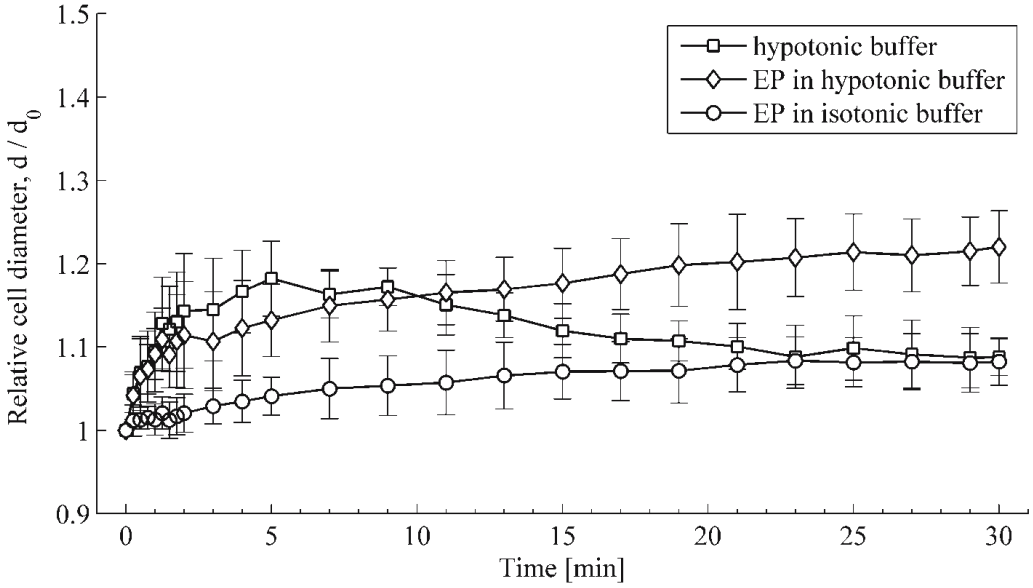

Figure 4. Time courses of relative cell diameter (d/d_0) during the hypotonic treatment for B16-F1 cells. Squares (□) represent cells in the hypotonic buffer. Rhombs (◊) represent cells in the hypotonic buffer, which were electroporated at t = 1 min. Circles (○) represent cells in the isotonic buffer, electroporated at the same time (t = 1 min). Cell diameter was obtained by directly measuring the size of the cells. Each data point represents the mean ± STD of at least three repetitions.

Electroporation was performed before a regulatory volume decrease took place.

In cell size dynamics we observe similar behaviour for cell lines B16-F1, V79 and CHO. In those cells as in many other mammalian cells the initial hypotonic swelling was accomplished within 2-5 min (Figure 2). [34,38-41] The maximal cell size increase was between 1.18 and 1.29 (Figure 2), the values that can be also found in the previously published studies.[33-35,38,40-42] The only exception was cell line NS1 that will be described later on. Observed cell swelling is a desired phenomenon in electrofusion. The increase in cell size requires the unfolding of undulations and invaginations of cell membrane.[42,43] This decrease in membrane undulations results in the decrease of undulation repulsive forces, yielding better cell-cell contacts.[31,44-47]

Differences among cell lines were found in regulatory volume decrease dynamics. The expression of a regulatory volume decrease depends on the type of sugar that is used as osmolyte. Cells can express a regulatory volume decrease in buffers containing disaccharide as major osmolyte whereas when monosaccharide is used, the regulatory volume decrease is inhibited. The different effect of sugars was explained by volume-sensitive channels in the plasma membrane that are selectively transporting monomeric sugars but are poorly permeable for oligosaccharides.[34,35,40] In our study such behaviour was observed for V79 and CHO cells that showed the fast regulatory volume decrease, mainly completed within 10-20 min (Figure 2).

In contrast to V79 and CHO, only a partial regulatory volume decrease was observed for B16-F1 cells (d_{end}/d_0=1.09, Figure 2). Similar phenomena was reported for the Jurkat leukemic cell line (d_{end}/d_0=1.11) and murine fibroblast (d_{end}/d_0=1.12).[33,40] A

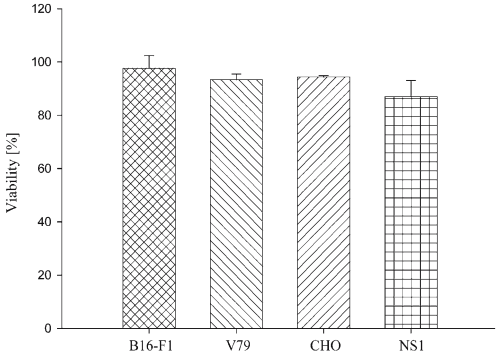

Figure 5. Viability of four different cell lines exposed to the hypotonic treatment (100 mOsm buffer containing sucrose as major osmolyte) for 30 minutes. Data represent mean ± STD of at least three independent experiments.

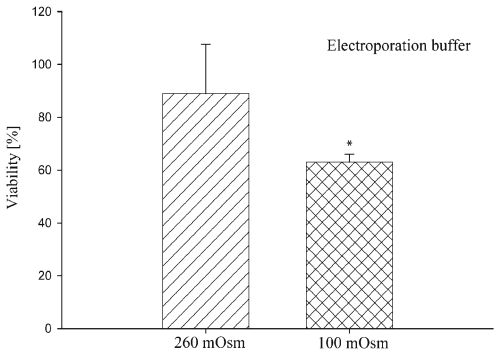

Figure 6. Effect of electroporation in both, hypotonic and isotonic buffer on viability of B16-F1 cells. Eight pulses of 100 μs duration at 1000 V/cm electric field intensity at 1 Hz pulse repetition frequency were applied to the cells in 100 mOsm and 260 mOsm buffer containing sucrose as major osmolyte. Data represent mean ± STD of at least three independent experiments. The Paired Sample T test showed that the viability of electroporated cells significantly decreased in the hypotonic buffer ($P = 0.002$) while in the isotonic buffer the viability was not affected ($P > 0.1$).

partial regulatory volume decrease in epithelial and cancerous cell lines was attributed to the fact that those lines may slowly uptake sucrose. This uptake of oligosaccharides[48,49] can explain the partial inhibition of regulatory volume decrease observed in B16-F1.

According to published literature the same hypotonic treatment causes different response in different cell lines due to their different (hypotonic) sensitivity.[33,40,41,43] In mouse myeloma cells NS1 no swelling and regulatory volume decrease were observed. Instead, NS1 cells started to bleb and express non-spherical shapes (Figure 3). A similar behaviour was reported earlier for different cell lines when too hypotonic treatment (30 to 60 mOsm) was used.[41,43] Therefore, we conclude that NS1 cells are more sensitive to a hypotonic treatment than other cell lines used in our study. Higher buffer osmolarities may have to be used for NS1 cells in order to obtain spherical cell shape with smooth membrane favourable for the electrofusion.

Regardless to immediately observed effect of the hypotonic treatment on cell size and shape, the cell viability was not affected (Figure 5). Those results show that the duration of hypotonic treatment and buffer hypoosmolarity were properly selected and are in agreement with literature.[40,50,51] Interestingly no significant decrease in cell viability was observed for NS1 cells, even though the hypotonic treatment caused cell blebbing.

In the second part of our study we determined the influence of the electroporation on B16-F1 cell size dynamics and the survival in the hypotonic buffer. Electroporation by itself causes swelling of electroporated cells in the isotonic buffer[36], which was also observed in our experiment with B16-F1 cells (Figure 4). Electroporation induced cell swelling was significantly amplified if the hypotonic buffer was used and, therefore, regulatory volume decrease was inhibited (Figure 4).

However, electroporation in the hypotonic buffer was associated with a significant loss of cell viability (Figure 6) that was also observed in the study of Golzio *et al*.[47] Electric field parameters for electroporation in the hypotonic buffer should be carefully selected in order to preserve cell viability since hypotonic treatment causes a higher

susceptibility due to the increase in cell size.[16,28,30-33,39,52]

In conclusion, cell size dynamics should be carefully analyzed and observed for each cell line in order to obtain all potential benefits of using hypotonic buffer for electrofusion. Electroporation should be performed when cells are close to their maximal size *i.e.* before the regulatory volume decrease starts. In addition, electroporation parameters should be adjusted to hypotonically induced changes on cells in order to preserve the cell viability.

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case report

Cisplatin-induced non-convulsive posterior reversible encephalopathy syndrome in a 41-year-old woman with metastatic malignant melanoma

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Background. *Cisplatin, a widely used antineoplastic agent usually induces peripheral neuropathy, but can rarely also complicate with encephalopathy, with or without seizures.*
Case report. *We report a case of a young patient with metastatic malignant melanoma with signs and symptoms of cisplatin-induced non-convulsive posterior reversible encephalopaty syndrome. Within the days shortly after the first cycle of cisplatin based chemotherapy the patient suffered from nausea, vomitus, headache, severe pain at the site of sub-cutaneous metastases and confusion. She later experienced somnolence, cortical blindness and aphasia, but without epileptic seizures.*
Conclusions. *Cisplatin is an effective chemotherapeutic drug but also very toxic one and physicians using it must also be aware of possible encephalopathy.*

Key words: neurotoxicity; cisplatin; posterior reversible encephalopathy syndrome

Introduction

Cisplatin is an effective and also widely used chemotherapeutic drug; it is part of numerous chemotherapeutic schedules used in the treatment of many solid tumours. Most common side-effects that patients experience are nausea, vomiting, nephrotoxicity, ototoxicity and peripheral neurotoxicity.[1,2] Central nervous system disorders such as seizures, cortical blindness, aphasia, coma, also hemiparesis are rare, but have already been reported many times,[3] cortical blindness and seizures being reported first time in 1980's.[4]

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Case report

A 41-year-old female patient with metastatic malignant melanoma was admitted to our hospital because of deterioration of her mental and physical state after the first cycle of the second line chemotherapy (ChT) in combination with cisplatin.

Primary malignant melanoma on the left lumbal part of the back was surgically removed in September 2005 (Clark IV,

Breslow 1.68, ulceration 2 mm, with signs of regression, without microsatelites, without vascular invasion, TNM stage T2bN1a(1/14)M0, stage IIIB). The sentinel node biopsy presented with a 2.5 mm wide metastasis; therefore the dissection of axilliary lymph nodes was performed.

From February 2006 to May 2007 she was receiving interferon in an adjuvant setting. During the adjuvant treatment there was clinically a locoregional relapse with a 12 cm bulky mass on the site of the primary tumour, further examination (CT, RTG, and UZ) has shown the dissemination in the lungs and pleura with multiple lung metastases.

She started her first line ChT with dakarabazine-DTIC (1000 mg/m^2 i.v., day 1, 21 days) in May 2007. After two cycles the further progression of the disease was observed, locoregionally and systemically. The bulky mass on the left lumbal part of her back was now clinically 15 cm in diameter and was at least 3 cm above skin level.

The second line treatment was started in June 2007, she received the first cycle of ChT with cisplatin (80 mg/m^2 i.v., day 2., 21 days), lomustin-CCNU (80 mg/m^2 p.o., day 1., 21 days) and vinblastin (3 mg/m^2 24 h infusion, day 1, 21 days) with premedication with dexamethason 20 mg i.v., granisetron 3 mg i.v., manitol 500 ml i.v. and $MgSO_4$ 5 ml i.v.. She was also hydrated with 3000 ml of 0.9% NaCl. She ended her first cycle without acute toxic event.

Shortly after she got home, she started suffering from nausea, vomitus, headache, severe pain at the site of subcutaneous mass and confusion. On the day of the admission she deteriorated even more, followed by somnolence, cortical blindness and aphasia, but without epileptic seizures. At home she was continuing with buprenorfin transdermal patch (Transtec®) 35 μg, short acting oral morphine (Sevredol®) 10 mg for breaking through pain, diclofenak tbl. (Olfen SR®) 100 mg/day, pregabalin tbl. (Lyrica®) 75 mg/day, pantoprazol tbl. (Controloc®) 1 tbl/day, metoklopramid tbl. (Reglan®) 3x1 tbl/day in granisetron tbl. (Kytril®) 2 mg when needed.

WHO performance status on admission was 3-4, the patient was affected, she was in pain, aphasic, somnolent, almost comatose. Blood pressure was 110/70 mmHg, heart rate was 80/min, she was afebrile. Meningeal signs were negative, her breathing and heart signs were normal. Abdomen was soft, without pathologic mass or liver palpable, without tenderness. The bulky subcutaneous mass on the site of primary tumour was smaller, only about 10 cm in diameter and has flattened.

The laboratory report has shown mild hypokaliemia (3.7) and hyponatriemia (132), without any signs of dehydration, with elevated WBC (14.9) and CRP (39). The laboratory report did not explain the patient's state; electrolytes, magnesium, calcium were within limits, her blood sugar level was normal.

Differential diagnosis

At first we suspected that she developed metastasis in the CNS, as CNS is frequent site of dissemination in malignant melanoma. She was presented with possible signs of increased intracranial pressure with severe headache and vomitus, confusion, aphasia, somnolence and later stupor. So, she was started on antioedematic therapy with corticosteroids and manitol solution, but her state was improving slower than expected in increased intracranial pressure.

To exclude the possibility of morfine intoxication, morphine therapy was discontinuated.

Urgent computer tomography (CT) of her brain did not show any proof of intracranial dissemination; bedsides, the neurologic exam showed that the patient was deeply somnolent, without speech contact, though

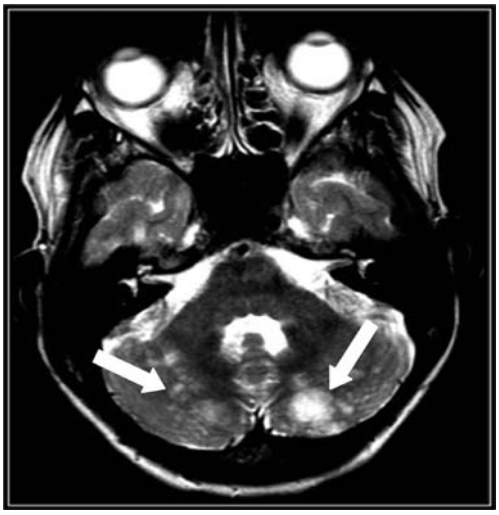

Figure 1a. Oedema in the cerebellum (T2, without contrast).

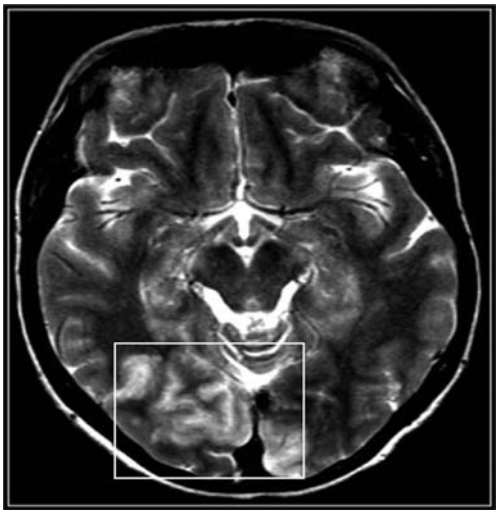

Figure 1b. Oedema in the occipital cortex (T2, without contrast).

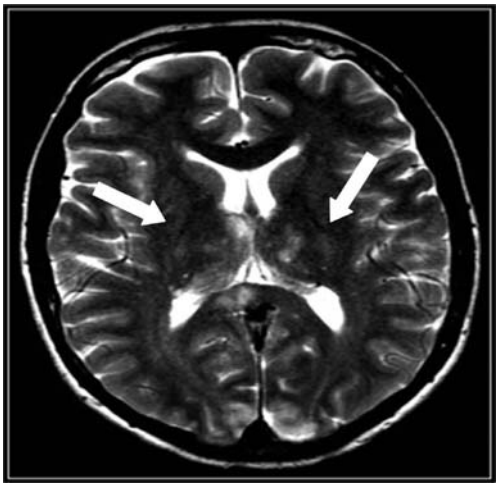

Figure 1c. Oedema in the corpus callosum (T2, without contrast).

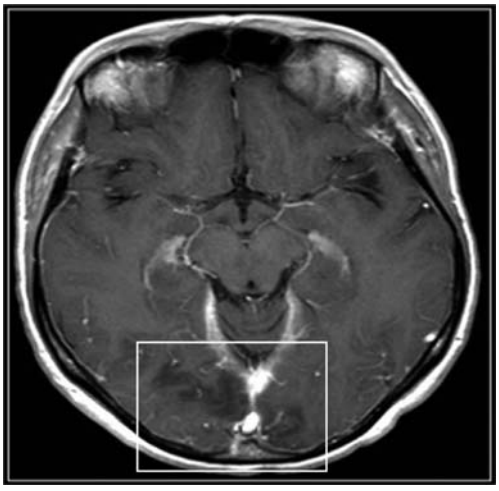

Figure 1d. Occipital cortex (T2, with contrast).

she did react on a call with movements and restlessness, but she did not open her eyes. There were neither focal signs of central nervous damage nor signs of pyramidal tract lesion; meningeal signs were negative. The lumbar punction was performed; cerebrospinal fluid was entirely normal, without malignant cells and with negative tests on bacterial and viral examination.

MRI presentation

At that time MRI of her brain was performed. She had typical neuroradiological changes in the form of oedema in supra- and infra-tentorial areas with hyperintensity on T2-weighted MRI imaging, seen in the occipital cortex, cerebellum, basal ganglia and corpus callosum (Figures 1a, 1b, 1c, 1d).

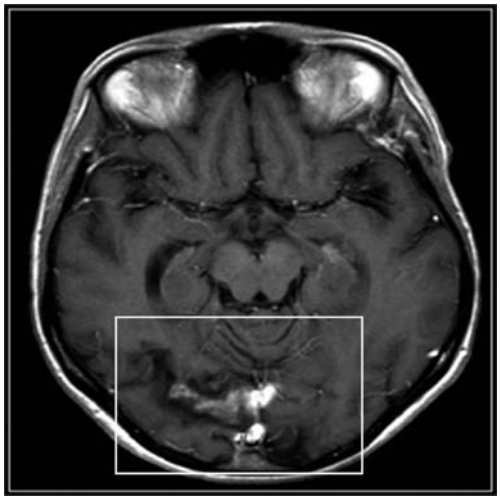

Figure 3. Control MRI-occipital cortex (T1, with contrast), infarction on the site of previous oedema.

EEG presentation

Electroencephalogram showed diffuse slow-wave activity with frequent generalized paroxysms of sharp waves and slow delta activity (1.5-2 Hz) (Figure 2), EEG findings were compatible with reversible non-convulsive encephalopathy.

Further course of disease

During hospitalization we continued with the symptomatic treatment, her condition slowly improved, on the third and fourth day she was more alert, but we disclosed blindness, she didn't complain about. The ophthalmologic examination at bedside was normal, except the possibility to test vision and visual fields. Her vision and other symptoms slowly improved and so did MRI (Figure 3) and EEG findings (Figure 4). Repeated MRI disclosed an ischemic lesion in the occipital cortex, otherwise rarely seen complication of PRES, with partly completed regression of other changes. After fifteen days she was discharged from the hospital, fully conscious, with much improved vision, and without dysphasia.

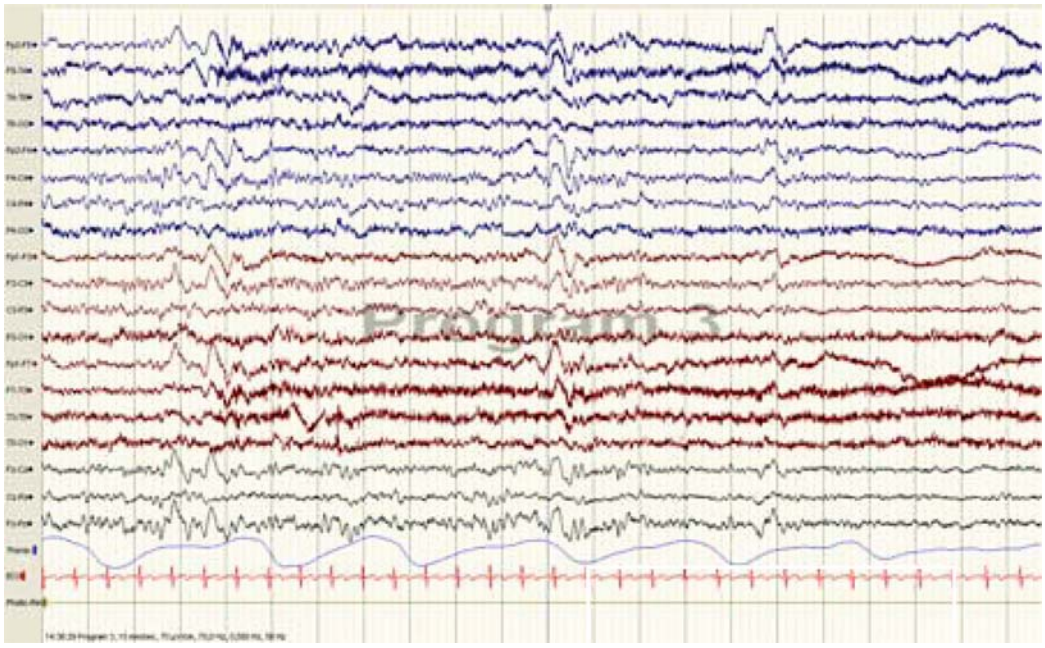

Figure 2. EEG two weeks after admission with diffuse slow wave activity and generalised paroxysms of sharp waves and slow delta activity.

She received next two cycles of ChT with cisplatin after twelve days and the third one later on, both times in split doses. No central neurotoxicity reoccurred, but after the malignant disease progressed, ChT was stopped and the patient died three and a half months after starting cisplatin-based ChT.

Discussion

Cisplatin side effects are often predictable in terms of their onset and duration, being most common nausea, vomitus, kidney toxicity and peripheral neuropathy with numbness, paresthesias, and occasionally pain, which usually begins in the toes and fingers, spreading proximally to affect the legs and arms.[5] In the presented patient, clinical signs and symptoms, MRI and EEG findings were compatible with rare central nervous toxicity of cisplatin-induced non-convulsive posterior reversible encephalopathy syndrome that occurred after the first dose of cisplatin-based ChT. The causal relationship to the agent was made after excluding other causes of the condition, such as progression of malignant disease, metabolic, iatrogenic, infectious and vascular causes. As in other cerebral diseases the MRI was essential in differential diagnosis,[6] but we found the main reason for the neurological disorder with contextual understanding of other clinical findings.

Of the other concurrent medications that are potentially central neurotoxic she also received lomustine, but according to data that we found, nitrosoureas are associated with little neurotoxicity at conventional doses, toxic effect is possible after cumulative and high iv doses,[7,8] which is not the case in our patient.

According to published data the exact mechanism for the emergence of cisplatin-induced encephalopathy is not known,

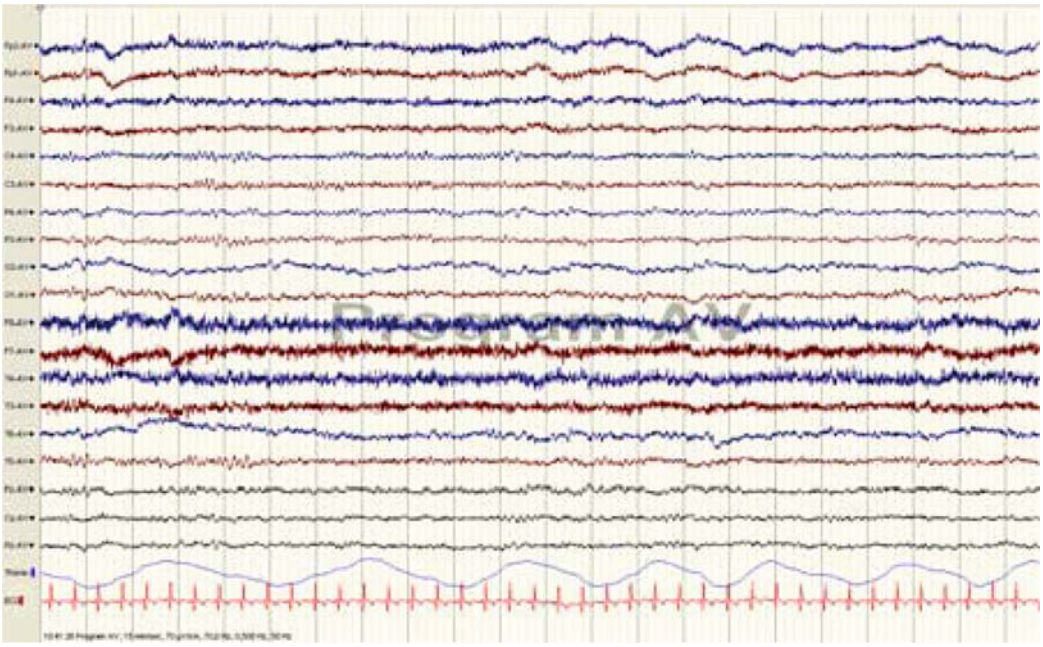

Figure 4. Control EEG after three months indicating improvement with disappearance of sharp and slow wave delta activity.

one hypothesis is that it may be caused by vascular events, other is that mechanism may be toxic encephalopathy, e.g. heavy metal toxicity and demyelinization.[9] Fever, thrombocytopenia, neutropenia, renal insufficiency, metabolic disturbances also contribute to CNS toxicity of cisplatin, but that was not the case in our patient. Neurotoxicity typically starts within two weeks after the treatment with cisplatin, with or without seizures, accompanied by acute or sub acute confusional state, central visual problems (even blindness) and often headache.[10] Our patient suffered from nausea, vomitus, headache, severe pain at the site of sub-cutaneous metastases and confusion, she later experienced somnolence, cortical blindness and aphasia, but without epileptic seizures, a combination characteristic of a less common, non-convulsive encephalopathy. Her symptoms resolved, she received the treatment with another two cycles, both times with split doses of cisplatin, decided by intending medical oncologist according to good clinical practice for minimizing the side effects. Both cycles were administered according to the same schedule and adverse events did not repeat. On control MRI, an ischemic lesion, a rarely seen complication to be present also at autopsy of such patients, was found.

Conclusions

According to published cases, cisplatin-induced central nervous toxicity is a relatively rare complication in cancer patients, but with the widespread use of cisplatin this rare disorder should be considered, especially if neurologic symptoms as described in this article occur. When diagnosed at an early stage it is usually a reversible condition, which requires adequate treatment of seizures, symptomatic treatment and withdrawal or cautiousness with cisplatin therapy.[11] It is important do differentiate neurotoxicity of cisplatin from signs of tumour progression.[12]

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case report

Angiosarcoma of the liver after multimodality therapy for gallbladder carcinoma

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Background. *Treatment-induced angiosarcoma is a rare complication of cancer therapy and is best known for arising on the chest wall after a mastectomy. We report an unusual case of a treatment-induced angiosarcoma developing in the liver after adjuvant radiotherapy for gallbladder carcinoma.*
Case report. *Radiation-induced angiosarcoma of the liver developed in a 46-year-old woman after radiotherapy for stage IIB (T3N1M0) adenocarcinoma of the gallbladder in 1991. The patient subsequently underwent resection, postoperative external beam radiotherapy, and multiagent chemotherapy. Several severe late adverse effects developed, including duodenal obstruction with fistula formation and chronic mesenteric ischemia secondary to occlusion of the superior mesenteric artery. Six years after her gallbladder resection and adjuvant treatment, a fatal grade 4 of 4 angiosarcoma of the liver developed within the radiation field.*
Conclusions. *To our knowledge, this is the first case of radiation-induced angiosarcoma of the liver after radiotherapy for gallbladder carcinoma. Normal organ dose-volume limits should be considered carefully when delivering a course of external beam radiotherapy in the upper abdomen.*

Key words: angiosarcoma; gallbladder carcinoma; liver; radiotherapy toxicity

Introduction

Angiosarcomas are rare malignant tumors arising from the vasculature.[1] Angiosarcomas of the liver occur even less commonly; about 25 cases are reported in the United States every year. Most angiosarcomas arising from the liver are secondary to long-term exposure to thorium dioxide, arsenic, or vinyl chloride.[2] To our knowledge, radiotherapy-induced liver angiosarcomas have not been previously reported. The purpose of this paper is to describe the first case of a radiotherapy-induced liver angiosarcoma.

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Conflict of interest: The authors have no conflicts of interest to disclose.

Case report

A 46-year-old woman presented with acute pain in the right upper quadrant of her

abdomen in 1991. Ultrasonography at that time revealed a thickened gallbladder wall. A computed tomographic (CT) scan of the abdomen demonstrated a mass arising in the gallbladder and extending beyond the gallbladder wall into the porta hepatis and the left hepatic lobe. Her staging work-up, which included a barium enema study, sigmoidoscopy, and pelvic ultrasonography, revealed no sites of metastatic disease. Levels of α-fetoprotein and cancer antigen 125 were within normal limits. Carcinoembryonic antigen (CEA) and alkaline phosphatase values were slightly elevated at 4.4 ng/mL and 126 units/L, respectively. The patient had a 20-pack-year history of smoking. There was no familial history of cancer or other genetic conditions.

The patient underwent a cholecystectomy and partial hepatectomy at another institution. Pathologic review of the resected specimen revealed a well- to moderately differentiated mucinous adenocarcinoma of the gallbladder, extending beyond the gallbladder wall to involve the liver capsule, stage IIB (T3N1M0). Three of 3 evaluated lymph nodes were involved with tumor. The patient's postoperative recovery was unremarkable and adjuvant therapy was recommended, given the primary tumor's adherence to the liver and the involved lymph nodes. The patient elected to receive adjuvant therapy. A course of external beam radiotherapy to a total dose of 54 Gy in 1.8-Gy daily fractions was administered using a 4-field technique. The patient received adjuvant chemotherapy consisting of fluorouracil, leucovorin, and mitomycin C concurrently with her radiotherapy. Her radiotherapy course was marked by grade 3 gastrointestinal tract toxicity, and she required hospital admission and support with intravenous hydration secondary to nausea, vomiting, and dehydration.

Over the next year, the patient had progressive upper abdominal pain with concomitant weight loss. Approximately 1 year after radiotherapy, an esophagogastroduodenoscopy revealed friable gastritis involving the distal antrum along with duodenitis involving the duodenal bulb, secondary to the radiation effect. In the next year, the patient continued to experience symptoms of persistent gastritis and duodenitis, and the following year she underwent a vagotomy and gastrojejunostomy to bypass a stricture of the duodenum, with an associated fistula. Four years after radiotherapy, in 1995, a diagnosis of chronic mesenteric ischemia was made. Mesenteric angiography revealed a 3-cm proximal occlusion of the superior mesenteric artery. She underwent aortic-to–superior mesenteric artery bypass at that time, and her symptoms resolved.

Six years after radiotherapy, in 1997, right upper quadrant pain and persistent nocturnal fevers up to 38.9°C developed. On clinical examination, she had right upper quadrant tenderness with abdominal distension and a palpable mass in the right upper quadrant. A CT scan subsequently revealed a 12-cm mass involving the anterior segment of the right hepatic lobe and medial left segment of the left hepatic lobe, with additional satellite lesions within the left lateral hepatic segment. The tumor extended into the porta hepatis, with occlusion and thrombus of the anterior segmental division of the right portal vein, and involved the prior gallbladder fossa. There was no evidence of neoplastic spread outside the liver. A biopsy revealed a spindle cell neoplasm consistent with angiosarcoma, grade 4 of 4. Immunohistochemical stains for CD31 and vimentin were positive and negative for keratin, CEA, and S-100.

The patient was referred to Mayo Clinic in Rochester, Minnesota, for evaluation and treatment at this point. She was not considered a surgical candidate because of the multifocal presence of tumor within the liver. A symptomatic thrombus of her supe-

rior mesenteric artery bypass graft developed approximately 14 days after the diagnosis of angiosarcoma. A course of salvage chemotherapy, consisting of ifosfamide, mitomycin C, doxorubicin, and cisplatin, was recommended, but the patient succumbed to fatal mesenteric ischemia before initiation of any systemic therapy.

Discussion

Gallbladder carcinoma is a rare malignancy. The prognosis is often poor because of the advanced stage at the time of diagnosis. Surgery remains the treatment of choice for gallbladder carcinoma.[3] For gallbladder carcinoma that is limited to the gallbladder mucosa, cholecystectomy is often sufficient to eradicate all malignant disease. However, for locally and regionally advanced disease, a radical cholecystectomy is recommended. For patients in whom an incidental carcinoma is found at the time of routine cholecystectomy, re-resection with abdominal exploration and lymph node dissection is often considered.

Given the high risk for systemic as well as regional recurrence in locally advanced tumors, we consider adjuvant therapy after resection of tumors that are locally extensive, invade adjacent organs, or involve regional lymph nodes. No randomized trials have been performed to quantify the benefit of such adjuvant therapy, however.[4] Retrospective studies have suggested that postoperative chemoradiotherapy has a survival benefit in patients with high-risk tumors, ie, tumors that show extensive local infiltration outside the wall of the gallbladder or those with regional lymph node involvement, in the absence of metastatic disease.[4-6] A study from Mayo Clinic, published in 2002, described 12 patients who underwent adjuvant chemoradiotherapy for gallbladder cancer after resection with negative margins and had 5-year survival of 64%.[5] Historical surgical controls from Mayo Clinic and other institutions had 5-year survival of 33% for patients treated with surgery alone.[5] A more recent evaluation at Mayo Clinic showed survival rates were equivalent for patients undergoing surgery and for those undergoing surgery and adjuvant radiotherapy.[4] However, the majority of patients who underwent resection alone did so for stage I gallbladder carcinoma (88%), whereas patients receiving adjuvant therapy had more advanced stages. Despite having more advanced stages, patients receiving adjuvant therapy, primarily concurrent fluorouracil and external beam radiotherapy, had a survival rate comparable to those undergoing surgery alone for stage I disease.[4] A recent large Surveillance, Epidemiology, and End Results analysis showed a benefit in overall survival rates for selected patients undergoing postoperative adjuvant chemotherapy.[7] Given the relative rarity of the disease in the Western world, however, it is unlikely that a definitive randomized trial will address this question. Retrospective studies from Mayo Clinic and elsewhere suggest the benefit of this treatment, but these studies are limited by known biases regarding patient selection with respect to performance status and other factors.

The difficulties encountered in case studies highlight the potential pitfalls in delivering external beam radiotherapy in the right upper quadrant. Modern image-guided radiotherapy techniques, coupled with 4-dimensional CT-based treatment planning, now provide radiation oncologists with tools to avoid excess doses in critical organs. The chronic adverse effects in the patient described here—duodenal strictures, chronic gastritis, and arterial ischemia—are known complications resulting from high-dose and large-volume irradiation of the organs in the gastrointestinal tract. Also,

the risk of chronic, radiation-induced liver disease must be considered in treatment planning.[8]

The long-term adverse effects of radiation on the stomach may include gastritis with stenosis, dyspepsia, and ulceration. Gastritis may appear from 1 to 12 months after radiation treatment and is usually accompanied by antral stenosis. Endoscopy may also reveal atrophy of the mucosal wall. The onset of dyspepsia varies and may occur up to 4 years after treatment. Radiation-induced gastric ulcerations may heal spontaneously but are usually accompanied by fibrosis. Management of chronic gastritis has not been extensively studied. Some physicians elect to prescribe antiulcer medications such as H_2-receptor blockers.[9]

The volume of small bowel receiving high doses of radiotherapy is typically the limiting factor in radiotherapy planning for the upper abdomen. Radiation effects include fibrosis and chronic ischemia after vascular injury. Multifocal adhesions and areas of stenosis can occur in a dose-dependent fashion.[9] Radiotherapy can decrease the number of mucosal stem cells in the small intestine, causing inflammation and shortening of the villi and leading to a decrease in intestinal absorption. Additionally, radiation can induce, independent of injury to the villi, an inflammatory cytokine cascade in which proinflammatory cytokines such as tumor necrosis factor α and interleukins 1 and 6 contribute to a cycle of initiation of injury, upregulation with generation of messenger agents, and ulceration with further inflammation.[10] Vascular damage caused by radiation may involve hyalinized thickening and foam cell deposition in small-sized (<100 μm) and medium-sized (100-500 μm) arteries. Lymphocytic infiltration can occur in the tunica media and tunica adventitia. These changes are typical of small-vessel injury from radiotherapy and may, in rare cases, occur in larger vessels.[11] Symptoms of enteritis may take years to develop after irradiation of the abdomen, but the reported median is 8 to 12 months.[9] Toxicity is strongly correlated with the volume of small bowel irradiated in conjunction with the dose.[9,12,13]

At Mayo Clinic, our current approach to postoperative treatment of locally advanced gallbladder carcinomas involves careful reevaluation of the patient's nutritional status and cancer staging status approximately 1 month after surgery, followed by consideration of concurrent radiotherapy and chemotherapy. For patients who have adequately recovered from their resection and who have no evidence of metastatic disease, either at surgery or at the time of restaging after surgery, consideration is given to systemic chemotherapy with gemcitabine as initial treatment. This is followed by concurrent continuous infusional fluorouracil chemotherapy with 50.4 Gy in 1.8-Gy daily fractions of conformal external beam radiotherapy to the gallbladder fossa and regional lymphatics. Then gemcitabine-based chemotherapy is resumed 1 month after completion of radiotherapy. This regimen is similar to the adjuvant therapy delivered postoperatively with gemcitabine for pancreatic cancer during the Radiation Therapy Oncology Group 97-04 trial[14], with the exception of a change in radiotherapy target volume. A total radiation dose of 50.4 Gy is delivered to the tumor bed and structures involved by the tumor at the time of resection, with a lower dose of 45.0 Gy delivered to uninvolved lymph node regions at risk in the porta hepatis, pancreatoduodenal lymph nodes, and celiac axis. The radiotherapy must be carefully planned to provide adequate planning target volume (PTV) coverage (95% of the PTV receiving ≥50.4 Gy) while respecting liver tolerance (mean liver dose in patients without preexisting liver disease ≤34 Gy; volume of liver

receiving ≥30 Gy is ≤50%); kidney tolerance (volume of kidney receiving ≥18 Gy is ≤33% for at least 1 kidney and preferably both); and limiting maximum small bowel dose to no more than 52 Gy. Radiotherapy beam arrangements typically consist of opposed laterals, 2 anterior oblique angles 45° off vertical, a noncoplanar anterior beam angled 30° superior, and a noncoplanar posterior beam angled 20° toward the feet, with the angle of the posterior beam limited by the linear accelerator and couch design. A 4-dimensional CT scan for radiotherapy planning is performed at the time of simulation to track the target volume at risk with the breathing cycle.

Ionizing radiation can produce sarcomas in irradiated tissues. Cahan *et al.*[15] have published a well-recognized set of criteria to describe radiotherapy-induced sarcomas. First, the sarcoma should arise in an area that was previously irradiated. Second, a period of latency must exist between the radiotherapy and onset of the sarcoma. Finally, histologic confirmation of the sarcoma should be obtained.[16] Applying the criteria of Cahan *et al.*[15], the angiosarcoma reported here would meet the criteria for a radiation-induced malignancy. Previously, angiosarcomas have been reported in organs such as the heart, liver, spleen, and adrenal glands and have had a poor prognosis. Angiosarcomas have also been reported after mastectomy in patients with breast cancer due to long-standing lymphedema (Stewart-Treves syndrome).[1]

In summary, the role of adjuvant therapy in the treatment of locally advanced, resected gallbladder carcinoma remains to be optimally defined. On the basis of limited retrospective evidence, chemotherapy, with or without concurrent chemoradiotherapy, may reduce the risk of recurrence for patients with risk factors for local and regional recurrence. The chronic adverse effects of treatment emphasize the need for careful consideration of normal-organ dose-volume limits when delivering a course of external beam radiotherapy in the upper abdomen.

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technical note

How well are clinical gross tumor volume DVHs approximated by an analytical function?

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The dose heterogeneity in the tumor is often described as being normally distributed. Besides the normal distribution we propose the Fermi function describing Fermi statistics as a possible dose heterogeneity descriptor. In order to demonstrate the adequacy of the proposed functions as dose distribution descriptors 30 clinical gross tumor volume (GTV) dose-volume histograms (DVHs) are gathered and fit with the examined functions.

Key words: dose-volume histograms; gross tumor volume; Gaussian and Fermi statistics

In order to theoretically investigate a given tumor control probability (TCP) model for the case of heterogeneous irradiation, it is often necessary to simulate tumor dose-volume histograms (DVHs) that closely resemble clinical ones. Some authors[1-3] have assumed that tumor dose inhomogeneities are normally distributed around the target dose. In this case the integral DVH, *iDVH*, is represented by the erfc function:

$$iDVH:\ v(d\mid\mu,\theta)=0.5erfc\left(\frac{1}{\theta\sqrt{2\pi}}(d-\mu)\right) \quad [1]$$

where ν is the relative tumor volume irradiated to a maximum dose d, μ is a parameter corresponding to the mean (target) dose delivered to the tumor, and θ is a parameter related to the slope of the erfc function. However, no investigations of how well this function describes the clinical tumor DVHs are reported in the literature.

We propose the parallel use of the *Fermi* statistics function for the description of clinical tumor DVHs:

$$iDVH:\ v(d\mid\mu,\theta)=\frac{1}{1+\exp\left(\frac{d-\mu}{\theta}\right)} \quad [2]$$

This function describes the filling up of free energy levels in a Fermi system. The parameters d, μ and θ have the same meaning as in eq. [1].

To investigate this problem, we gathered 30 clinical gross tumor volume (GTV) DVHs for different treatment sites – lung, head & neck, prostate, etc., that were either

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obtained in the treatment planning process at the Cross Cancer Institute (CCI) or reported in the literature.[4-14] They were fit with the erfc [Eq. 1] and Fermi [Eq. 2] functions.

The fit was performed using the χ^2 criterion for goodness of fit, presuming a log-log-normal distribution for the integral DVHs. Correspondingly, the function to be minimized is:

The log-log form of the χ^2 criterion was used to account for the fact that an integral DVH is defined in the interval [0,1], while the standard χ^2 criterion deals with normally distributed random variables defined in $(-\infty,+\infty)$.[15] The experimental error is $\sigma_{\text{experimental}}$, which unfortunately is not reported in the literature. Therefore, we substituted $\sigma_{\text{experimental}}$ with a percentage band

$$\chi^2 = \sum_i \left[\frac{-\ln\left(-\ln\left(v^i_{theoretical}\right)\right) + \ln\left(-\ln\left(v^i_{\text{experimental}}\right)\right)}{\sigma^i_{\log\text{ experimental}}} \right]^2 ; \sigma^i_{\log\text{ experimental}} = \frac{-\sigma^i_{\text{experimental}}}{v^i_{\text{experimental}} \ln\left(v^i_{\text{experimental}}\right)}$$

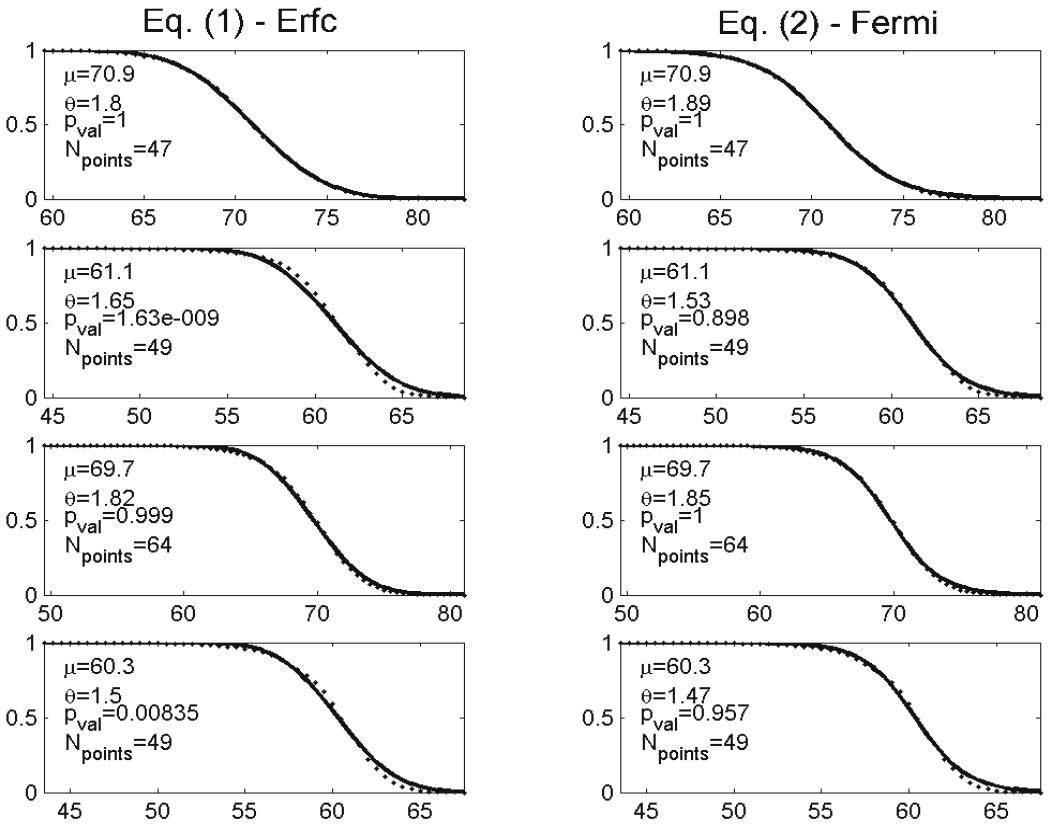

Figure 1. Fits to four clinical (head & neck) DVHs from CCI with the erfc function – a) and with the Fermi function – b) for the case of a 2% error band ($\sigma_{\text{experimental}} = 2\%$). On each subplot the p-value of the fit are shown, along with the statistics (number of data points, N_{points}) and the corresponding best fit values of the model parameters (μ and θ).

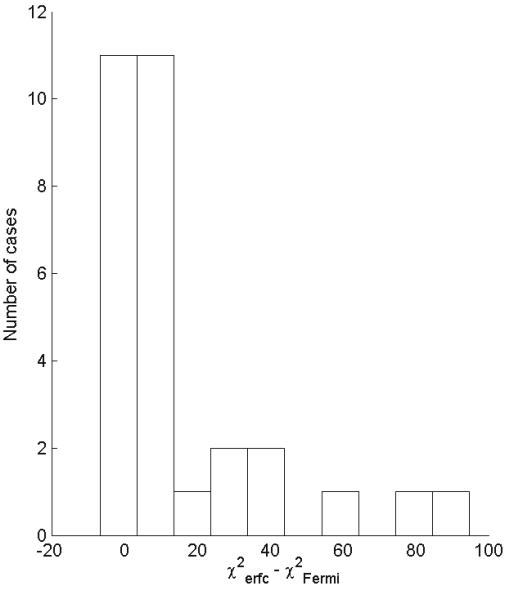

Figure 2. Distribution of the difference of χ^2 values for the fits obtained with the erfc and the Fermi functions, $\left(\chi^2_{erfc} - \chi^2_{Fermi}\right)$, built on the bases of fits to 30 clinical DVHs.

in which a statistically acceptable fit could be obtained. We initially presumed a 2% error band and found that in 24 out of 30 cases Eq. [2] produced acceptable fits, while Eq. [1] produced acceptable fits in 19 out of 30 cases. We also investigated a 4% error band and found out that Eq. [2] produced statistically acceptable fits in all considered cases, while Eq. [1] produced poor fits in 2 of all cases. As an illustration, fits of four head & neck DVHs obtained at CCI with the erfc function [Eq. 1] and with the Fermi function [Eq. (2] are shown in Figure 1a and Figure 1b, respectively. The fits correspond to the case of $\sigma_{\text{experimental}}$ =2%. As can be seen from the obtained χ^2 and p-values (shown in each subplot of Figure 1), the Fermi function produces excellent fits in all four cases, while the erfc function produces a poor fit in one case. It can be therefore concluded that the Fermi function describes clinical integral DVHs better than the erfc function. To further illustrate this conclusion a distribution of the difference of the χ^2 values of the fits obtained with the two proposed functions is shown in Figure 2. This distribution is constructed on the bases of the 30 clinical DVHs used in this study. As can be seen from Figure 2, the average of the distribution of $\left(\chi^2_{erfc} - \chi^2_{Fermi}\right)$ is greater than zero, showing that in most cases the *Fermi* function indeed produces better fits to clinical DVHs than the *erfc* function.

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Sindrom rakave kaheksije-anoreksije in izguba skeletno mišične mase

Jurdana M

Izhodišča. Sindrom rakave anoreksije-kaheksije je skupen in pomemben zaplet povezan z rakom. Pojavi se pri 30% do 80% vseh rakavih bolnikov. Beseda kaheksija pomeni „slabo stanje" in je lahko prisotna v zgodnji fazi rakavega razvoja, preden se pojavijo znamenja malignosti. Sindrom rakave kaheksije je postopno hujšanje telesa, za katerga je značilna izguba maščobnega tkiva in skletne mišične mase. Pri mnogih rakavih bolnikih je kaheksija povezana z anoreksijo, ki je posledica pomankljivega vnosa hrane. Tovrstno odklanjanje hrane, ki je pri raku povezano z energijsko porabo je ena temeljnih fizioloških motenj, ki vodi v izgubo telesne teže. Izguba teže pri pacientih s sindromom rakave anoreksije-kaheksije se razlikuje od izgube, ki je posledica stradanja in anoreksije nervoze. Patofiziologija rakave kaheksije še ni popolnoma pojasnjena, čeprav so študije pokazale pomembno vlogo citokinov v presnovi ogljikovih hidratov, maščob in beljakovin. Rak, dolgotrajno ležanje, HIV infekcija in staranje so označena kot stanja, katerih skupna lastnost je izguba mišične mase. Ukrep, s katerim bi lahko postopno zmanjšali izgubo mišične mase, je vadba proti uporu, definirana kot ponovitev statičnih in dinamičnih mišičnih kontrakcij. Na ta način želimo povečati mišično maso.

Zaključek. Ključni, sestavni deli patološkega stanja kaheksije so anoreksija in metabolne anomalije, kot sta izguba maščobe in katabolizem mišičnih beljakovin. Prihodnje raziskave bodo osredotočene na molekularne anomalije kaheksije ter na preiskovanje funkcijske koristi treninga z vajami proti uporu pri preprečevanju izgube mišične mase.

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Ksantogranulomatozni holecistitis ostaja izziv v medicinski praksi

Izkušnje s 24 bolniki

Yildirim M, Oztekin O, Akdamar F, Yakan S, Postaci H

Izhodišča. Ksantogranulomatozni holecistitis je redka, benigna, kronična vnetna bolezen žolčnika. S slikovnimi preiskavami pa tudi intraoperativno lahko vidimo tumorozno raščo. Raziskava je želela pri bolnikih, kjer smo načrtovali holecistektomijo, oceniti vrednost preoperativnih priskav in jih primerjati z intraoperativnim stanjem.
Bolniki in metode. Retrospektivno smo analizirali klinične podatke 24 bolnikov s ksantogranulomatoznim holecistitisom, ki so se zdravili v obdobju 7 let (srednja starost 53 let (32-68), razmerje med moškimi in ženskami 1:1,4).
Rezultati. Pogosti klinični simptomi so bile bolečine v trebuhu (79%), slabost (62%) in zlatenica (12%). Preoperativna ultrazvočna preiskava je pokazala žolčne kamne (95,8%) in žolčni drobir (8%) ter periholecistično tekočino (20%), polip (4%) in tumor žolčnika (4%). Stena žolčnika je bila zadebeljena (>3mm) pri 10 bolnikih. Računalniška tomografija je pri vseh bolnikih pokazala patološke spremembe. Intraoperativno pa smo našli: žolčne kamne pri vseh bolnikih (100%), kronični holecistitis pri več kot polovici bolnikov (54%), redkeje pa hidropični žolčnik, emfizematozni žolčnik, priraščanje žolčnika na sosednje organe in tumorske mase.
Zaključki. Ksantogranulomatozni holecistitis je težko ugotoviti pre- ali intraoperativno in ostaja izziv v klinični praksi. Končno diagnozo lahko postavimo le s histopatološko priskavo.

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Subduralni hematom hrbtenice pri von Willebrandovi bolezni

Franko A, Antulov R, Dunatov S, Antončić I, Miletić D

Izhodišča. Von Willebrandova bolezen je najpogostejša prirojena motnja strjevanja krvi, ki se najpogosteje manifestira s spontanimi krvavitvami. Bolniki s prirojenimi motnjami strjevanja krvi so neredko okuženi tudi z virusom hepatitisa C. Raziskave so pokazale, da je pri bolnikih, ki so okuženi z virusom hepatitisa C in prejemajo kombinirana zdravila, večja nevarnost nastanka znotrajmožganske krvavitve.

Prikaz primera. 44 letni moški je bil pripeljan v urgentni center z bolečinami v spodnjem predelu hrbta, glavobolom, bolečinami v vratu in z znaki spastične parapareze. Magnetnoresonančna preiskava prsnega dela hrbtenice je pokazala subduralni hematom v višini T8 do T 11.

Zaključki. Kolikor nam je znano, je to prvi opisani prikaz subduralnega hematoma v prsnem predelu hrbtenice pri bolniku z von Willebradovo boleznijo in s kronično okužbo z virusom hepatitisa C. Bolnik je prejemal kombinirano zdravljenje, ki je lahko dodatni dejavnik tveganja za nastanek krvavitve.

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Porast kronične poškodbe sečnika pri pooperativnem obsevanju karcinomov prostate z vključitvijo področnih bezgavk

Kragelj B

Izhodišča. Z obsevanjem ležišča prostate (PBRT) lahko pri bolnikih s karcinomom prostate, ki so zdravljeni s prostatektomijo, preprečimo ponovitev bolezni ali pa ob ponovitvi ponovno vzpostavimo remisijo bolezni. Uspešnost obsevanja lahko izboljšamo z dodatnim obsevanjem področnih bezgavk (WPRT).
Metode. Namen raziskave je bilo oceniti pozne posledice pooperativnega obsevanja pri 43 bolnikih (21 z dodatnim WPRT). Opazovali smo težave pri odvajanju urina in blata. Stopnjo zapleta smo ocenjevali s prilagojeno RTOG/LENT lestvico. Ležišče prostate je bilo obsevano z mediano dozo 64,8 (59,4-70,0) Gy in področne bezgavke z mediano dozo 50,4 (48,0-56,0) Gy.
Rezultati. Pozne posledice zdravljenja so bile predvsem okvare sečnika (poslabšanje delovanja za 1 stopnjo pri 33% in ≥2 stopnji pri 26% bolnikov) in manj črevesne okvare (poslabšanje delovanja za 1 stopnjo pri 54% in ≥2 stopnji pri 5% bolnikov). Okvare sečnika so bile pogostejše pri WPRT kot pri PBRT (67% in 50% bolnikov), zlasti, če je bila doza za WPRT ≥52 Gy (71% bolnikov).
Zaključki. Kljub temu, da so okvare sečnika po prostatektomiji in pooperativnem obsevanju verjetno posledica več dejavnikov, lahko z zmanjšanjem obsevanosti sečnika, kot ga omogočajo sodobne tehnike, pričakujemo zmanjšanje poznih posledic zdravljenja.

Radiol Oncol 2009; 43(2): 97-107.

Primerjava standardnega in modificiranega testa komet pri ugotavljanju poškodb DNA na humanih limfocitih po izpostavitvi ionizirajočemu sevanju

Mikloš M, Gajski G, Garaj-Vrhovac V

Izhodišča. Človeški organizem je zelo občutljiv na ionizirajoče sevanje, ki ima močno genotoksično delovanje na molekulo DNA. Namen raziskave je bil ugotoviti vrsto DNA poškodb in način umiranja celic po obsevanju z ionizirajočimi žarki ter primerjati senzitivnost standardiziranega in modificiranega testa komet.
Metode. Učinki sevanja gama (0,1 Gy in 4 Gy) so bili proučevani na humanih limfocitih s standardnim in alkalnim Fpg- modificiranim testom komet, kot tudi s testom DNA difuzije.
Rezultati. Parametri standardnega testa komet so pokazali značilno višje vrednosti pri vzorcih, ki so bili obsevani s 4 Gy, kot pri vzorcih, ki so bili obsevani z 0,1 Gy in kontrolnih vzorcih. Modificirani Fpg test komet je pokazal značilno višje vrednosti že pri nižji dozi obsevanja 0,1 Gy, zaradi oksidativne poškodbe DNA pri obsevanju in večje sensitivnosti testa. Test DNA difuzije je pokazal, da sevanje gama vodi celice v apoptozo bolj kot v nekrozo.
Zaključki. Raziskava nakazuje, da sta oba testa komet, standardni in modificirani, kot tudi test DNA difuzije, zanesljivi za oceno poškodb DNA in vrsto celične smrti povzročene z ionizirajočim sevanjem *in vitro*. Poleg tega je modificirani Fpg test komet bolj občutljiv za zaznavo poškodb DNA kot standardni test komet.

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Vpliv hipotoničnega pufra in elektroporacije na dinamiko nabrekanja in viabilnost celic za optimizacijo elektrofuzije

Ušaj M, Trontelj K, Hudej R, Kandušer M, Miklavčič D

Uvod. Na elektrofuzijo vplivajo različni parametri. Do sedaj objavljeni rezultati so pokazali, da hipotonični fuzijski pufer pripomore k večji učinkovitosti elektrofuzije. Takšna optimizacija elektrofuzije vključuje določitev primerne osmolarnosti pufra in inkubacijskega časa, pri katerem nabrekla celica še ne vzpostavi mehanizmov regulacije volumna. Osmolarnost pufra in inkubacijski čas morata biti določena za vsako celično linijo posebej, ne da bi vplivala na njihovo preživetje. V naši raziskavi smo določili dinamiko nabrekanja celic in njihovo viabilnosti v hipotoničnem pufru za štiri celične linije. Za izbrano celično linijo smo proučili tudi vpliv elektroporacije v hipotoničnem pufru.

Materiali in metode. Dinamiko nabrekanja celičnih linij CHO, V79, B16-F1 in NS1 smo določili z meritvami velikosti celic v 30 minutnem časovnem intervalu po začetku inkubacije v hipotoničnem pufru. Viabilnost celic po inkubaciji v hipotoničnem pufru smo določili 24 ur po poskusu z modificirano kristal vijolično metodo za določanje viabilnosti.

Rezultati. Pri naših eksperimentalnih pogojih je bila inkubacija v 100 mOsm pufru učinkovita za nabrekanje celičnih linij CHO, V79 in B16-F1. Najustreznejši inkubacijski čas celic v hipotoničnem pufru je bil od 2 do 5 minut. Enaki eksperimentalni pogoji pa niso povzročili želenega nabrekanja celic NS1. Za razliko od celic elektroporiranih v izotoničnem pufru, smo pri celicah elektroporiranih v hipotoničnem pufru opazili povečano nabrekanje, pri čemer pa po elektroporaciji celic v hipotoničnem pufru nismo opazili zmanjšanja volumna i.e. regulacije volumna.

Zaključek. Ker se različne celične linije razlikujejo po svoji občutljivosti na inkubacijo v hipotoničnih pogojih, moramo dinamiko nabrekanja v hipotoničnem pufru določiti za vsako celično linijo posebej. Povečan volumen celic v hipotoničnem pufru, ki je zaradi elektroporacije dolgotrajnejši, lahko prispeva k izboljšanju učinkovitosti elektrofuzije. Viabilnost celic se po inkubaciji v hipotoničnem pufru ni zmanjšala. Pri izbiri parametrov elektroporacije v hipotoničnem pufru moramo upoštevati tudi povečanje celic.

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Nekonvulzivna posteriorna reverzibilna encefalopatija povzročena s cisplatinom pri 41-letni bolnici z metastatskim malignim melanomom

Ocvirk J, Boc M, Reberšek M, Roš T

Izhodišča. Cisplatin, široko uporabljen cistostatik, v veliki večini povzroča periferno nevropatijo, redko pa lahko povzroči zaplete v obliki encefalopatije ali brez krčev.
Prikaz primera. Predstavljamo primer mlade bolnice z metastatskim malignim melanomom, z znaki in simptomi nekonvulzivne posteriorne reverzibilne encefalopatije povzročene s cisplatinom. Pri bolnici je v nekaj dneh po prvem krogu kemoterapije na osnovi cisplatina prišlo do slabosti, bruhanja, glavobola, hudih bolečin na mestu podkožnih metastaz in zmedenosti. Simptomi in znaki so se kasneje stopnjevali do somnolence, kortikalne slepote in afazije, brez epileptičnih krčev.
Zaključki. Cisplatin je učinkovit citostatik, vendar tudi zelo toksičen. Zdravniki, ki ga uporabljajo, morajo biti pozorni tudi na možnost encefalopatije.

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Angiosarkom jeter po kombiniranem zdravljenju raka žolčnika

Botros M, Quevedo JF, Miller RC

Izhodišča. Angiosarkom, ki je nastal zaradi zdravljenja raka, je redek zaplet obravnave bolnika. Najbolj pogosto je opisan v prsni steni po mastektomiji. Poročamo o redkem primeru z zdravljenjem povzročenem angiosarkomu, ki je nastal v jetrih po pooperativni kemoradioterapiji raka žolčnika.

Prikaz primera. Angiosarkom jeter se je razvil pri 46-letni bolnici, ki je bila leta 1991 operirana zaradi železnega raka žolčnika, stadij IIB (T3N1M0). Dodatno smo jo zdravili z obsevanjem in nato s kombinirano kemoterapijo. Po operaciji so se razvili pozni zapleti z zaporo dvanajsternika in fistulo ter zaradi zapore zgornje mezenterične arterije še kronična mezenterična ishemija. Šest let po operaciji in dodatnem zdravljenju smo odkrili lokalno napredovali angiosarkom jeter, gradus 4, ki se je razvil v področju obsevalnega polja.

Zaključki. Po našem vedenju je to prvi opisani primer angiosarkoma jeter, ki je bil povzročen s pooperativnim obsevanjem zaradi raka žolčnika. Pri obsevanju zgornjega dela trebuha moramo tako zelo skrbno načrtovati dozno-volumske omejitve zdravih organov.

Radiol Oncol 2009; 43(2): 132-135.

Kako dobro lahko DVH makroskopskega tumorskega volumna opišemo z analitično funkcijo?

Stavrev P, Schinkel C, Stavreva N, Fallone BG

Dozno heterogenost v tumorju pogosto opišemo z normalno porazdelitvijo. Kot drugo možnost za opis dozne heterogenosti predlagamo uporabo Fermijeve funkcije, ki opisuje Fermijevo statistiko. Da bi prikazali primernost predlagane funkcije, obe funkciji prilagodimo 30 dozno volumskim histogramom (DVH) makroskopskih tumorskih volumnov (GTV) in ju med seboj primerjamo.

Notices

*Notices submitted for publication should contain a mailing address, phone and/or fax number and/or e-mail of a **Contact** person or department.*

Oncology

July 2-5, 2009
The Educational Cancer Convention (ECCLU) will be held in collaboration with European Society for Medical Oncology in Lugano, Switzerland.
E-mail www.cmelcher@eso.net; or see www.eso.net

Lung cancer

July 31 - August 4, 2009
The "13th World Conference on Lung Cancer" will be offered in San Francisco, USA.
Contact Conference Secretariat International Conference Services Ltd., Suite 2101 - 1177 West Hastings Street, Vancouver, BC Canada V6E 2K3; or call +1 604 681 2153; or e-mail wclc2009@meet-ics.com; or see http://www.2009worldlungcancer.org/

Radiotherapy

August 30 – September 3, 2009
The 19th Biennial ESTRO Conference" will be held in Maastricht, the Netherlands.
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The "34th ESMO Congress" will take place in Vienna, Austria.
Contact ESMO Head Office, Congress Department, Via La Santa 7, CH-6962 Viganello-Lugano, Switzerland; or +41 (0)91 973 19 19; or fax +41 (0)91 973 19 18; or e-mail congress@esmo.org; or see http://www.esmo.org

Medical physics

September 7-12, 2009
The "World Congress 2009 – Medical Physics and Biomedical Engineering" will take place in Munich, Germany.
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The ESTRO teaching course ''3D Image-Based Brachytherapy for Gynaecological Malignancies'' will be offered in Amsterdam, The Netherlands.
Contact ESTRO office, Avenue E. Mounierlaan, 83/12, B-1200 Brussels, Belgium; or call +32 2775 93 40; or fax 32 2779 5494; or e-mail events@estro.org; or see http://www/estro-events.org

Radiation oncology

September 13-16, 2009
The "8th International Conference on Dose, Time and Fractionation in Radiation Oncology" will be held in Madison, Wisconsin, USA.
Contact ASTRO, 8280 Willow Oaks Corporate Dr., Suite 500, Fairfax, VA 22031; or call +1 703 502-1550; see http://www/astro.org

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Nuclear medicine

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Contact EANM Executive Secretariat and call +43 1 212 80 30; or fax +43 1 212 80 309; or e-mail office@eanm.org; or see http://www. eanm.org

Radiation oncology

October 11-16, 2009
The ESTRO teaching course Evidence Based Radiation Oncology: Methodological Basis and Clinical Application '' will be offered in Vienna, Austria.
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Lung Cancer

October 15-17, 2009
The ESTRO multidisciplinary teaching course on lung cancer will be held in Prague, Czech Republic.
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Radiobiology

October 18-23, 2009
The ESTRO teaching course on basic clinical radiobiology will be offered in Toledo, Spain.
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Therapeutic radiology and oncology

November 1-5, 2009
The "American Society for Therapeutic Radiology and Oncology Annual Meeting ASTRO" will take place in Chicago, USA.
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Radiotherapy

November 15-19, 2009
The ESTRO teaching course on IMRT and other conformal techniques in practice will take place in Gliwice, Poland.
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PET in radiation oncology

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PET in radiation oncology

December 13-17, 2009
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Head and neck cancer

February 25-27, 2010
The multidisciplinary symposium on head and neck cancer will be offered in Chandler, Arizona, USA.
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Clinical oncology

June 4-8, 2010
The American Society of Clinical Oncology Conference (ASCO 2010) will be offered in Chicago, USA.
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Nuclear medicine

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Therapeutic radiology and oncology

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The "American Society for Therapeutic Radiology and Oncology Annual Meeting ASTRO" will take place in San Diego, California, USA.
Contact ASTRO, 8280 Willow Oaks Corporate Dr., Suite 500, Fairfax, VA 22031; or call +1 703 502-1550; or see http://www.astro.org

Clinical oncology

June 3-7, 2011
The American Society of Clinical Oncology Conference (ASCO 2010) will be offered in Chicago, USA.
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Lung cancer

July 3-7, 2011
The "14th World Conference on Lung Cancer" will be offered in Amsterdam, The Netherlands.
See http://www.iaslc.org

Oncology

September 23-27, 2011
The "16th ECCO and 36th ESMO Multidisciplinary Congress" will be offered in Stockholm, Sweden.
See http://www.ecco-org.eu

Nuclear medicine

October 15-19, 2011
The "EANM'11 Annual Congress of the European Association of Nuclear Medicine" will take place in Birmingham, United Kingdom.
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Oncology

September 27 – October 1, 2013
The "17th ECCO and 38th ESMO Multidisciplinary Congress" will be offered in Amsterdam, The Netherlands.
See http://www.ecco-org.eu

Lung cancer

2013
The "15th World Conference on Lung Cancer" will be offered in Sydney, Australia.
See http://www.iaslc.org

As a service to our readers, notices of meetings or courses will be inserted free of charge.
Please send information to the Editorial office, Radiology and Oncology, Zaloška 2, SI-1000 Ljubljana, Slovenia.

Fundacija "Docent dr. J. Cholewa"
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Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – a report for the second quarter of 2009

Dr. J. Cholewa Foundation for Cancer Research and Education is of opinion that excellent and unorthodox ideas in cancer research and education must not be prevented to succeed for the simple lack of funding. The Foundation thus supports cancer research and education activities in Slovenia and continues to assess the requests for research grants and scholarships submitted by Slovenian experts in oncology and other associated scientific activities. As much as possible, the Foundation helps putting resulting advances in cancer therapy in practice. One of the goals of the Foundation is to transmit the latest diagnostic and therapy methods and knowledge to everyday research and clinical environment in Slovenia. This activity is directly beneficial for the increasing number of patients with various types of cancer in Slovenia, since the incidence rates of many types of cancer, like breast and prostate carcinoma, have kept rising in this country in recent decades.
The "Dr. L. Cholewa Foundation for Cancer Research and Education« continues to support the regular publication of "Radiology and Oncology" international medical scientific journal, that is edited, published and printed in Ljubljana, Slovenia. This support emphasizes the need for the spread of information about advances in experimental and clinical oncology to professionals and interested individuals in public in Slovenia and elsewhere. "Radiology and Oncology" is an open access journal, available free of charge on its own website, thus allowing its users and readers to access it free of expense. In this way the Foundation promotes cancer education in general and among scientists with a particular interest in cancer research.
The Foundation also supports the publication of results of cancer research in Slovenia and from Slovenian authors elsewhere in international scientific journals and other means of communication worldwide. It is important to know that results of cancer research, supported by the Foundation, have in many cases found its way to the practical application in hospital wards across Slovenia in a significantly easier manner in recent years than before. Careful assessment of requests and proposals for research grants and scholarships submitted by experts in oncology and other scientists is an essential part of the Foundation activities in spreading advanced knowledge of therapy and education in cancer.

Andrej Plesničar, MD
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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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Odmerjanje in način uporabe: Odrasle (tudi starejše) bolnice: 1 tableta po 1 mg peroralno, enkrat na dan. Odmerka zdravila ni treba prilagajati pri bolnicah z blago ali zmerno ledvično odpovedjo ali blagim jetrnim odpovedovanjem. Pri zgodnjem raku je priporočljivo trajanje zdravljenja 5 let.
Glavni neželeni učinki: Zelo pogosti (≥ 10 %): navali vročine, običajno blagi do zmerni. Pogosti (≥ 1 % in < 10 %): astenija, bolečine/okorelost v sklepih, suhost vagine, razredčenje las, izpuščaji, slabost, diareja, glavobol (vsi običajno blagi do zmerni)
Posebna opozorila in previdnostni ukrepi: Uporabe Arimidexa ne priporočamo pri otrocih, ker njegova varnost in učinkovitost pri njih še nista raziskani. Menopavzo je potrebno biokemično določiti pri vseh bolnicah, kjer obstaja dvom o hormonskem statusu. Ni podatkov o varni uporabi Arimidexa pri bolnicah z zmerno ali hudo jetrno okvaro ali hujšo ledvično odpovedjo (očistek kreatinina manj kakor 20 ml/min (oziroma 0,33 ml/s)). Pri ženskah z osteoporozo ali pri ženskah s povečanim tveganjem za razvoj osteoporoze je treba določiti njihovo mineralno gostoto kosti z denzitometrijo, na primer s slikanjem DEXA na začetku zdravljenja, pozneje pa v rednih intervalih. Po potrebi je treba začeti z zdravljenjem ali preprečevanjem osteoporoze in to skrbno nadzorovati. Ni podatkov o uporabi anastrozola z analogi LHRH. Arimidex znižuje nivo estrogena v obtoku, zato lahko povzroči zmanjšanje mineralne kostne gostote. Trenutno ni na voljo ustreznih podatkov o učinku bifosfonatov na izgubo mineralne kostne gostote, povzročene z anastrozolom, ali njihovi koristi, če se uporabijo preventivno. Zdravilo vsebuje laktozo.
Kontraindikacije: Arimidex je kontraindiciran pri: ženskah pred menopavzo, nosečnicah in doječih materah, bolnicah s hujšo ledvično odpovedjo (očistek kreatinina manj kot 20 ml/min (oziroma 0,33 ml/s)), bolnicah z zmernim do hudim jetrnim obolenjem, bolnicah, ki imajo znano preobčutljivost za anastrozol ali za katerokoli pomožno snov. Zdravila, ki vsebujejo estrogen, ne smete dajati sočasno z Arimidexom, ker bi se njegovo farmakološko delovanje izničilo. Sočasno zdravljenje s tamoksifenom.
Medsebojno delovanje z drugimi zdravili in druge oblike interakcij: Klinične raziskave o interakcijah z antipirinom in cimetidinom kažejo, da pri sočasni uporabi Arimidexa in drugih zdravil klinično pomembne interakcije, posredovane s citokromom P450, niso verjetne. Pregled baze podatkov o varnosti v kliničnih preskušanjih pri bolnicah, ki so se zdravile z Arimidexom in sočasno jemale druga pogosto predpisana zdravila, ni pokazal klinično pomembnih interakcij.
Imetnik dovoljenja za promet: AstraZeneca UK Limited, 15 Stanhope Gate, London, W1K 1LN, Velika Britanija
Režim predpisovanja zdravila: Rp/Spec
Datum priprave informacije: april 2007

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in na spletnih straneh:
www.arimidex.net
www.bco.org
www.breastcancersource.com

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ONKOLOGIJA

ARI 03/07 P

*adjuvant [ae´džuv*nt]*

1. *adjective* pomagljiv, koristen; *~ treatment with Arimidex:* Adjuvantno zdravljenje žensk po menopavzi, ki imajo zgodnji invazivni rak dojke s pozitivnimi estrogenskimi receptorji.

*advanced [*dva:nst]*

1. *adjective* napreden; zvišan (cene); to be ~ napredovati; ~ in years visoke starosti; *treatment of ~ breast cancer with Arimidex:* Zdravljenje napredovalega raka dojke pri ženskah po menopavzi. Učinkovitost pri bolnicah z negativnimi estrogenskimi receptorji ni bila dokazana razen pri tistih, ki so imele predhodno pozitiven klinični odgovor na tamoksifen.

switch [swič]

1. *transitive verb* udariti, bičati s šibo (z repom); šibati z, hitro mahati z; naglo pograbiti; railway ranžirati, zapeljati (usmeriti) (vlak) na drug tir; electrical vključiti, vklopiti; spremeniti (pogovor), obrniti drugam (tok misli); to ~ back to figuratively (v mislih) vrniti se na;
~ to Arimidex: Adjuvantno zdravljenje zgodnjega raka dojke s pozitivnimi estrogenskimi receptorji pri ženskah po menopavzi, ki so se dve do tri leta adjuvantno zdravile s tamoksifenom.

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SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Gemcitabin Lek 200 mg in 1 g prašek za raztopino za infundiranje
Sestava: Vsaka viala vsebuje 200 mg ali 1000 mg gemcitabina (v obliki gemcitabinijevega klorida). Vsak ml zdravila vsebuje 40 mg gemcitabina po redčenju na 5 ml (Gemcitabine Lek 200 mg) ali 40 mg gemcitabina po redčenju na 25 ml (Gemcitabiln Lek 1 g). **Indikacije:** Zdravilo prve izbire za zdravljenje bolnikov z lokalno napredovalim ali metastazirajočim nedrobnoceličnim pljučnim rakom. Za zdravljenje lokalno napredovalega ali metastazirajočega adenokarcinoma trebušne slinavke. Za zdravljenje lokalno napredovalega ali metastazirajočega raka na mehurju v kombinaciji z drugimi citostatičnimi zdravili. V kombinaciji s paklitakselom za zdravljenje bolnikov z neoperabilnim, lokalno ponavljajočim se ali metastazirajočim rakom na dojki, pri katerih se je bolezen ponovno pojavila po adjuvantni/neoadjuvantni kemoterapiji, ki je morala vključevati antraciklin, razen če ni bil klinično kontraindiciran.
Odmerjanje in način uporabe: Zdravljenje mora začeti zdravnik, ki ima precej izkušenj z zdravljenjem s citotoksičnimi zdravili. *Nedrobnocelični pljučni rak pri odraslih: Kombinirana uporaba*: Pri tritedenskem načrtu gemcitabin v odmerku 1250 mg/m² v 30-minutni intravenski infuziji prvi in osmi dan vsakega 21-dnevnega ciklusa. Pri štiritedenskem načrtu gemcitabin v odmerku 1000 mg/m² v 30-minutni intravenski infuziji prvi, osmi in petnajsti dan vsakega 28-dnevnega ciklusa. Cisplatin v odmerkih med 75 in 100 mg/m², enkrat na vsake tri ali štiri tedne. *Uporaba enega samega zdravila:* Priporočeni odmerek gemcitabina znaša 1000 mg/m² v 30-minutni intravenski infuziji, ponavljajoče enkrat tedensko v obdobju treh tednov, čemur sledi en teden premora. Štiritedenski ciklus se nato ponovi. *Rak trebušne slinavke:* Priporočeni odmerek znaša 1000 mg/m² v 30-minutni intravenski infuziji, ponavljajoče enkrat tedensko v obdobju do sedem tednov, čemur sledi en teden premora. Naslednji ciklusi morajo biti sestavljeni iz injiciranj enkrat tedensko v obdobju treh zaporednih tednov izmed vsakih štirih tednov. *Rak na mehurju:* Priporočeni odmerek znaša 1000 mg/m² v 30-minutni infuziji. Odmerek je treba dati prvi, osmi in petnajsti dan vsakega 28-dnevnega ciklusa v kombinaciji s cisplatinom. Cisplatin se daje v priporočenem odmerku 70 mg/m² prvi dan po dajanju gemcitabina oziroma drugi dan vsakega 28-dnevnega ciklusa. Ta štiritedenski ciklus se zatem ponovi. *Rak na dojki:* Priporočljiva je uporaba gemcitabina v kombinaciji s paklitakselom, pri čemer se paklitaksel (v odmerku 175 mg/m²) uporabi prvi dan v tri ure trajajoči intravenski infuziji, čemur sledi gemcitabin (v odmerku 1250 mg/m²) v 30 do 60 minut trajajoči intravenski infuziji prvi in osmi dan vsakega 21-dnevnega ciklusa. Pred začetkom dajanja kombinacije gemcitabin + paklitaksel mora pri bolnikih absolutno število granulocitov znašati najmanj 1,5 (× 10⁹/l). *Preverjanja:* Pri bolnikih, ki prejemajo gemcitabin, je treba pred dajanjem vsakega odmerka preverjati število trombocitov, levkocitov in granulocitov. Če je potrebno, se odmerek gemcitabina ob prisotnosti hematološke toksičnosti lahko zmanjša ali se ga preneha uporabljati. Treba je izvajati redne klinične preglede in preverjati delovanje jeter in ledvic, da bi lahko zaznali nehematološke škodljive vplive.
Kontraindikacije: Preobčutljivost za gemcitabin ali katerokoli pomožno snov. Uporaba med dojenjem pri ženskah, ki otroke dojijo. Sočasna uporaba s cepivom proti rumeni mrzlici. Kombinacija gemcitabina s cisplatinom pri bolnikih s hudo ledvično okvaro.

Posebna opozorila in previdnostni ukrepi: Gemcitabin lahko kratkotrajno zavre delovanje kostnega mozga, kar se kaže v levkopeniji, trombocitopeniji in anemiji.
Gemcitabin je treba uporabljati previdno pri bolnikih z okvarjeno ledvično funkcijo. Z uporabo gemcitabina je treba prenehati ob prvem pojavu kakršnihkoli znakov mikroangiopatske hemolitične anemije, kot je na primer hitro padajoča raven hemoglobina s spremljajočo trombocitopenijo, povečanje koncentracije bilirubina in kreatinina v serumu, povečanje ravni sečninskega dušika v krvi ali LDL, kar lahko nakazuje razvoj hemolitičnega uremičnega sindroma. Odpoved ledvic je lahko tudi po prenehanju zdravljenja ireverzibilna in lahko je potrebna dializa. Ne glede na to, ali zdravilo uporablja moški ali ženska, je treba med zdravljenjem upoštevati ukrepe za preprečevanje nosečnosti.
Medsebojno delovanje z zdravili in druge oblike interakcij: Sočasno zdravljenje z obsevanjem (ki se izvaja obenem ali s časovnim presledkom ≤ 7 dni). Zaporedno zdravljenje z obsevanjem (ki se izvaja s časovnim presledkom > 7 dni). Gemcitabin deluje radiosenzitizirajoče. Zaradi povečanega tveganja za trombozo pri bolnikih z rakom je uporaba antikoagulacijskega zdravljenja pogosta. Velika razlika v koagulacijskem statusu med posamezniki v času bolezni in možnost medsebojnega delovanja oralnih antikoagulantov in kemoterapije zahteva bolj pogosto spremljanje INR-ja v primeru uporabe antikoagulantov. *Kontraindicirana sočasna uporaba*: cepivo proti rumeni mrzlici. *Nepriporočljiva sočasna uporaba:* živa, oslabljena cepiva (razen rumene mrzlice). *Sočasna uporaba, ki zahteva premislek:* ciklosporin, takrolimus.
Vpliv na sposobnost vožnje in upravljanja s stroji: Gemcitabin lahko povzroči blago do zmerno zaspanost. Bolnike je zato treba posvariti pred vožnjo ali upravljanjem s stroji, dokler se ne izkaže, da zdravilo nanje nima omenjenega vpliva.
Neželeni učinki: Na pogostnost in hudost neželenih učinkov vplivajo odmerek, hitrost infundiranja in časovni presledki med odmerki. *Zelo pogosti* (> 1/10): anemija, levkopenija, trombocitopenija, nevtropenija, dispneja, navzea, bruhanje, povečane vrednosti jetrnih transaminaz (AST in ALT) in alkalne fosfataze, alergijski kožni izpuščaj, ki ga pogosto spremlja srbenje; plešavost – običajno blaga, hematurija, proteinurija, edemi/periferni edemi, gripi podobni simptomi (povečana telesna temperatura, glavobol, bolečine v hrbtu, drgetanje, bolečine v mišicah, astenija, pomanjkanje teka, kašelj, rinitis, občutek slabosti, znojenje, motnje spanja). *Pogosti* (> 1/100 do < 1/10): febrilna nevtropenija, zaspanost, stomatitis in razjede v ustih, driska, zaprtje, povečana koncentracija bilirubina, povečana telesna temperatura, astenija. Neželeni učinki, zaradi katerih je treba odmerek omejiti, so zmanjšanja števila trombocitov, levkocitov in granulocitov.
Oprema: Škatla z eno vialo s praškom.
Način izdaje zdravila: H
Imetnik dovoljenja za promet: Lek farmacevtska družba d.d., Verovškova 57, 1526 Ljubljana, Slovenija.
Informacija pripravljena: julij 2008

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Temodal 20 mg, 100 mg, 140mg, 180 mg, 250 mg.

Sestava zdravila: Vsaka kapsula zdravila Temodal vsebuje 20 mg, 100 mg, 140 mg, 180 mg ali 250 mg temozolomida.

Terapevtske indikacije Temodal kapsule so indicirane za zdravljenje bolnikov z:

- za zdravljenje novo diagnosticiranega glioblastoma multiforme, sočasno z radioterapijo in kasneje kot monoterapija
- malignim gliomom, na primer multiformnim glioblastomom ali anaplastičnim astrocitomom, ki se po standardnem zdravljenju ponovi ali napreduje.

Odmerjanje in način uporabe Temodal smejo predpisati le zdravniki, ki imajo izkušnje z zdravljenjem možganskih tumorjev. **Odrasli bolniki z novo diagnosticiranim glioblastomom multiforme** Temodal se uporablja v kombinaciji z žariščno radioterapijo (faza sočasne terapije), temu pa sledi do 6 ciklov monoterapije z temozolomidom. **Faza sočasne terapije** Zdravilo Temodal naj bolnik jemlje peroralno v odmerku 75 mg/m^2 na dan 42 dni, sočasno z žariščno radioterapijo (60 Gy, danih v 30 delnih odmerkih). Odmerka ne boste zmanjševali, vendar se boste vsak teden odločili o morebitni odložitvi jemanja temozolomida ali njegovi ukinitvi na podlagi kriterijev hematološke in nehematološke toksičnosti. Zdravilo Temodal lahko bolnik jemlje ves čas 42-dnevnega obdobja sočasne terapije do 49 dni, če so izpolnjeni vsi od naslednjih pogojev: absolutno število nevtrofilcev ≥ 1,5 x 109/l, število trombocitov ≥ 100 x 109/l, skupni kriteriji toksičnosti (SKT) za nehematološko toksičnost ≤ 1. stopnje (z izjemo alopecije, slabosti in bruhanja). Med zdravljenjem morate pri bolniku enkrat na teden pregledati celotno krvno sliko. **Faza monoterapije** Štiri tedne po zaključku faze sočasnega zdravljenja z zdravilom Temodal in radioterapijo naj bolnik jemlje zdravilo Temodal do 6 ciklov monoterapije. V 1. ciklu (monoterapija) je odmerek zdravila 150 mg/m^2 enkrat na dan 5 dni, temu pa naj sledi 23 dni brez terapije. Na začetku 2. cikla odmerek povečajte na 200 mg/m^2, če je SKT za nehematološko toksičnost za 1. cikel stopnje ≤ 2 (z izjemo alopecije, slabosti in bruhanja), absolutno število nevtrofilcev (AŠN) ≥ 1,5 x 109/l in število trombocitov ≥ 100 x 109/l. Če odmerka niste povečali v 2. ciklusu, ga v naslednjih ciklusih ne smete povečevati. Ko pa odmerek enkrat povečate, naj ostane na ravni 200 mg/m^2 na dan v prvih 5 dneh vsakega naslednjega ciklusa, razen če nastopi toksičnost. Med zdravljenjem morate pregledati celotno krvno sliko na 22. dan (21 dni po prvem odmerku zdravila Temodal). **Ponavljajoči se ali napredujoči maligni gliom Odrasli bolniki** Posamezen ciklus zdravljenja traja 28 dni. Bolniki, ki še niso bili zdravljeni s kemoterapijo, naj jemljejo Temodal peroralno v odmerku 200 mg/m^2 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/m^2 enkrat na dan, v drugem ciklusu pa se poveča na 200 mg/m^2 enkrat na dan 5 dni, če ni bilo hematoloških toksičnih učinkov (glejte poglavje 4.4). **Pediatrični bolniki** Pri bolnikih starih 3 leta ali starejših, posamezen ciklus zdravljenja traja 28 dni. Temodal naj jemljejo peroralno v odmerku 200 mg/m^2 enkrat na dan prvih 5 dni, potem pa naj sledi 23-dnevni premor (skupaj 28 dni). Otroci, ki so že bili zdravljeni s kemoterapijo, naj prejmejo začetni odmerek 150 mg/m^2 enkrat na dan 5 dni, s povečanjem na 200 mg/m^2 enkrat na dan 5 dni v naslednjem ciklusu, če ni bilo hematoloških toksičnih učinkov (glejte poglavje 4.4). **Bolniki z motnjami v delovanju jeter ali ledvic** Pri bolnikih z blagimi ali zmernimi motnjami v delovanju jeter je farmakokinetika temozolomida podobna kot pri tistih z normalnim delovanjem jeter. Podatki o uporabi zdravila Temodal pri bolnikih s hudimi motnjami v delovanju jeter (razred III po Child-u) ali motnjami v delovanju ledvic niso na voljo. Na podlagi farmakokinetičnih lastnosti temozolomida obstaja majhna verjetnost, da bo pri bolnikih s hudimi motnjami v delovanju jeter ali ledvic potrebno zmanjšanje odmerka zdravila. Kljub temu je potrebna previdnost pri uporabi zdravila Temodal pri teh bolnikih. **Starejši bolniki:** Analiza farmakokinetike je pokazala, da starost ne vpliva na očistek temozolomida. Kljub temu je potrebna posebna previdnost pri uporabi zdravila Temodal pri starejših bolnikih. **Način uporabe** Temodal mora bolnik jemati na tešče. Temodal kapsule mora bolnik pogoltniti cele s kozarcem vode in jih ne sme odpirati ali žvečiti. Predpisani odmerek mora vzeti v obliki najmanjšega možnega števila kapsul. Pred jemanjem zdravila Temodal ali po njem lahko bolnik vzame antiemetik Če po zaužitju odmerka bruha, ne sme še isti dan vzeti drugega odmerka. **Kontraindikacije** Temodal je kontraindiciran pri bolnikih, ki imajo v anamnezi preobčutljivostne reakcije na sestavine zdravila ali na dakarbazin (DTIC). Temodal je kontraindiciran tudi pri bolnikih s hudo mielosupresijo. Temodal je kontraindiciran pri ženskah, ki so noseče ali dojijo. **Posebna opozorila in previdnostni ukrepi** Pilotno preskušanje podaljšane 42-dnevne sheme zdravljenja je pokazalo, da imajo bolniki, ki so sočasno prejemali zdravilo Temodal in radioterapijo, še posebej veliko tveganje za nastanek pljučnice zaradi okužbe s Pneumocystis carinii (PCP). Profilaksa proti tovrstni pljučnici je torej potrebna pri vseh bolnikih, ki sočasno prejemajo zdravilo Temodal in radioterapijo v okviru 42-dnevne sheme zdravljenja (do največ 49 dni), ne glede na število limfocitov. Če nastopi limfopenija, mora bolnik nadaljevati s profilakso, dokler se limfopenija ne povrne na stopnjo ≤ 1. Antiemetična terapija: Z jemanjem zdravila Temodal sta zelo pogosto povezana slabost in bruhanje. **Laboratorijske vrednosti**: Pred jemanjem zdravila morata biti izpolnjena naslednja pogoja za laboratorijske izvide: ANC mora biti ≥ 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 nad 1,5 x 109/l in število trombocitov nad 100 x 109/l. Če med katerimkoli ciklusom ANC pade na < 1,0 x 109/l ali število trombocitov na < 50 x 109/l, morate odmerek zdravila v naslednjem ciklusu zmanjšati za eno odmerno stopnjo. Odmerne stopnje so 100 mg/m^2, 150 mg/m^2 in 200 mg/m^2. Najmanjši priporočeni odmerek je 100 mg/m^2. **Moški bolniki** Temozolomid lahko deluje genotoksično, zato morate moškim, ki se zdravijo z temozolomidom svetovati, da naj ne zaplodijo otroka še šest mesecev po zdravljenju. **Interakcije** Sočasna uporaba zdravila Temodal in ranitidina ni povzročila spremembe obsega absorpcije temozolomida ali monometiltriazenoimidazol karboksamida (MTIC). Jemanje zdravila Temodal s hrano je povzročilo 33 % zmanjšanje Cmax in 9 % zmanjšanje površino pod krivuljo (AUC). Ker ne moremo izključiti možnosti, da bi bila sprememba Cmax lahko klinično pomembna, naj bolniki jemljejo zdravilo Temodal brez hrane. Analiza populacijske farmakokinetike v preskušanjih druge faze je pokazala, da sočasna uporaba deksametazona, proklorperazina, fenitoina, karbamazepina, ondansetrona, antagonistov receptorjev H2 ali fenobarbitala ne spremeni očistka temozolomida. Sočasno jemanje z valprojsko kislino je bilo povezano z majhnim, a statistično značilnim zmanjšanjem očistka temozolomida. Uporaba zdravila Temodal v kombinaciji z drugimi mielosupresivnimi učinkovinami la hko poveča verjetnost mielosupresije. **Nosečnost** Študij na nosečih ženskah ni bilo. Predklinične študije na podganah in kuncih z odmerkom 150 mg/m^2 so pokazale teratogenost in/ali toksičnost za plod. Zato naj noseče ženske načeloma ne bi jemale zdravila Temodal. Če pa je uporaba v času nosečnosti nujna, morate bolnico opozoriti na možne nevarnosti zdravila za plod. Ženskam v rodni dobi svetujte, naj med zdravljenjem z zdravilom Temodal preprečijo zanositev. **Dojenje** Ni znano, ali se temozolomid izloča v materino mleko, zato ženske, ki dojijo ne smejo jemati zdravila Temodal. **Neželeni učinki** V kliničnih preskušanjih so bili najpogostnejši neželeni učinki, povezani z zdravljenjem, prebavne motnje, natančneje slabost (43 %) in bruhanje (36 %). Oba učinka sta bila ponavadi 1. ali 2. stopnje (od 0 do 5 epizod bruhanja v 24 urah) in sta prenehala sama, ali pa ju je bilo mogoče hitro obvladati s standardnim antiemetičnim zdravljenjem. Incidenca hude slabosti in bruhanja je bila 4 %. Laboratorijski izvidi: Trombocitopenija in. nevtropenija 3. in. 4. stopnje sta se pojavili pri 19 % in. 17 % bolnikov, zdravljenih zaradi malignega glioma. Zaradi njiju je bila potrebna hospitalizacija in/ali prekinitev zdravljenja z zdravilom Temodal pri 8 % in. 4 % bolnikov. Mielosupresija je bila predvidljiva (ponavadi se je pojavila v prvih nekaj ciklusih in je bila najizrazitejša med 21. in 28. dnem), okrevanje pa je bilo hitro, ponavadi v 1 do 2 tednih. Opazili niso nobenih dokazov kumulativne mielosupresije. Trombocitopenija lahko poveča tveganje za pojav krvavitev, nevtropenija ali levkopenija pa tveganje za okužbe. **Imetnik dovoljenja za promet** SP Europe 73, rue de Stalle B-1180 Bruxelles Belgija. **Način in režim izdaje** Zdravilo se izdaja samo na recept, uporablja pa se pod posebnim nadzorom zdravnika specialista ali od njega pooblaščenega zdravnika. **Datum priprave informacije** marec 2009.

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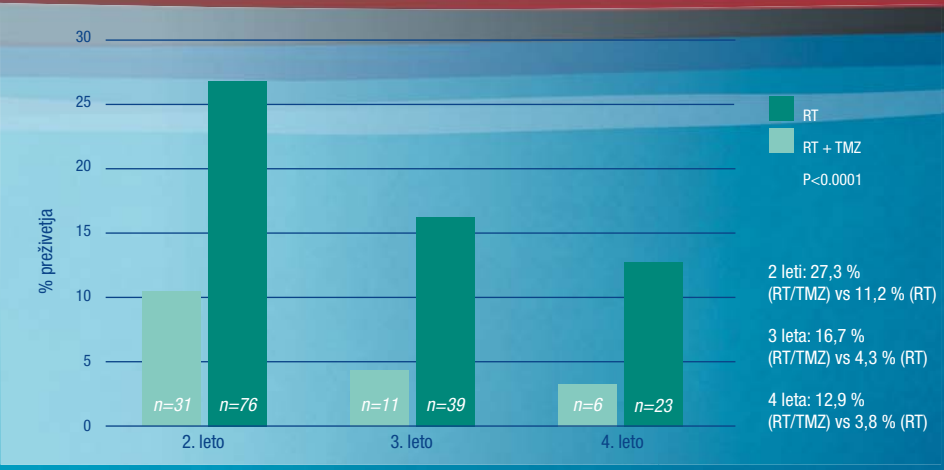

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Kakovostna in količinska sestava: Ena filmsko obložena tableta vsebuje 25 mg, 100 mg ali
150 mg erlotiniba (v obliki erlotinibijevega klorida).

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. 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 poraščenosti moškega tipa pri ženskah, spremembah trepalnic/obrvi, krhkih nohtih, odstopanju nohtov od kože, resni intersticijski pljučni bolezni, vključno s smrtnimi primeri.

Režim izdaje zdravila: H/Rp.

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

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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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