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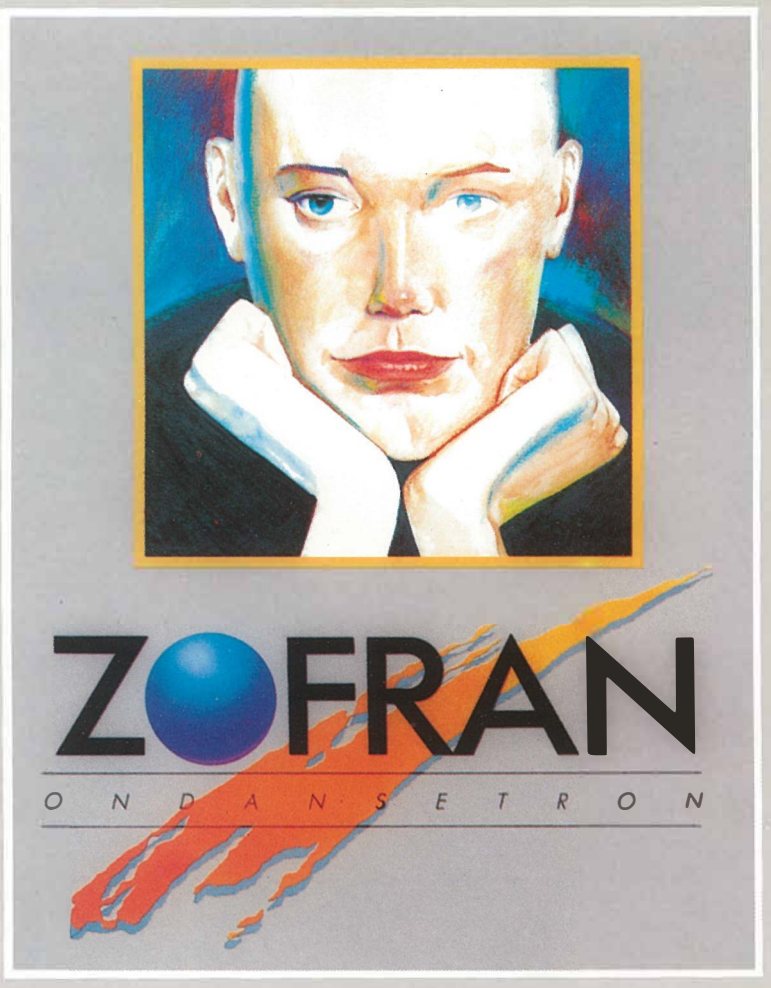
SLABOST IN BRUHANJE STA NAJHUJŠA STRANSKA UČINKA KEMOTERAPIJE IN RADIOTERAPIJE



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Complementariness of the radiological finding and transbronchial lung biopsy for definitive diagnosis of diffuse interstitial lung diseases

Ivica Mažuranić and Zlata Ivanovi-Herceg

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In this study 52 patients admitted to hospital for transbronchial biopsy of the lungs (TBB) due to anamnestically, clinically and radiologically suspected diffuse interstitial lung disease (DILD) were examined. Using classical radiological symptomatology tele radiograms were recorded (F-F 180 cm) of the chest PA and profile, by high voltage technique (125 kV). The patients were selected with regard to contraindications for general anaesthesia, and TBB. TBB was carried out under general anaesthesia with combined use of a rigid and flexibile bronchoscope, without radiological control. The percentage of adequate biopsy findings was very satisfactory (94 %), as was also the case with regard to sufficient pathoanatomic (51 %) and radiological (42 %) findings, by which a definitive clinical diagnosis can be made without the need for other tests. In the same way the percentages of adequately sufficient pathoanatomic (16 %) and radiological (39 %) findings were very statisfactory, by which, combined with other tests, definitive clinical diagnosis can be concluded. In no single disease were both findings insufficient, which indicates their complementariness, and in this way they are sufficient judging by the clinical status, BAL and biochemical findings, with regard to the final clinical diagnosis.

Key words: lung diseases, interstitial-pathology; biopsy; thoracic radiography

Introduction

Interstitial lung diseases are a heterogeneous group of diseases characterized by damage to the supportive structure of the lung units for gas exchange, periaveolar and alveoral tissue. In the acute phase alveolitis occurs, accompanied by prolonged, unrestrained inflammation of neighbouring parts of the interstitium and blood-vessels, and after a long period the inflammatory changes and consequent interstitial lung fibrosis damage the lung parenchyma, leading to impairment of gas exchange and ventilation. This diverse group of approximately 125 diseases has many common characteristics, including similar clinical symptomatology, graded radiological findings, consistent alterations in lung functions and typical cytological and histological appearance.

Bronchoscopy demonstrated its great value by facilitating the use of biopsy forceps to take tissue samples for histological and cytological analysis. Probably no other diagnostic or therapeutic technique changed pulmological work so radically in such a short time.

Interstitial disease have recently become the subject of great interest for radiologists. On the chest X-rays and imaging techniques this special group of diseases is characterized by multiple diffusely

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distributed lesions. According to these radiological criteria, complex clinical symptomatology, consistent changes in lung function, bronchoalveolar lavage (BAL), approximate pathoanatomic diagnosis can be made. The interstitial sample is an antithesis for the consolidatory alveolar process. It is a disorder within the lung architecture caused by an abnormal accummulation of tissue in the parenchymal lung interstitium.

In this study we compared the findings of transbronchial lung biopsy (TBB), which is accepted as suitable in histopathological diagnosis of diffuse interstitial lung diseases (DILD), with the radiological finding. The aim of the study was to determine the specific diagnostic value of each method separately and the possible additional benefit when both methods are interpreted simultaneously.

Patients and methods

In this investigation 52 hospitalized patients were examined (27 women and 25 men, range 16–76 years) in the Bronchoscopy unit at The Clinical Hospital for Lung Diseases – Jordanovac.

The patients were admitted for bronchological examination with transbronchial lung biopsy (TBB) because of anamnestically, clinically and radiologically suspected disease of the lung interstitium.

Teleradiograms (F-F 180 cm) of the chest PA and profile were recorded by high voltage technique (125 kV) on a Thoramat Siemens aparatus and, when necessary, tomography was performed. Such a radiological technique facilitated the taking of a biopsy sample in the area of the pathological process, with maximum accuracy, and without the use of a discope. As patients with DILD are regularly followed up long-term, it is essential to reduce the dose of exposure to ionizing radiation. Namely, during one minute of diascopy patients receive a dose which is equivalent to one hundred chest X-rays.

Although unrelated to ILO classification, the interstitial lesions were considered as patognomonic for certain diffuse interstitial lung disease.

Prior to bronchoscopy the following tests were carried out: gas analysis of arterial blood, coagulogram, haemogram, electrolytes, urea, creatinine clearence and an ECG.

The patients were selected with regard to contraindications for this examination (pulmonary hypertension, hemorrhagic diathesis, asthma, hypoxia where pO2 is less than 8,7 kPA inspite of oxygen administration, acute hypercapnia where pCO2 is more than 6 kPA, severe arhithmias, status within 3 months after the myocardial infarction, bad general condition, haemogram, electrolytes, local finding of the bronchial mucosa).

Following premedication with 0.8–1 mg Atropin, the anesthesized (narcotic, hypnotic and depolarizing muscle relaxants) patient was intubated with a rigid bronchoscope ("Wolf"). During the bronchoscopy the patient was ventilated according to Venturi's method, and a fiberbronchoscope (Olympus BS 10") was then introduced by which 2–3 biopsy samples were taken (by a standard technique) of TBB from the pathologically changed lung parenchyma, without using radiological control. The biopsy sample was placed in 10 % formalin and sent to the Institute for Pathology, Clinical Hospital Centre Rebro for pathoanatomical analysis. After the procedure the patient rested for 25 hours, with an obligatory control X-ray within 8 hours of the procedure.

Table 1. Dependence of succesful TBB on examination techniques. Comparison of the results of this study with the results of other authors.

Authors	Attempted biops. N	Adequate biops. N	%	Examination techniqe
Anderson i Fontana	450	378	84	Fiberbronchoscopy + dia. + local anaesthesia
R. Petit	66	47	71	Fiberbronchoscopy + dia. + local anaesthesia
J. Malenić	66	59	89	Combined + dia. + general anaesthesia
Wal	53	50	94	Fiberbronchoscopy + dia. + local anaesthesia
Own results	52	49	94	Combined without dia. + general anaesthesia
Levin	22	21	95	Fiberbronchoscopy + dia. + local anaesthesia

Mean (apart from own results)

Table 2. Complimentariness of radiological and pathoanatomic findings in the diagnostics of DILD.

	Pathoanatomic finding		Radiological finding	
	N	%	N	%
Sufficient	25	51	21	43
Adequately sufficient	8	16	19	39
Insufficient	16	33	9	18
Total	49	100	49	100

Table 3. Complimentariness of radiological and pathoanatomic findings in the final diagnosis of DILD.

Final dg. DILD	N	Sufficient PA	Sufficient RTG	Adequately sufficient PA	Adequately sufficient RTG	Insufficient PA	Insufficient RTG
Fibrosis	20	16	5	2	9	2	6
Sarcoidosis	16	5	11	2	4	9	1
Pneumonia interstitial	2	2			2		
Vasculitis necrotisans	2	1		1		1	1
Hypersensitive pneumonitis	2				1	2	1
Microlithiasis	1	1	1				
Granulomatosis Wegener	1		1	1			
Granulomatosis Lymphomatoides	1		1		1		
TBC milliaris	1				1		
TBC + sarcoidosis	1	1			1		
Silicosis	1		1	1			
Asbestosis	1			1	1		
Total	49	25	21	8	18	17	9

Results

By simultaneous use of priliminary radiological diagnostic treatment and combined TBB technique, the number of satisfactory samples was very high, up to 94 %. This result is comparable with the percentages of other techniques (bronchofiberscopy with radiological control, combined techniques with radiological control) in approximately the same number of cases (Table 1).

The percentages of sufficient interpretations of pathoanatomic (51 %) and radiological findings (43 %) were very satisfactory and would enable a definitive diagnosis, without other clinical data, and also adequately sufficient pathoanatomic (16 %) and radiological findings (39 %) with which, together with clinical data, the final diagnosis could be made. The percentage of insufficient pathoanatomic findings was higher (33 %) than radiological findings (18 %) (Table 2).

As to the pathoanatomic criteria, the initial diffuse interstitial fibrosis is a dominant finding. Pulmonary fibrosis, which is in fact compatible with the definitive stage of other interstitial diffuse lung diseases, was found in 18 patients, while radiological findings of sarcoidosis was found in 15 patients.

In interstitial pneumonia the radiological finding was sufficient while the pathoanatomical finding was not. Both the pathoanatomic and radiologic findings were sufficiently adequate in pulmonary alveolar microlithiasis, as well as sufficient in asbestosis. In hypersensitive pneumonitis only the radiological finding was sufficient. In other diagnoses both pathoanatomic and radiological findings were adequately sufficient and therefore in no disease were they both insufficient, which indicates their complementariness (Table 3).

Discussion

Interstitial lung diseases are currently the object of lively interest because of their great frequency, both due to air pollution and other harmful effects in the environment and because of the increasing number of circulating harmful agents. They have become a great health problem because of their acute stadium, different dynamism and response to therapy and finally their chronic forms, with regard to invalidity, i.e. remaining work ability.[1-3]

The protective reaction of the lungs which can include all types of immunological responses, can occasionally lead to one of many diffuse diseases of the lung interstitium.[4–5] Diffuse diseases of the lung interstitium are more frequent in their final stadium of pulmonary fibrosis with lung function impairment of prognosis. They are extremely difficult to classify as there are approximately 125 different diseases, the features of which are often common.

Diseases can be classified into known and unknown etiology. Of the diseases of known etiology the largest group comprises occupational diseases due to exposure to atmospheric pollutants. In the case of diseases of unknown etiology the largest group includes idiopathic lung fibrosis, collagen-vascular diseases (CVD), and granulomatous sarcoidosis. In many diffuse diseases of the lung interstitium similar conditions are present during their immunopathological course. They are judged by the clinical status, characteristic radiological finding, histological samples, bronchoalveolar lavage, scintigraphy with Ga-67 and biochemical findings.[6–12]

By using bronchoscopy it is possible, by a special technique, to obtain a sample of the lung parenchyma, avoiding open-lung biopsy.[13] Transbronchial lung biopsy was first carried out by Andersen[14] in 1965 an later in 1975. It was improved by Zavala and became the most modern technique in the diagnostics of DILD.[15] Current research in this field has shown that the piece of tissue obtained by TBB is usually small, and there is a possibility that widespread changes are not always gripped by the biopsy forceps. Consequently the biopsy sample is frequently taken 3 to 6 times, compared to only 2 to 3 times in our patients. The greater the number of pieces of tissue taken increases the chance of a sucessful diagnosis.[16–17]

Pneumothorax is the most frequent complication of the lung biopsy, (1–5 %, Herf, Zavala).[18, 19] In our series. there were two cases of pneumothorax (3,8 %), which required a conservative treatment. Significant hemorrhage (more than 50 ml of blood) is the second most frequent complication (1.3 % Herf, 5–9 % Zavala).[18, 19] We had a moderate hemorrhage, which ended spontaneously, in two patients.

Interstitial diseases are also currently the object of lively interest of radiologists. According to radiological criteria this particular group of diseases differs with regard to the multiple diffusely disseminated lesions on the chest X-rays and imaging techniques, and within the complex of clinical symptomatology, consistent changes in lung function, bronchoalveolar lavage and cell study approximates pathoanatomic diagnosis. These diseases may, primarily, be lung diseases or they may be a "reflection" of systemic diseases. They were initially described as milliary diseases, because the lesions were the size of a grain of millet,[20, 21] later as "diffuse disseminated",[21] or "nodular and reticular".[22] They are currently known as interstitial diseases. However, as these diseases encompass occasionally histologically determined both the interstitium and alveolar area some authors consider that "chronic diffuse infiltrative disease" is more appropriate. Radiological criteria for these anatomic locations was developed by Felson and later Fraser and Pare as alveolar or acinous lesions, and interstitial lesions.[23–25] The finest interstitial pattern is "ground-glass", consisting of discretely swollen connective structure and inflammatory infiltration without bronchiectasis, not covering the outline of the blood vessels. Nodular opacities are interstitial, 1–2 mm in size, which cover the outline of the blood vessels (apart from the interstitium they can also be in the alveolars and bronchioles). The reticular pattern relates to swollen interlobular septa with exudation – early stage of fibrosis – chronic stage. The linear pattern – honey combing – typical areas 5–10 mm in diameter. The reticulonodular pattern corresponds to the size of the nodular opacities and is characteristic for granulomatosis.[24] Theve have been many classifications, particularly for sarcoidosis, since 1940, King.[26] Wurn, Reindell and Heimeyer, classified sarcoidosis in three radiological stadia, taking into account the course and prognosis of disease, which was the case in our patients.[27]

As there is in fact no correlation between different parameters with regard to disease activity (lung function tests, BAL, ACE, scintigraphy with Ga-67) the radiological stage remains the most sensitive in the prognostics of these diseases.[27]

Because of the need to systematically evaluate radiological changes caused by inhalation of silicia dusts in various parts of the world the first International Classification for Pneumoconiosis was produced in 1930 by the International Bureau for Work in Geneva and reviewed in 1955, 1958, 1968, 1971 and 1980.[28] The ILO classification with standard proposals is clearly written and does not adopt pathoanatomic hypotheses. It only describes the round and irregular opacities according to size and profusion and does not go into the morphology of lesions which can be misleading.[29, 30] McLoud et al. were the first to apply the modified ILO classification to

other diffuse diseases of the lung interstitian, added as a description of reticulonodular pattern, characteristic for granulomatosis.[31] In our patients reticular and reticulonodular patterns were dominant in the radiograms, although without ILO classification of lesion.

In our investigation the percentage of satisfactory biopsies during which an adequate sample was obtained, was very high (94 %). As such biopsy material contains at least one piece of tissue with diffuse pathological changes of the lung, this sample was considered representative. This percentage agrees with the tests performed by the techniques of other authors[14, 32–35] with approximately the same number of patients with an average (apart from individual results) of 85 % (Table 1).

The same percentage of reliable pathoanatomic (51 %) and radiological (43 %) findings (39 %) compared to adequately sufficient pathoanatomic findings (16 %) and greater percentage of insufficient pathoanatomical findings (33 %) compared to insufficient radiological findings (18 %) (Table 2).

The most frequent were definitive clinical diagnoses of interstitial lung fibroses and sarcoidosis. Both pathoanatomically and radiologically reliable findings were of the same etiology. Pathoanatomically reliable findings were most frequent in interstitial lung fibroses (18 patients) and radiologically reliable findings in sarcoidosis (15 patients) (Table 3). In no single disease were they both insufficient, which indicates their complementariness, and they are therefore sufficient judging by the clinical status, BAL, and biochemical finding, with regard to the final clinical diagnosis. With respect to immunopathogenesis, in many diffuse diseases of the lung interstitium similar conditions are combined in their immunopathological course, which is another complicating factor in their differentiation. Definitive evaluation of the evolutiveness of DILD is facilitated by bronchoalveolar lavage (BAL) and cell analysis, and in the complex of clinical symptomatology and consistent changes of lung functional findings it is possible to make an approximate pathoanatomic diagnosis, and thus TBB, in this case, is an inferior test.

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Effects of nonionic radiographic contrast media on renal function after cardiac catheterisation

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Purpose: *The present study was undertaken to elucidate the effects of nonionic radiographic contrast media on systemic and renal function after routine cardiac catheterisation.*
Materials and methods: *In 108 consecutive patients, undergoing elective diagnostic or interventional cardiac catheterisation, several clinical and laboratory parameters were determined at baseline and 24 hours after the contrast study.*
Results: *The baseline serum creatinine level was 94.99 ± 19.44 nmol/l and increased to 96.33 ± 21.08 nmol/l (P = 0.72). Acute renal failure occured in only 1.9 percent of patients. The increase in serum creatinine level depended on the dose of the radiocontrast administered (P = 0.061), but was not predicted by gender, pre-existing renal insufficiency, hypertension, diabetes mellitus or congestive heart failure. In addition, a marked decrease in body weight was observed (from 78.8 ± 12.7 kg to 78.4 ± 12.9 kg, P= 0.001).*
Conclusion: *The use of nonionic contrast media for cardiac catheterisation appears to be safe and is associated with a low incidence of acute renal complications. No predicting factors of nephrotoxicity were observed. However, the increase in the serum creatinine and serum creatinine clearance 24 hours after the procedure depended largely on the dose of radiocontrast agent. We also observed a significant decrease in body weight and concluded that prophylactic pre-hydration is necessary to prevent the nephrotoxic effect of radiocontrast agents.*

Key words: heart catheterization, contrast media; kidney function tests

Introduction

The administration of radiographic contrast media continues to be a common cause of hospital-acquired acute renal failure.[1,2] During the past decade, nonionic (low-osmolality) contrast media have been increasingly employed in radiographic procedures, using intravascular contrast media, as they are associated with a decreased incidence of systemic and organ toxicity compared to conventional ionic (high-osmolality) contrast agents[3-5]. A variety of underlying conditions (renal insufficiency, diabetes mellitus, congestive heart failure, hypertension and a high dose of contrast medium) have been incriminated for the increased risk of renal toxicity after the administration of radiographic material. It is still unknown, however, whether these same predisposing factors can predict complications occurring after the use of nonionic contrast media.[6-11]

Although the pathogenesis of acute renal insufficiency induced by radiocontrast media is not fully understood, it appears to be due to medullary ischemia caused by reduced renal blood flow, resulting from an imbalance between vasodilative and vasoconstrictive factors.[12-14] In keeping with these observations, some recent studies have demonstrated a prophylactic effect of saline pre-hydration, especially in patients with increased risk for renal dysfunction after cardiac catheterisation.[15-18]

The role of nonionic contrast media on systemic and renal function in patients investigated in our

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catheterisation laboratory has not been evaluated yet. We therefore designed a prospective study of possible complications after routine cardiac catheterisation in 108 consecutive patients.

Material and methods

One hundred and eight consecutive patients, undergoing elective diagnostic or interventional cardiac catheterisation at the Department of Cardiovascular Diseases, Univesity Medical Centre were enrolled in the prospective study between January 23 and June 20, 1995. The indications for angiography were determined by each patient's cardiologist. Most patients were studied because of symptomatic coronary ishemia. All interventional procedures involved percutaneous transluminal coronary angioplasty were performed separately after diagnostic angiography.

One hour before, catheterisation hydration status was clinically assessed (central venous pressure, turgor, peripheral oedema). All patients were sedated (oral diazepam 10 mg), while antibiotics (oral amoxycillin 3 g or clindamycin 600 mg) were given only to patients at high risk for infective endocarditis. All catheterisation procedures were performed using the femoral approach. During the diagnostic or interventional catheterisation all inserted catheters were perfused with heparinised isotonic saline. The fluid intake before or during the procedure was not monitored. Nonionic contrast media iopamidol (Iopamiro 370, Bracco, Milano, Italy) or iohexol (Omnipaque 350, Nycomed Imaging, Oslo, Norway) were used exclusively in our contrast study. The average amount of nonionic contrast medium was 132.7 ± 72.8 ml (range 40 to 440 ml).

All blood and urine specimens were obtained one day before and 24 hours after cardiac catheterisation. Hematocrit, the serum concentration of sodium, potassium, creatinine and urea were determined in the Central laboratory, University Medical Centre. The content of protein in the urine and the sediment were also analysed. Creatinine clearance (ml/s) was calculated using the equation by Cockcroft and Gault:[19]

(140-age) x weight / 0.81 x serum creatinine

A radiocontrast-induced decrease in renal function was defined as an increase in the baseline serum creatinine concentration of ≥ 44 μmol/l (0.5 mg/dL) within 24 hours of the administration of radiocontrast medium.[5]

All numeric continuous data were analysed as the mean ± 1 standard deviation (SD). Descriptive statistical parameters were expressed as proportions. Continuous pairwise variables were assessed by the two-tailed paired t-test. The differences among subgroups were obtained by the analysis of variance. The influence of clinical and laboratory parameters, and the effect of the amount of radiocontrast medium on the decrease in renal function were assessed using the Spearman correlation coefficient. The obtained differences were considered significant at $P < 0.05$. All analyses were performed with the SPSS release 6.0 statistical package.

Results

In 108 consecutive patients undergoing elective diagnostic or interventional cardiac catheterisation, no serious events, such as acute myocardial infarction, aggravation of congestive heart failure, major arrhythmias or extensive peripheral bleeding, demanding blood transfusion, were observed neither during the procedure nor 24 hours afterwards.

The mean age of patients was 56.3 ± 10.4 years (range 23 to 78 years). Seventy-four percent of patients were males. Arterial hypertension was present in 66 (61.1 %) patients, pre-existing renal insufficiency (serum creatinine level above 132 mmol/l) in 4 (3.7 %), diabetes mellitus in 22 (20.4 %) and history of congestive heart failure in 22 (20.4 %) patients. Normal turgor was noticed in 106 (98.1 %) patients and peripheral oedema in 3 (2.8 %) patients. The mean central venous pressure was 7.8 ± 2.2 cm H_2O.

Table 1. Clinical and laboratory characteristics (mean ± SD) at baseline and 24 hours after cardiac catheterisation with nonionic contrast media (P, two-tailed paired t-test).

parameters	baseline (mean ± SD)	after 24 h (mean ± SD)	P
weight (kg)	78.80 ± 12.70	78.40 ± 12.90	0.001
hematocrit	0.42 ± 0.04	0.41 ± 0.04	0.058
Na^+ (mmol/l)	142.12 ± 3.09	141.69 ± 2.51	0.140
K^+ (mmol/l)	4.46 ± 0.33	4.31 ± 0.32	0.0001
urea (mmol/l)	6.55 ± 1.73	6.45 ± 1.78	0.540
creatinine (mmol/l)	94.99 ± 19.44	96.33 ± 21.08	0.720
creatinine clearance (ml/sec)	89.79 ± 23.81	88.43 ± 23.29	0.210

Clinical and laboratory data observed before and 24 hours after cardiac catheterisation with nonionic contrast media, are presented in Table 1. The baseline serum creatinine level was 94.99 ± 19.44 mmol/l and increased to 96.33 ± 21.08 mmol/l (P = 0.72). Twenty-four hours after the administration of contrast a slight decrease in the creatinine clearance, serum urea concentration and sodium was observed, but the differences were not statistically significant (P = 0.21, P = 0.54, P = 0.14, respectively). A trend of hematocrit reduction was also detected (P = 0.058). The decrease in the serum potassium from the baseline level was statistically significant (84.46 ± 0.33 mmol/ to 4.31 ± 0.32, P = 0.0001). No change in proteinuria or urinary sediment occurred after the administration of radiocontrast agent.

A rise in serum creatinine ≥ 44.2 mmol/l occurred in only two patients (1.9 %). Neither of them had pre-existing renal insufficiency, congestive heart failure or diabetes mellitus. In one patient, who received 210 ml of nonionic contrast agent, the serum creatinine level increased from 78 mmol/l to 136 mmol/l. In another, the administration of 370 ml of contrast medium, resulted in the elevation of serum creatinine level from 103 mmol/l to 148 mmol/l. No patient developed anuria or oliguria, or needed treatment by haemodialysis.

The studied subgroups did not differ significantly as concerns the increase in their serum creatinine (Table 2). Most notably, the gender, congestive heart failure, arterial hypertension, diabetes mellitus, underlying renal insufficiency and chronic diuretic therapy did not predict a rise in serum creatinine.

Table 2. Increase in serum creatinine (mean ± SD) 24 hours after the administration of radiocontrast by specific subgroups of patients (P, analysis of variance).

subgroups		N (%)	mean ± SD	P
gender	M	80 (74.1)	1.2 ± 11.9	0.77
	F	28 (25.9)	0.4 ± 14.1	
congestive heart failure	Y	22 (20.4)	-1.2 ± 11.9	0.36
	N	86 (79.6)	1.6 ± 12.7	
arterial hypertension	Y	66 (61.1)	1.9 ± 3.1	0.36
	N	42 (38.9)	-0.4 ± 11.5	
diabetes mellitus	Y	22 (20.4)	-2.8 ± 8.4	0.11
	N	86 (79.6)	1.9 ± 13.2	
renal insufficiency	Y	4 (3.7)	4.7 ± 13.6	0.54
	N	104 (96.3)	0.9 ± 12.5	
diuretic therapy	Y	29 (26.9)	-1.0 ± 9.6	0.84
	N	79 (73.1)	-1.5 ± 11.7	

The correlations between the dose of radiocontrast (ml/kg body weight) and some laboratory indicators of renal function, are shown in Table 3. A positive correlation between the radiocontrast dose and the increase in serum creatinine was observed (R = 0.18, P = 0.061) (Figure 1a). A negative association between the dose of radiocontrast and creatinine clearance 24 hours after cardiac catheterisation was also detected (R = -0.17, P = 0.086) (Figure 1b).

Table 3. Correlations between the dose of radiocontrast (ml/kg body weight) and some laboratory indicators of renal function in patients undergoing diagnostic or interventional cardiac catheterisation (P, R = Spearman correlation coefficient).

parameters	R	P
serum creatinine after 24 h	0.02	0.846
difference in serum creatinine	0.18	0.063
creatinine clearance after 24 h	-0.17	0.086
difference in creatinine clearance	-0.05	0.579
serum urea after 24 h	0.11	0.247
difference in serum urea	-0.09	0.350

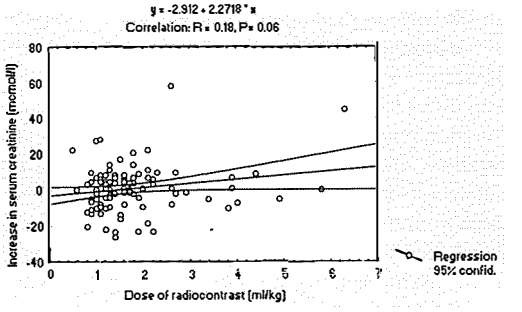

Figure 1a. The dose of radiocontrast and the increase in serum creatinine 24 hours after the administration of radiocontrast agent.

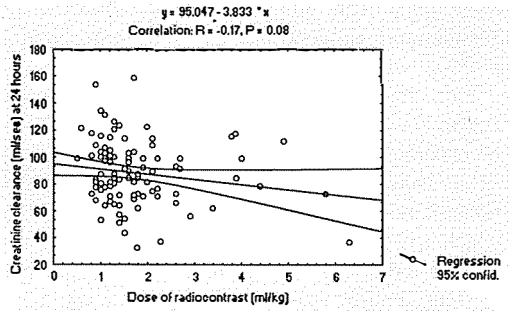

Figure 1b. The dose of radiocontrast and creatinine clearance 24 hours after the administration of radiocontrast agent.

Before the administration of radiocontrast media the mean body weight was 78.8 ± 12.7 kg and it decreased significantly to 78.4 ± 12.9 kg (P = 0.001). In 29 (26.9 %) patients receiving diuretic therapy, the changes in body weight 24 hours after angiography were less expressed than in patients without it (-0.2 ± 0.9 kg versus -0.4 ± 1.0 kg), but the difference was not statistically significant (P = 0.34).

Discussion

Nephrotoxicity due to administration of contrast material is reported to be one of the most common causes of acute renal failure acquired in hospital.[1, 2] In recent studies, the incidence of nephrotoxicity of contrast agents has shown wide variability (from 2 % to about 50 %).[1-10] The role of newer and much more expensive nonionic and lower osmolality contrast media remains to be defined. We therefore designed a prospective study involving 108 consecutive elective patients in order to investigate eventual systemic and renal complications occurring 24 hours after a routine diagnostic and interventional cardiac catheterisation.

Prospective studies have shown that a small rise in the plasma creatinine concentration (18 mmol/l) is a common occurrence after a radiocontrast study.[7] However, a more marked decline in renal function can occur by mechanisms which are not well understood. Contrast media are powerful renal vasoconstrictors, causing intense, though usually transient, reductions in renal blood flow.[13, 14, 22] At sufficiently high concentrations they appear to be toxic to isolated kidney cells studied in vitro. Furthermore, they can produce tubular obstruction and immunologic reactions.[22] The renal failure induced by contrast media typically begins immediately after its administration.[15, 22] In most patients, the serum creatinine usually begins to increase within the first 24 to 48 hours of the administration of contrast agent.[7] We therefore assumed that our short term sampling protocol was appropriate.

This study has clearly shown that cardiac catheterisation using nonionic contrast media has little or no nephrotoxic potential. Acute renal failure, defined as an increase in serum creatinine level of ≥ 44 mmol/l, was observed in only 2 patients (1.9 %), which is in agreement with some previous studies.[4, 5, 7] Neither did we detect any other laboratory parameters of contrast induced nephropathy, such as significant changes in serum urea level, creatinine clearance, urinary sediment or proteinuria.

We were unable to define any factors that would predict acute renal failure after the administration of nonionic contrast media. There was no significant difference in the serum creatinine increase in patients with pre-existing renal disease, congestive heart failure, hypertension and diabetes mellitus. Several investigators have found that pre-existing renal insufficiency alone or combined with diabetes mellitus significantly increases the risk of acute renal failure.[3, 5-7, 11] The discrepancy between our study and the earlier investigations is most likely due to the differences in the sample size and study design. In our group of 108 nonselected patients, the number of subjects with pre-existing renal insufficiency or other risk factors was considerably small.

The low osmotic pressure of solutions, the nonionic nature of the molecule and its inherently low chemotoxicity contribute to the exceptionally good local and systemic tolerability of nonionic contrast media. Some, but not all studies, have demonstrated a dose-dependent risk of renal dysfunction, with lower doses being safer, but not necessarily safe.[4, 8, 9, 11] Though we did not observe a significant rise in serum creatinine after cardiac catheterisation, the increase in serum creatinine and creatinine clearance 24 hours after the radiocontrast study correlated with the dose of radiocontrast. Thus, we concluded that the dose of radiocontrast medium used in our study was maintained within the range of relative safety.

In addition, the present study showed a significant decrease in body weight in patients 24 hours after cardiac catheterisation (P = 0.001). This finding may be due to osmotic diuresis caused by the administration of contrast agent. Intravenous fluids given before the administration of contrast agents may be beneficial by correcting plasma volume depletion, which leads to renal vasoconstriction and active sodium reabsorption, and by minimizing the consequences of osmotic diuresis after the administration of contrast agents.[23] Some up-to-date studies have demonstrated a prophylactic effect of saline pre-hydration, especially in patients at increased risk of developing renal dysfunction after cardiac catheterisation.[10, 15-18] So far we have not used special hydration methods before cardiac catheterisation. In view of the above mentioned observations it would be resonable to introduce a preventive hydration protocol with 0.45 percent saline in our catheterisation laboratory, especially in patients with chronic renal insufficency.

Surprisingly, our study showed a greater, yet not significantly greater, decrease in body weight in patients receiving no diuretics compared to patients on long-term diuretic therapy. Although we can not exactly explain this finding, it may be that patients on diuretic therapy were initially less hydrated than patients receiving no diuretics.

In summary, the use of nonionic contrast media in cardiac catheterisation appears to be a safe method, associated with a low incidence of acute renal complications (1.9 %). In contrast to previous studies, we failed to define any predisposing factors, such as pre-existing renal insufficiency, hypertension, diabetes mellitus or congestive heart failure, that would predict acute renal failure secondary to the use of nonionic contrast media. However, the increase in serum creatinine level and serum creatinine clearance 24 hours after the procedure were well correlated with the dose of radiocontrast. In addition, our study demonstrated a significant decrease in body weight, which might be due to osmotic diuresis, caused by the administration of contrast agent. We therefore strongly recommend the use of preventive saline hydration and careful control of body fluid balance before and during the catheterisation procedure.

Acknowledgement

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Percutaneous drainage of a pancreatic pseudocyst into the stomach

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This paper reports on a patient who had a pancreatic pseudocyst, drained externally operatively in May 1995. The pseudocyst wall was too thin to make pseudocystogastric internal anastomosis. Since this treatment failed, a percutaneous cystogastric double pigtail catheter was introduced with the assistance of ultrasound and gastroscopy. Two days after intervention the patient left the hospital without troubles. We followed him every month by clinical, biochemical, and US control. At first US control the pseudocyst was not seen. Percutaneously US guided internal drainage is an elegant and less traumatic alternative method to the patient compared with surgical procedure.

Key words: pancreatic pseudocyst; drainage; stomach

Introduction

Pancreatic pseudocyst is localized collection of fluid retroperitoneally confined by fibrous membrane without endothelial lining. It appears in 2 to 8 percent after acute pancreatitis. The patient complains of upper abdominal pain, early satiety and vomiting. Clinically there is a palpable mass in epigastrum. Diagnostic accuracy of ultrasonography is over 90 per cent. There are three nonsurgical alternative methods of drainage:

a) percutaneous external drainage

b) endoscopic internal (cystogastro-, cistoduodeno) drainage

c) internal cystogastric drainage with double "J" catheter (see Figure 1).

Cystogastric drainage with double pigtail catheter under ultrasonographic and gastroscopic control in selective cases is less traumatic to the patient and more comfortable than external drainage. There is greater intraluminal pressure of pseudocyst that enables flow of its contents through catheter to stomach or duodenum. This method was published by Hancke in 1985.[1] Nowadays interventional radiology developed many procedures alternative to classical, patient less friendly surgical procedures. Cooperation between different medical disciplines facilitates less aggressive procedures.

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Case report

A 58-year-old man (P.J.) was operated earlier due to gallbladder stones.

In August 9th 1994 he was operated due to acute pancreatitis in another hospital. Postoperatively there was pancreatic fistula, and secretion spontaneously stopped. Control US showed collection of fluid 6.1 x 5.5cm area in October 10th 1994. In January 11th 1995 control US due to abdominal pains confirmed 14 x 9cm great pseudocyst. Percutaneous US guided punction was not successful, and the patient was operated on May 12th 1995. The wall of pseudocyst was too thin to perform pseudocystogastroanastomosis. It was drained externally through mesocolon. Twelve days after operation the patient left the hospital. One month later

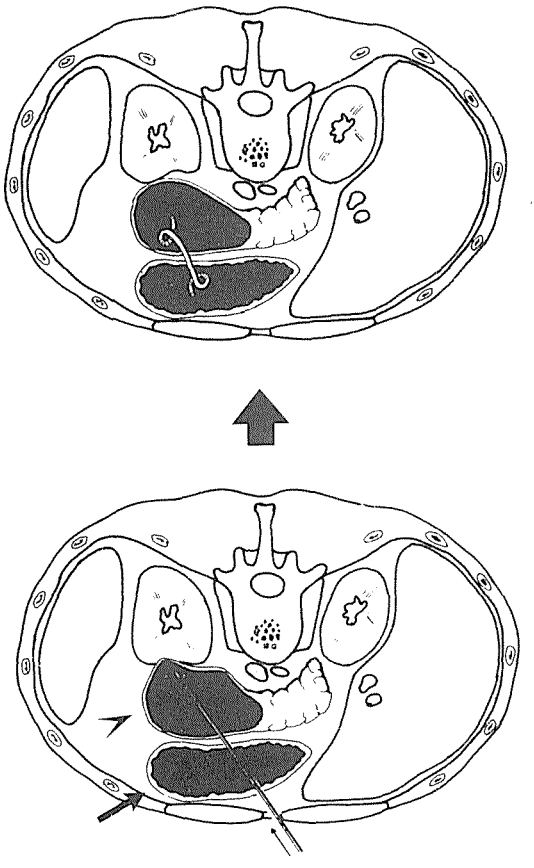

Figure 1. Schematic illustration of introducing percutaneous cystogastric double pigtail catheter.

the pains returned with palpable resistance in epigastrium. Control US showed fluid collection in bursa omentalis (see Figure 2). Our radiologists decided to make percutaneously US guided internal cystogastric drainage with double pigtail catheter and endoscopic assistance.

The purpose and the manner of performing the procedure was explained to the patient in detail, so that the patient's consent was obtained and the patient was reassured. The procedure was performed in an intervention-radiology room. The patient was lying supine on an X-ray table. Prior to the procedure, the patient was re-examined using ultrasound in order to determine the location and direction of access. The patient was then given 5 ml of noraminophenazone and 5 mg of diazepam i.v.. A flexible gastroscope was introduced into the patient's stomach. The chosen area in the epigastric region

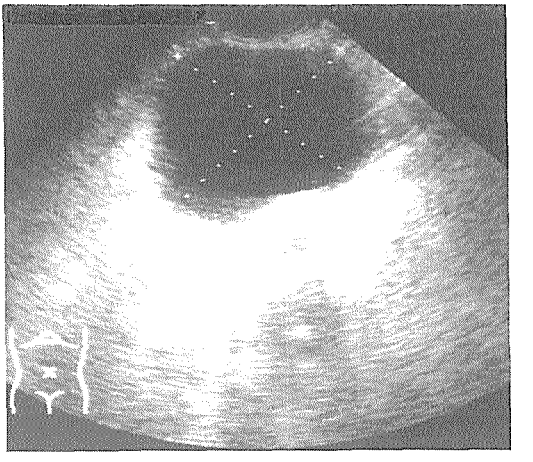

Figure 2. Ultrasonogram (US) of fluid collection in bursa omentalis.

was washed sterile and lined with sterile surgical sheets. The location and direction of puncturing were finally determined with a sterile-enclosed 3.5 MHz probe with an attachment for puncturing. The skin on the anterior abdominal wall was infiltrated with 10 ml of 2 % Xylocaine and a small incision was made. Puncturing was performed with a 20 cm long 18-gauge needle with mandrel. The penetration of the needle into the pseudocyst through the anterior and posterior stomach walls was observed on an ultrasound monitor and through a gastroscope. Gastric access is a condition for the connection of the pseudocyst with the stomach through a double-pigtail catheter in order to allow drainage. The success of puncturing was checked by aspiration of cystic contents and a short fluoroscopic control after the administration of the contrast medium, while its passage through the stomach was checked with a gastroscope. A J-type 0.035" tephlon guide wire was introduced through the needle cannula. The cannula was removed and dilation of the channel was performed with 7F and 8F plastic dilators. A double-pigtail catheter (8.5 F thick and 8 cm long) with side openings at both ends was introduced. It was placed at the tip of a 35 cm long needle with 16-gauge thick mandrel. On the needle behind the pigtail is a pusher with which the tip of the pigtail catheter was pushed from the cannula into the pseudocyst, while its base remained in the stomach. The needle cannula, the wire and the thread which serves for the regulation of the position (depth) of the drainage catheter, and the pusher were removed.

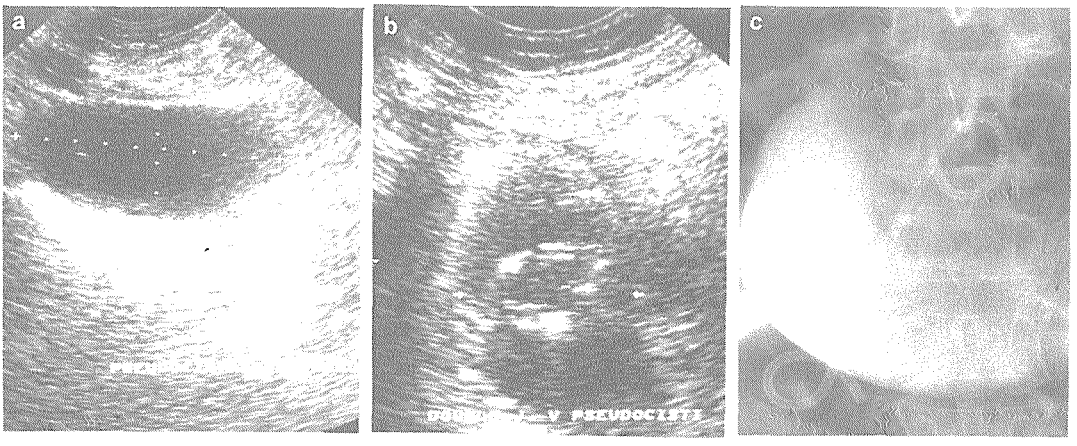

Figure 3. (a) US shows smaller pseudocyst diameter after percutaneous drainage and (b) final position of the drainage catheter seen on US, and (c) Xray.

The position of the pigtail catheter is monitored with an endoscope and adjusted, and the thread is cut if its spontaneous removal is impossible. Ultrasonography revealed rapid drainage of the pseudocyst (see Figure 3a). The position of the drainage catheter could be seen on an X-ray taken after the completion of the procedure (see Figure 3 b, c). One month after the procedure the pseudocyst was not visible on ultrasonography (see Figure 4 a, b), and the patient showed no clinical symptoms.

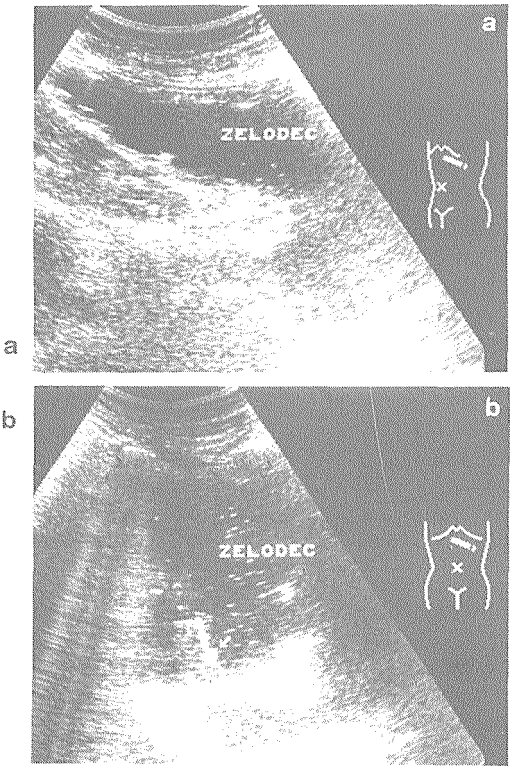

Figure 4a, b. The pseudocyst is not visible on US ane month after the procedure.

Discussion

In the past pancreatic pseudocyst was treated only operatively. The introduction of ultrasonography and various catheters enabled other less invasive methods of treatment. Percutaneous catheter external drainage is less comfortable to the patient than endoscopic drainage (cystogastro - or - cystodudeno -) or internal cystogastric drainage with double "J" catheter. Percutaneous double pigtail catheter internal drainage of pancreatic pseudocyst to stomach with ultrasonographic and gastroscopic guidance described first by Hancke[1] is less traumatic to the patient, than operative procedure by laparotomy.[2] There must be proper selection of patient: the cyst must be mature (6-8 weeks old to get thick wall) and in close contact with duodenum or stomach. Too small residual of stomach following surgery and bleeding to pseudocyst or infection of its contents does not permit double "J" catheter drainage. The diameter of pseudocyst must be at least 5 cm. This selection is possible by US examination. High concentration of amilase and lipase of pseudocyst content prevents occlusion of catheter lumen.

This procedure is reported to be tolerated by patient better than external drainage.[3] The condition of success is good cooperation between interventional radiologist and endoscopist.[4] There are reports of worth results in infected pseudocyst and

immature pseudocyst.[5,6] After the procedure we control the patient every month (blood amilase, clinical status, US). The drain is usually removed by gastroscope after 1-6 months.

The results are good if there is a proper selection of patients. There are reports of reacutisation of inflammation, due to alcohol drinking that demanded earlier extraction of catheter, which stimulated inflammation as a "foreign body".

Internal drainage with double pigtail "J" catheter is minimal invasive method which can be made in local anaesthesia, especially in patients with prohibitive operative risk.

Surgical treatment should be performed when endoscopical and percutaneous procedures are impossible or if malignancy is suspected.

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Insulin dependency of F-18-fluorodeoxyglucose accumulation in breast carcinoma cells compared to Tl-201 uptake

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The effect of euglycemic hyperinsulinism on ^{18}F-fluorodeoxyglucose (FDG) uptake in cultures of breast cancer was determined, in comparison to ^{201}Tl. Measurements of both tracers were performed on 168 cell culture tubes, with incubation intervals ranging from 1 to 240 min. Linear accumulation of FDG over time was observed both with and without insulin. A significant increase from 3.52 ± 0.74 % to 5.10 ± 0.32 % over 240 min was attained after adding insulin. In contrast, ^{201}Tl revealed only a slightly significant increase after insulin. By extrapolating these results to FDG PET tumor imaging, a markedly improved tumor targeting might be obtained by providing a state of euglycemic hyperinsulinism, i.e. replacing the commonly used single FDG injection by a continuous FDG/glucose/insulin infusion. An optimum imaging period of 150 min after starting the infusion can be derived from our data, considering the decay of ^{18}F.

Key words: breast neoplasms; tumor cells, cultured; euglycemic hyperinsulinism; deoxyglucose, ^{18}F-fluorodeoxyglucose; thallium radiosiotopes, ^{201}Tl

Introduction

Increased glycolysis is an important characteristic of cancer cells.[1, 2] Positron emission tomography (PET) with [^{18}F]-2-fluoro-2-deoxy-D-glucose (FDG) is used as a suitable indicator of the glycolytic activity of tumors. FDG is rapidly transported into the tumor cells and phosphorylated by hexokinase to FDG-6-phosphate, but is not further metabolized,[3, 4] meanwhile the physiologic substrate glucose enters the glycolytic pathway. Increased FDG uptake imaged by PET has been reported in many types of human tumors, e.g., head and neck cancer,[5] lung,[6] colon,[7, 8] liver[9, 10] and breast cancer.[11, 12]

^{201}Tl SPECT imaging might work as well as FDG PET in the detection of viable tumor tissue based on the relatively enhanced tumor blood supply. In neoplastic cell cultures the ^{201}Tl uptake increases in conjunction with the cell's metabolic activity, thereby confirming that it might also reflect tumor growth rather than just tumor perfusion.[13]

In the in-vivo and in-vitro studies so far available[5, 12, 14–19] controversial effects of plasma insulin and/or glucose levels on FDG tumor targeting have been reported. Agreement consists in most studies that elevated cold glucose levels seem to compete with FDG, thus hampering FDG accumulation.

The purpose of this study was to determine the effect of euglycemic hyperinsulinism on the cellular uptake of FDG in cultures of breast cancer cells, and to compare it with the uptake of ^{201}Tl under identical conditions, since insulin effects on ^{201}Tl tumor accumulation are also hardly known.

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Materials and methods

Radiopharmaceuticals

FDG (BTZ Beschleuniger- und Tomographiezentrum Hamburg, Germany) was obtained with a radiochemical purity higher than 98 %. Impurities like ethanol, acetonitrile and acetone were less than

0.06 mg/ml and the glucose concentration was less than 0.6 mg/ml.

A commercially available ^{201}Tl thallous chloride was used (Mallinckrodt, Hennef, Germany) as a reference tracer.

Cell cultivation

The human breast carcinoma cell line (MCF 7, dkfz – Tumorbank, Heidelberg, Germany) chosen for examination was maintained in a medium containing 5 mmol/l glucose, supplemented with 10 % fetal calf serum (Boehringer Mannheim, Mannheim, Germany), 0.5 % L-glutamine 20 mmol/l (Biochrom, Berlin, Germany) and 1ml gentamycin 0.1 mg/ml (Merck, Darmstadt, Germany). The cell line grew well in vitro as a monolayer and had a doubling time of approximately 40 h. A Fuchs-Rosenthal counting chamber was used for cell counting and the trypan blue exclusion method for viability determination was conducted using an inverted Leitz microscope.

The total number of cells ranged from 7.9 to 9.2 million cells per tube with a median of 8.5 million. Cell viability was higher than 90 %.

Uptake measurements

30 MBq FDG and 3 MBq ^{201}Tl were added to 250 ml medium, thus maintaining a continuous supply during the whole incubation period. For each tube, 3 ml medium was used, and the exponentially growing cells were incubated with FDG and ^{201}Tl at 37 °C. Influx was stopped at different incubation intervals ranging from 1 minute to 4 hours, by removing the medium. Subsequently, cells were washed rapidly 3 times with 4 °C saline solution for a total of 15 seconds and harvested with trypsin-EDTA (Sigma Chemie, Deisenhofen, Germany).

The radioactivity was measured with a gamma well counter in a definite geometry. Total activity per culutre tube was attained by measuring the tube before removing the medium. The cellular uptake was determined after the washing phase. FDG measurements were performed first and samples of medium and cells were stored for two days to determine the ^{201}Tl uptake, after the complete decay of ^{18}F. All results were corrected for physical decay.

To assure adequate measurement statistics, six tubes per exposure period were used, totaling 84 culture tubes in all. The experiment was repeated exactly under the same conditions, but with an additional administration of insulin, in a quantity of 0.5 IU to the medium, i.e. 2 mIU/ml, providing a complete receptor saturation.

Uptake results are presented as the percentage of the activity accumulated within the cells, related to the activity added in each tube normalized to 1 million cells, and expressed as mean ± 1 s.d. For statistical comparison, Student's t-test for unpaired data was used.

Results

As shown in Figure 1, linear accumulation of FDG over time was observed in nearly all the exposures, up to a maximum of 240 min. With the longest exposure period of 240 min an uptake of 3.52 ± 0.74 % was measured under baseline conditions. A further significant increase of up to 5.10 ± 0.32 % was attained after adding insulin, with $p \leq 0.02$ already beginning after an incubation time of 20 min.

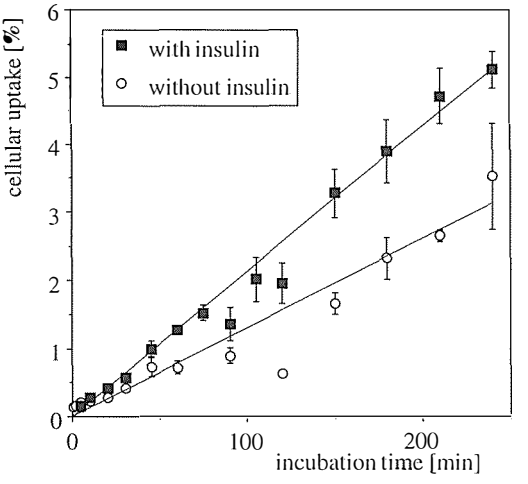

Figure 1. Cellular uptake of ^{18}FDG expressed as % of medium activity per 1 million breast carcinoma cells with and without insulin (0.5 mIU/ml medium) at various incubation time intervals. Results are given as mean of 6 culture tubes ± 1 SD. Additional insulin: filled squares; without insulin: open circles.

In contrast, ^{201}Tl accumulation occured at a high rate during the first 10 to 20 min and then reached a plateau. Adding insulin did not induce such marked effects, as illustrated in Figure 2.

By lumping all the tubes, with and without insulin, together, the groups differ with 0.33 ± 0.06 % and 0.25 ± 0.05 %, respectively. Significant differences with $p < 0.02$ might be obtained in the initial phase, such as at 10, 30, 45 or 90 min, while after a 150 min exposure time, both curves converge on each other.

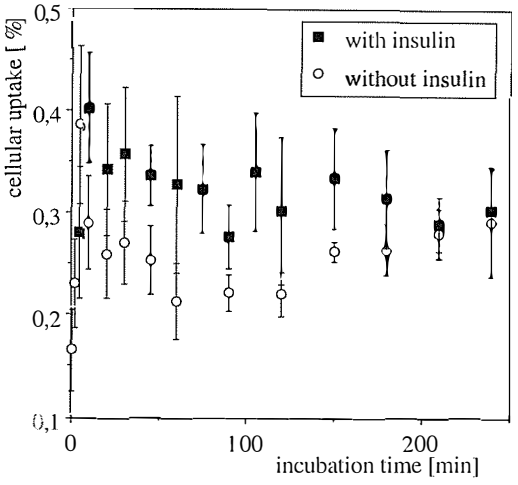

Figure 2. Cellular uptake of ^{201}Tl expressed as % of medium activity per 1 million breast carcinoma cells with and without insulin (0,5 mIU/ml medium) at various incubation time intervals. Results are given as mean of 6 culture tubes ± 1 SD. Additional insulin: filled squares; without insulin: open circles.

Discussion

Tumor targeting and quantitative evaluation of tumor tissue viability with FDG and PET have shown encouraging results, with high tumor/background ratios. Attempts have been made to further increase FDG accumulation in tumor tissue for even better scintigraphic tumor delineation, mainly by using additional glucose administration on induce a state of hyperglycemic hyperinsulinism.[14–17, 20] In agreement with in-vitro studies, examing the same issue, FDG accumulation mainly decreased with elevated plasma or media glucose concentrations[12, 15, 21] except in brain tumor imaging.[17] This was interpreted as a competing effect between cold and labeled glucose leading to the conclusion that FDG tumor imaging ought to be performed under conditions of food abstinence.[14, 15, 18]

The purpose and the results of this study have to be seen in that context. Because of the obviously hampering FDG accumulation at increased plasma glucose levels, the approach was different to all previously performed studies. The experimental design simulated a state of euglycemic hyperinsulinism in conjunction with a relatively constant supply of FDG, maintained for up to 240 min. Providing this environment to breast cancer cells a significant increase of FDG uptake in tumor tissue was observed as seen in Figure 1. This result seems to support the above cited hypothesis of competition between cold and labeled glucose.

The effect of the same study design on ^{201}Tl cell accumulation was considerably less, and completely concomitant to recent results.[19] Particularly during the initial exposure period of up to 150 min, however, insulin increased the ^{201}Tl uptake by 50 %. Provided this also holds true for in vivo scintigraphy, an increase of the tumor/background ratio by a factor of 1.5 would still represent a great progress in scintigraphic tumor targeting.

Based on this data it seems thus justified to propose a modified application protocol for both ^{201}Tl and FDG tumor imaging, suggesting euglycemic hyperinsulinism and maintaining constant tracer supply for a longer period of time. In the case of ^{201}Tl, an approximately 60 min infusion might be suggested, which causes no additional problem, since insulin clamping requires a continuous infusion anyway. In the case of FDG, the short tracer half life has to be considered. As seen in Figure 1 constant tracer supply results in linear accumulation, if corrected for physical decay. Considering ^{18}F decay an accumulation type of curve is obtained, as shown in Figure 3, with maximum tracer concentration and, thus, a postulated optimum for imaging, at approximately 150 min after starting the FDG/insulin infusion. The dose of insulin should be moderate, i.e. 20–30 IU/h, so as not to induce significant hypoglycemia and/or clinical symptoms, but to raise the plasma insulin level.

Clinical trials are needed to validate this postulated approach. The outcome of such trials is difficult to predict, since the effect of euglycemic hyper-

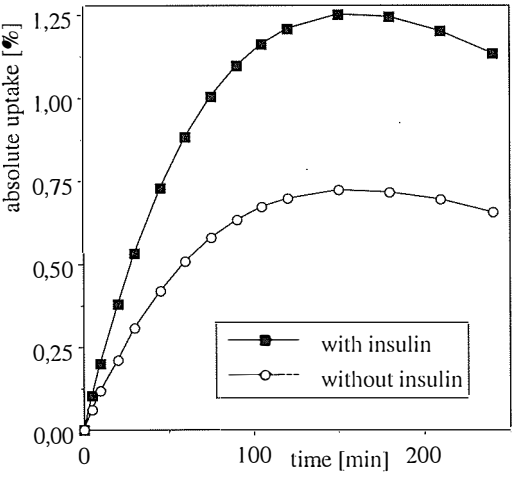

Figure 3. Cellular uptake of ^{18}FDG with (filled squares) and without (open circles) insulin considering physical decay of ^{18}F, as calculated from the regression curves of Figure 1.

insulinism on both the tumor and the surrounding tissue in vivo is uncertain because of the various distribution of the, at least five different, glucose transporting molecules, and the unknown magnitude as to whether these glucose transporters are involved in the facilitated transmembraneous glycolytic flux.[12, 14–18]

Conclusion

The accumulation of FDG and, to a lesser extent, that of ^{201}Tl, in breast carcinoma cells can be significantly increased by insulin and euglycemia. The potential improvement of PET and SPECT tumor targeting ought to justify further clinical testing.

Acknowledgements

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Positron emission tomography (PET) in ischemic heart disease

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The key substrates of any biochemical pathway may be labelled by positron emitting nuclides, without interfering with their biological behaviour. These nuclides desintegrate with two gamma rays in opposite directions. Different kinds of PET cameras, their advantages and disadvantages are discussed in terms of geometric resolution, nuclides useable, costs, and logistic problems. Latest camera technology deals with SPECT camera capable of coincidence detection, thus allowing to perform PET images without large expenses for dedicated PET systems. This could turn FDG imaging towards just another simple nuclear medicine procedure. The main clinical benefit of this method lies in the proof of tissue viability in akinetic, hybernating myocardium prior to therapeutic interventions. Thus, for patient management PET will help to select the appropriate therapeutical procedure and thereby will increase the benefit-risk ratio for the patients.

Key words: myocardial ischemia; tomography, emission-computed, positron emission tomography, PET; myocardial metabolism

Introduction

Imaging procedures in nuclear medicine tend to be non-invasive, simple to perform once the equipment is available, and they produce a macroscopic display of the organ under investigation with a somewhat limited geometric resolution. The key message of nuclear medicine is visualizing both (patho-) physiology and metabolism.

In order to image pathophysiology and to charactarize the tissue under investigation small amounts of radioactive substances are incorporated into the patients and their distribution in the body is detected and analyzed over time. Single-photon emitters like technetium-99m, thallium-201, iodine-131, iodine-123, and indium-111 are the most often used radionuclides for labelling procedures. Once these nuclides are bound to a carrier the physicochemical properties of these carriers are altered, and therefore their metabolism is somewhat unphysiological. Thus, the challenge for the radiochemist with single-photon emitting nuclides is to produce radiopharmaceuticals, which despite of their unphysiological nature will detect clinically useful signals. This is the main limiting factor in the development of new tracers for conventional gamma camera techniques in nuclear medicine.

In contrast, with positron emitting nuclides completely physiological tracers may be developped, as shown in this paper.

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Positron emitters

The main advantage of these positrons radiated from the nucleus is their fate in tissue. Within a very short distance of about 1 mm the positrons collide with an electron and both corpuscles annihilate, vanishing completely. Their energy is transformed to electromagnetic radiation in a characteristic pattern. Two photons of exactly 511 keV each radiate from the site of collision in almost opposite directions. (To be more precise, the angle between the two photons is about 179 degrees. This fact together with the average length of radiation of the positrons

define the lowest possible limit of geometric resolution for physical reasons to about 2 mm). This allows for comparatively high resolution metabolic imaging with positron emission tomography (PET). Full width at half maximum is 5 mm for PET studies. In comparison, realistic data on full width at half maximum in SPECT studies is some 15 mm.

The most widely used positron emitting nuclides in PET-centers are given in Table 1. Obviously, positron emitting nuclides from nitrogen, oxygen and carbon are ideally suited for the design of radiopharmaceuticals with completely physiological behaviour ("make as small a change in the molecule to be traced as possible"). This opens tremendous possibilities for non-invasive, in-vivo autoradiographic analysis. In a specialized radiochemical laboratory any organic substrate of interest might be labelled, e.g. metabolites including analog substances, receptor ligands and drugs will react chemically and biologically in exactly the same way as their non-radioactive counterparts, due to their identical physico-chemical properties.

Table 1. Half-life of positron emitting nuclides commonly used.

Nuclide	half-life [min]
Rb-82	1.26 min
O-15	2.07 min
N-13	9.96 min
C-11	20.40 min
F-18	109.70 min
Rb-81	274.80 min

A characteristic feature of positron emitting nuclides is their short half-life in the range of minutes as depicted in Table 1. Therefore, for full utilisation of the PET technology an onsite cyclotron for the generation of short-lived nuclides and a radiochemical laboratory are required. The radiochemistry needed may be characterized by extremely fast synthesis and labelling techniques, essential for these (ultra-) short-lived tracers. Because of these expensive installations, costs have been reduced tentatively by supplying several tomographs by one cyclotron only. However, the main benefit of this shipment of short-lived nuclides might be for the inital phase of a newly installed PET-center.

On the other hand, the short half-life of these positron emitters puts a very small radiation burden on the patient, and investigations may be repeated in a short time before and following medication or therapeutic interventions. This fact is of special interest in diagnostic and therapeutic procedures in cardiology. By applying different tracers myocardial perfusion and blood pool, fatty acid metabolism, glucose utilisation (a marker of ischemia plus myocardial vitality) and the receptor status may be visualized successfully as shown in Table 2. An ideal tracer should clear fast from the background, should have a high myocardial uptake of sufficient duration for imaging, and shoult not influence metabolic pathways.

Competing PET technologies

The common axis of the two photons of 511 keV may be seen by scintillation detector blocks with electronics, which is able to detect the corresponding pair of counts by their coincidence. These emitted projection data is then backprojected in a similar way as is done in X-ray computed tomography. Moreover, both machines look quite similar.

In modern PET systems a series of detector rings acquire three-dimensional data with high sensitivity, i.e. within a given angle any part of any ring may interact with any other part of any other ring for the coincident detection of the paired gamma rays. This technique will acquire simultaneously all data to cover an organ like the heart. Transmission

Table 2. Positron emitting nuclides in cardiology.

Circulation	N-13 NH_3 (ammonia)	blood flow
	Rb-82	blood flow
	O-15 H_2O	blood flow
	O-15 and C-11 CO	blood pool
Metabolism	C-11 palmitate	lipid acid metabolism
	F-18 deoxyglucose	glucose uptake
	C-11 acetate	Kreb's cycle / oxygen consumption
	C-11 amino acids	protein synthesis
	N-13 amino acids	protein synthesis
Neuronal receptors	C-11 quinucyclidine	ß-receptor ligand
	F-18 metaraminol	adrenergic innervation
	C-11 hydroxyephedrine	adrenergic innervation
Varia	F-18 misonidazol	detection of hypoxia
	Rb-81	potassium pool

Table 3. Comparison of tomographic systems in nuclear cardiology.

	PET	SPECT + coincidence	SPECT
Tracer	physiological	physiological	non-physiological
Nuclides	short-lived	short-lived	long-lived
Nuclide distribution	restricted	restricted	widespread
Repeated studies	within hours	within hours	next day
Acquisition time (heart)	10 min	40 min	20 min
Whole body capability	yes	no	limited
Resolution (FWHM)	5 mm	5 mm	15 mm
Quantitation	precise	poor	poor
Availability	restricted	potentially widespread	widespread
Installation	10 Mill US$	1 Mill US$	1 Mill US$
Costs per investigation	1200 US$	600 US$	300 US$

data are acquired for physically exact absorption correction. This allows to display the tracer distribution in Becquerel per volume and, therefore, serves as a basis for the calculation of quantitative physiological parameters.

In contrast, this is quite different in SPECT measurements. With single photons the count distribution correlates poorly with the activity distribution, and proves by experience only to be clinically useful as given in Table 3. However, the latest generation of multi-headed SPECT systems makes it possible to acquire transmission data as well. This SPECT transmission system allows for sufficient absorption correction while scatter remains a major problem. Superb images have been shown, but the clinical value of absorption correction in SPECT still remains to be evaluated.

Positron emission tomography is now around for about 15 years. However, the limited number of PET centers currently installed will not allow routine patient management on a broad basis. The high costs of the systems are due to rather sophisticated hardware required, especially when combining an on-site cyclotron and a radiochemistry with the PET scanner. The initial investment of a complete PET center will require 6–8 Mill US$ and the reimbursement for one investigation will approximate 1200 US$. These financial considerations will limit the technology to clearly defined clinical problems and especially to cardiological and brain research. To overcome these limitations, a new generation of low cost PET scanners are introduced by industry, e.g. ART-PET, which may change the benefit-cost ratio towards PET in the near future.

The latest camera technology came up with a machine in a somewhat intermediate position between PET and SPECT.[1] A double head gamma camera designed for excellent SPECT studies was equipped with high countrate capability and coincidence detection. Thus, PET and SPECT are achievable in a single gamma camera. After a potentially widespread installation this may allow tomographic examinations with physiological PET-tracers, i.e. fluordeoxyglucose (FDG), with the intrinsic good geometric resolution but without huge expenses necessary for dedicated PET centers. Since neither transmission measurements and consecutive quantification nor whole body imaging are feasable so far this system may become a worthwhile alternative in imaging of small organs as the heart and the brain.

In these organs, SPECT cameras equipped with high energy 511 keV collimators have been used. However, these images lack quality due to the rather limited geometric resolution of this system, with a possible role in cardiac studies only.

Ischemic heart disease

Up to now only in a few PET centers worldwide basic research on myocardial ischemia in animal models has been performed. Subsequent clinical work has concentrated on myocardial ischemia and cardiomyopathies.[2, 3] Conclusive results using PET in ischemic heart disease are available only for the last two years with about 20–30 original papers based on studies with less than 200–300 patients in total, mostly performed in the US.

Normal myocardium utilizes fatty acids for its energy requirements during rest. At stress lactate acid from the skeletal muscle is taken additionally. During fasting state there is definitely no uptake of glucose in the myocardium. In contrast, the postprandial endogenous insulin load will result in glucose uptake of the myocardium as well. This pattern changes quite dramatically during ischemia. Even in the fasting state myocardial cells will switch towards anerobic energy production using glucose. This glucose uptake signals ischemic, but still viable myocardium. On the other hand, in scar tissue

there is very little uptake of any tracer because of its bradythophic metabolism.

In patients with ischemic heart disease PET may image non-invasively blood flow and metabolic parameters.[4] During hypoxia fatty acid metabolism is stopped and subsequently switched over to aerobic and anaerobic glycolysis, as desribed above. Using F-18 labelled FDG, increased glucose utilization may be detected in regional myocardial ischemia. This metabolic imaging may be combined with blood flow studies. Rb-82[5] and N-13-ammonia are commonly used blood flow tracers. With a double tracer technique of FDG and N-13-ammonia it seems possible to differentiate normal, scarred, and ischemic myocardium. In the latter there is decreased uptake of blood flow tracer while glucose utilization is enhanced, thus a "mismatch" between the two tracer patterns occurs. Infarcted myocardium may be identified by FDG (and C-11-palmitate) as a region of abolished metabolism.

Myocardial vitality

Unexpected and partially spectacular results concerning demonstration of remaining vitality in akinetic myocardial regions, where no Tl-201 uptake could be shown in SPECT studies, have been reported. In one study, up to 58 % of persisting Tl-201 stress and rest perfusion defects interpreted as scar tissue showed metabolic residual activity with FDG in PET studies.[6] According to these results PET seems to allow prognostic statements concerning the prediction of contraction function of akinetic but still vital myocardial tissue after revascularization. This prediction was true in one study for 85 % of the patiens, whereas regions identified as scar tissue by N-13-ammonia and FDG-PET showed functional improvement in 8 percent only.[7] Therefore, the specificity for scar detection is high for PET, quite in contrast to SPECT studies performed with Tl-201.

However, two principal drawbacks underlying FDG-PET should be mentioned. There is no way to differentiate aerobic from anaerobic glycolysis, i.e. postprandially even normal myocardium shows FDG uptake. This has led to a variety of different acquisition protocols with no commonly accepted procedure so far. Furthermore, following myocardial infarction a solid block of scar tissue may be missing. Histologically, a mixture of scar fibres and still viable myocardial cells is demonstrated in these patients. Although these cells will show an increased FDG uptake, they remain immobilized by surrounding scar tissue. Therefore, following revascularization cardiac contraction will not be enhanced. Beside this clearly defined value for patients with ischemic heart disease in cardiological diagnostics, PET has a unique importance for clinical research due to the nearly unlimited possibilities of non-invasive in vivo investigations.[8]

Ventricular tachycardia following myocardial infarction

One example will be given for current PET research in patients with ischemic heart disease. Following myocardial infarction some patients develop high risk ventricular tachycardias. The site of the arrhythmogenic substrate may be delineated by PET in two different ways. First, at the border of a myocardial scar ischemic myocardium is sometimes found. These areas are characterized by a perfusion – metabolism mismatch, i.e. reduced perfusion and enhanced glucose uptake. In exactly these areas, localized by PET, the electric focus during episodes of venticular tachycardia could be confirmed by elektrophysiologic studies.[9] Second, in a more specific approach the reuptake of adrenergic substances in nerve fibres of the myocardium may be documentated by PET.[10] Disturbances of this reuptake of adrenergic substances may signal membrane instabilities and, thus, a tendency towards arhythmia. In carefully controlled clinical studies it may be possible to link these findings of scintigraphically proven cardiac neuropathy with ventricular tachycardias and with the problem of sudden cardiac death.

Conclusions

The main clinical benefit of PET in cardiology is to facilitate the prognosis of the success of any revascularization. By using a glucose derivate the viability of hybernating myocardium and thereby the curability may be proven. Otherwise, the impact of PET technology is concentrated mainly on basic research.

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Papillary thyroid cancer in two sisters

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Case reports on two sisters with histologically confirmed papillary thyroid cancer are presented. Gastroenterological examinations were normal in both patients, Gardner's syndrome excluded. It was concluded that family history, clinical presentation free of underlying cancer syndrome, and histopathologic examination are not adequate for reliable differentiation between occasional and familial forms, unless genetic markers are available.

Key words: thyroid neoplasms; Gardner's syndrome, thyroiditis, autoimmune

Introduction

In contrast to medullary thyroid carcinoma (MTC) which, in part, shows familial occurrence as a part of multiple endocrine neoplasia syndrome type 2 (MEN 2), the nonmedullary forms of thyroid cancer are not generally thought to present familial occurrence. However, evidence from literature[1-6] on thyroid cancer associated with familial adenomatous gastrointestinal polyposis (Gardner's syndrome) suggests possible inheritance. In a majority of familial cases, papillary cancer has been described, almost exclusively in young females, as being twice more often multicentric in origin, affecting both lobes of the thyroid gland, diagnosed before the age of 30, and with excellent outcome.[2-6]

In this case report, we present two sisters with papillary thyroid cancer. The aim of evaluation was to determine whether these cases could be considered a familial disease.

Case report 1

In 1982, P.A., a 42-year-old woman, presented at our Department for suspect hyperthyroidism. The patient's mother had died from leukemia. There were no other severe diseases or patients with gastrointestinal disease in the family.

The patient complained of palpitations, nervousness, inappetence, nausea, diarrhea and weight loss. She was not aware of thyroid gland enlargement. On physical examination, a solitary 1.5 x 1.5 cm palpable nodule was found in the left thyroid lobe. The right thyroid lobe was enlarged but free of any palpable nodules. Scintigraphy using Tc-99m and I-131 showed the palpable nodule to be a "cold" one. Ultrasonography of the thyroid was not performed. Serum thyroid hormone values were normal, thyroglobulin and thyroid microsomal autoantibody levels were negative. Fine-needle aspiration biopsy (FNAB) of the nodule was cytologically diagnosed as papillary carcinoma. The cytologic finding indicated surgical ablation of the left thyroid lobe. Histologic examination of the surgical specimen showed macroscopically a peripheral gray solid tissue, 1.8 cm in diameter, with an encapsulated nodule of 1.6 cm in diameter. Microscopically, tumorous tissue was found to be composed of papillae with thin fibrovascular core, covered with one layer of tumor cells with ground-glass nuclei. The nemerous follicles were covered with large cells with cytoplasmic pseudoinclusions. After one month, surgical ablation of the right lobe was performed. Hystology showed no malignant elements. There was no infiltration of the lymph nodes either.

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After total thyroidectomy, an ablative dose of iodine-131 of 1.85 Gbq (50 mCi) was administered. In 1984, total body imaging showed enhanced accumulation of iodine-131, located in the projection of the right thyroid lobe, which was considered a local recurrence. An ablative dose of 4.44 GBq (120 mCi) of iodine-131 was administered.

In 1987, her sister (Case 2) was examined at our Department. She had enlarged thyroid gland and a cytologic finding suspect of medullary thyroid carcinoma. In view of the possibility of familial medullary thyroid cancer also considered in the former patient, histologic findings of both surgical specimens were demanded again. However, papillary carcinoma was verified on reexamination. Serum calcitonin levels measured before and after stimulation with ethanol were normal.

Other close family members were also examined. In a third sister, a discretely enlarged thyroid gland, normal concentrations of triiodothyronine and thyroxine, and positive titres of microsomal autoantibodies were found. Iodine-131 scintigram showed a homogenous activity distribution, while FNAB pointed to chronic lymphocytic thyroiditis. Gastroenterological examination was also performed for possible association with Gardner's syndrome. Gastroscopic and colonoscopic findings were normal in all the three sisters.

Case report 2

Š.A., a sister 7 years younger than the latter patient, presented at our Department at the end of 1984, with enlarged thyroid gland. During the preceding four years, she had elevated blood pressure reaching 220/130 mmHg, irregularly treated with antihypertensives, accompanied by hot flushes. She often suffered from nausea, vomiting and diarrhea, regardless of the food taken. On physical examination, an enlarged left lobe and isthmus were found, with no markedly palpable nodules. Scintigraphy with Tc-99m and I-131 showed an inhomogeneous activity distribution in the silhouette of the enlarged gland. Ultrasonography of the thyroid was not performed. The FNAB cytologic finding was suspect of medullary carcinoma. Total thyroid hormone levels were normal, and thyroglobulin and thyroid microsomal autoantibody levels were increased. The basal serum calcitonin level was normal, whereas after stimulation with ethanol and pentagastrin it was twice above the baseline. Surgery was advised, but she refused operation. She did not present at the Department until 1986. At that time, an enlarged thyroid was found, with two separate palpable nodules in the left lobe and isthmus, sized 1.5 x 2.0 cm each, but not "cold" on scintigraphy,Repeated FNAB was suspect of medullary carcinoma again. Repeated measurements of serum calcitonin level before and after stimulation with pentagastrin and ethanol were normal. As medullary thyroid cancer was cytologically suspected, and considering the possibility of MEN 2 syndrome, further evaluation was undertaken. The urinary excretion rate of vanillylmandelic acid value measured in a 24-hour collection was normal. Computed tomography scanning of the abdomen was normal. Total thyroidectomy was performed in 1987. The histologic section showed two nodules, 1.5 x 2.0 x 1.0 cm and 3.0 x 3.0 x 3.0 cm in dimension. Histologically, the both were tumorous tissue of papillary structure, the papillae were covered with one layer of cylindrical epithelial cells with light and bullous nuclei. In the right lobe, numerous follicles of different size were found, covered with one layer of epithelial cells filled with dense eosinophilic colloid. A lymphocytic infiltration with numerous germinative centers was observed, and follicles in these areas were destroyed. After the surgery, an ablative dose of 3.7 GBq (100 mCi) of iodine-131 was administered.

Both sisters presented for clinical examination every 6 months, with annual chest x-rays and whole body iodine-131 scans. Until 1995, control examinations did not show any sign of propagation of the disease in either of them. Thyroglobulin levels measured annually were normal. Hypothyroidism was oversubstituted with 200 μg of L-thyroxin daily in order to completely suppress TSH secretion.

Discussion

The diagnosis of papillary thyroid cancer was undoubtedly confirmed histologically after thyroidectomy in both sisters. Due to familial occurrence of thyroid cancers, the patients were examined for hereditary MTC, which is the best known form of familial thyroid cancer. When MTC was excluded, the familial non-medullary form of the disease was established. Review of the literature suggested that there may be two groups of familial non-medullary thyroid cancer: one in which association of non-medullary thyroid cancer with inherited cancer syndrome (Gardner's syndrome and Cowden's disease)[2–7] could be documented, and another one in which familial papillary cancer is independent of the un-

derlying syndromes.[8-14] The incidence of papillary carcinoma in Gardner's syndrome is estimated to be 160-fold that in normal population.[6] The association of papillary carcinoma and possible hereditary cancer syndrome was not confirmed in our patients. As DNA analysis was not performed in our patients, it was difficult to conclude that our cases had a hereditary basis independent of the association with the underlying syndrome.

At the time of making the diagnosis, both sisters were middle-aged, while in other studies of familial occurrence of papillary carcinoma the patients almost exclusively were younger females. The malignant disease did not affect the contralateral thyroid lobes in our patients, although other authors found dissemination into both lobi of the gland in cases of familial papillary carcinoma.[2-5, 7] There were no distant metastases or increased thyroglobulin levels during the postoperative follow-up, suggesting a good outcome, which is consistent with other reports.[2, 3, 5, 7] In one sister, chronic lymphocytic thyroiditis was found. It was also diagnosed in the third sister. Lote and al. examined the thyroid surrounding tissue in their patients with familial papillary thyroid cancer, looking for possible focal thyroiditis with no confirming results.[12] On the other hand, others report on a significantly increased incidence of primary hypothiroidism in families with a familial form of papillary thyroid cancer, as a consequence of chronic lymphocyte thyroiditis.[9, 15, 16]

We accept suggestions of most authors that examinations should also include other close relatives when two or more members of the family have papillary carcinoma.[3, 4, 9] Families with papillary thyroid cancer should also be gastroenterologically examined in order to find possible associated polyposis, and *vice versa*, familial gastrointestinal polyposis should be examined for possible associated papillary thyroid cancer.

We conclude that family history, clinical presentation without underlying syndrome, and histopathologic examination are not adequate for reliable differentiation between occasional and familial forms, unless genetic markers are available.

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Effect of the type of application of Newcastle disease virus on the Ehrlich ascites tumor

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Newcastle disease virus (NDV) has been shown to have an inhibitory effect on the tumours. Most authors use peritumoral application of virus. The purpose of our study was to compare the effects of the ip in contrast to sc application of the virus on the ip and sc transplanted Ehrlich ascites tumor (EAT) in CBA/H mouse. We measured the length of survival, the tumor cure rates, the metastatic rate, and the frequency of ascites and sc tumors in the site of ip EAT injection.

Prolongation of survival after the therapy with NDV in ip transplanted EAT was found. The average time of survival in control group was 70.5 days, and 107 and 79.9 days with ip and sc NDV virus therapy respectively. The differences were significant only between control group and the group treated with ip application of NDV. Tumor cure rates were: ipNDV group 30%, scNDV group 20 % and control group 5 %. NDV therapy in sc transplanted EAT prolonged the time of survival; in control group it was 63.3 days, and 75.2 and 65.9 days with ip and sc NDV therapy respectively.

NDV therapy inhibited metastatic rate of ip transplanted EAT. Inhibition was more effective with ip application of NDV. Virus therapy also lowered the frequency of appearance of ascites and sc tumour in the site of ip EAT injection. In sc transplanted EAT ip application of NDV inhibited the metastatic rate while in sc applied NDV some stimulation of metastasation was found.

Ip application of NDV was found to be superior in contrast to sc application in all its therapeutic effects against EAT. Our results show that the tumor inhibition of NDV, in the system we used, has the characteristics of the biological response modifiers.

Key words: Carcinoma, Ehrlich tumor; Newcastle disease virus; survival rate; mice

Introduction

Newcastle disease virus (NDV) is a paramyxovirus pathogenic to birds and only slightly to men.[1, 2] As an inhibitor of tumor growth it has commanded continuous attention ever since the '50s until recently. The tumour inhibition was found in *in vitro* systems, on experimental animals, and in men after incidental infection,[3] or in therapeutic trial.[4, 5] Reichard et al.[6] have found the selective effect on tumor in contrast to normal cells of live NDV *in vitro*.

In *in vivo* experiments the authors use various modes of virus application but no study compares the effect of different modes. In our experiment we tried to find out if there is a difference in the effect between sc and ip virus application on sc and ip transplanted EAT of mouse. In the case that the differences in the effect are found it would to some degree explain the mechanism of NDV's tumor inhibition.

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Abbreviations: BRM-biological response modifier(s), EAT Ehrlich ascites tumor, ipEAT-intraperitonealy transplanted Ehrlich ascites tumor, scEAT-subcutaneously transplanted Ehrlich ascites tumor, NDV-Newcastle disease virus, ipNDV-intraperitonealy applied Newcastle disease virus, scNDV-subcutaneously applied Newcastle disease virus.

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Materials and methods

Experimental animals

We used 120 inbred mice, 8 to 10 weeks old, males of CBA/H strain, which were obtained from the Institute Ruđer Bošković, Zagreb. The animals were

provided pelleted Knapka food and tap water *ad libitum*. The light regime was natural.

Experimental tumor

We used EAT, composed of predominantly hyperdiploid cells, in ascitic form. EAT was transplanted ip to a donor animal 14 days before. Tumor cells were counted in a hemocytometer with Trypan blue exclusion test.

The same number of tumor cells was implanted in both experimental and control groups. For ip transplantation we used 7.9 x 10^3 cells in 0,5 ml of sterile 0.9% NaCl while for sc transplantation we used 18.9 x 10^4 cells in 0.3 ml of sterile 0.9% NaCl per animal. Tumor cells were inoculated into the right inguinal region.

Virus

Wild type NDV strain was used. Virus was obtained and titrated by the Viral laboratory, Faculty of Veterinary Medicine, Ljubljana. It was cultured in chorioallantoic fluid of 10-day old embryonated SPF chicken eggs, EID_{50} was $10^{7.5}$. It was stored at -70°C until application. Before application it was diluted in Hanks' solution in the ratio 1:15. For ip and for sc application 0.2 ml of viral solution was used. Sc application in scEAT groups of animals was in the peritumoral region.

Experimental course

We used 120 mice which were divided in 6 groups, each consisting of 20 animals. EAT was transplanted ip to group 1, 2 and 3 and sc to group 4, 5, and 6. The first and the fourth group were control, and the other 4 were experimental groups, which received NDV either ip or sc (Table 1).

The therapy of the groups with ipEAT started on the 7th day after transplantation, and of scEAT groups when the tumor reached the average diameter of 8 mm. The therapy was applied twice a week, during the total length of the experiment. Mice died spontaneously until 149th day when we finished the experiment. The animals which survived were sacrificed by the method of cervical dislocation.

Morphological Techniques

All the animals were autopsied to check the presence, site, and location of the tumor growth and presence of ascitic fluid. The organs, except the brain, were removed and fixed in 10 % buffered formalin for macroscopic evaluation of tumor growth in fixed tissues and for the histologic examination. The presence of tumor tissue was confirmed histologically in all animals in at least one specimen. In the cases where animals were sacrificed and the tumor was not found macroscopically, we examined histologically all the organs.

Statistical Methods

The result were statistically evaluated with computer statistical package SOLO (BMDP), and Log-rank test.

Results

The effect of the site of NDV application on survival

In ipEAT groups evident differences in survival of animals were found (Fig.1). In control group the average survival was 70.15 days, in ipNDV group 106,15 days and in scNDV group 79.9 days. The difference between control group and ipNDV group is statistically significant (p=0.008), but there is no such difference between control group and scNDV group (p=0.36).

The influence of application site is also reflected in the number of animals which survived the whole length of experiment (149 days) and no tumor was found at morphological analysis: there were 30% such animals in ipNDV group, 20 % in scNDV group and 5 % in the control group.

Table 1. Groups of CBA/H mice and experimental design of NDV treatment.
* 7.9 x 10^3 EAT cells for ip and 18.9 x 10^4 EAT cells for sc transplantation were used per animal. The original virus titer was EID $10^{7.5}$; 0,2 ml of NDV diluted 1:15 with Hanks' solution was applied per animal twice weekly.

Group No., (20 mice in each)	Mode of EAT transplantation	Solution applied	Mode of solution application
1 (control)	ip	0,9% NaCl	ip
2	ip	NDV	ip
3	ip	NDV	sc
4 (control)	sc	0,9% NaCl	sc
5	sc	NDV	sc
6	sc	NDV	ip

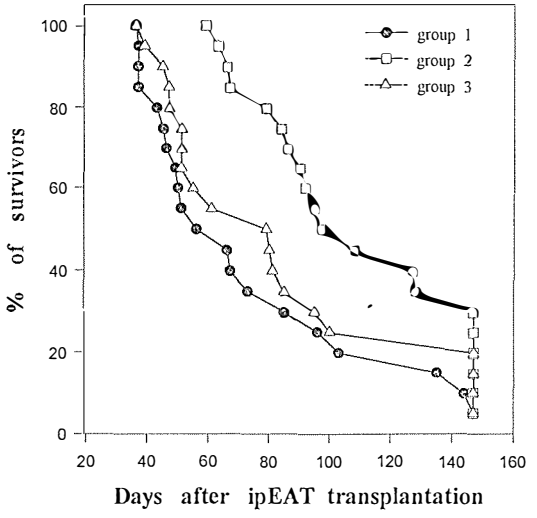

Figure 1. Survival of ipEAT groups (group 1-control, group 2-ipNDV, group 3-scNDV). The animals that survived 147th day were sacrificed and showed histologically no tumor growth. 7,9 x 10^3 EAT cells were transplanted per animal. The original virus titer was EID $10^{7.5}$; 0,2 ml of NDV diluted 1:15 with Hanks' solution was applied per animal twice weekly.

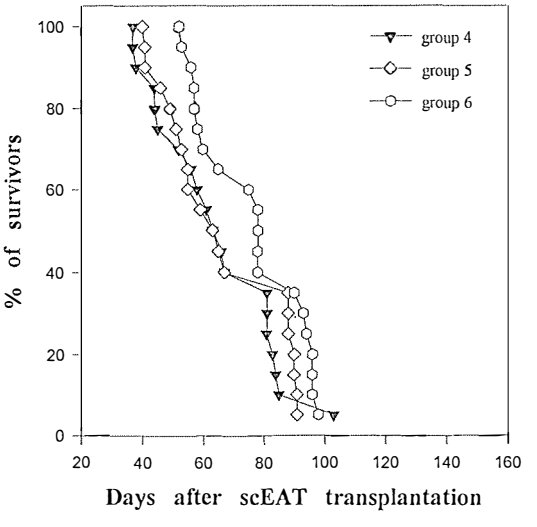

Figure 2. Survival of scEAT groups (group 4-control, group 5-ipNDV, group 6-scNDV). The animals died spontaneously. 18,9 x 10^4 EAT cells were transplanted per animal. The original virus titer was EID $10^{7.5}$; 0,2 ml of NDV diluted 1:15 with Hanks' solution was applied per animal twice weekly.

Among scEAT groups the differences in survival were smaller than in ipEAT groups (Fig. 2). The average time of survival in control group was 63.3 days, and in ipNDV group 75.2 days and in scNDV group 65.9 days. The differences are not significant. All the animals died spontaneously with tumour by 103rd day of the experiment.

The effect of the site of NDV application on the number of metastases

In ipEAT the greatest number of tumors was found in mesentery, pancreas and respiratory diaphragm, and smaller number in organs of small pelvic cavity, kidneys, suprarenal glands and liver. Metastases outside abdominal cavity were found in the lungs, and the lymph nodes (inguinal, axillary). The total number of tumors and metastases found by groups was: control 44, ipNDV 25 and scNDV 34. Ascitic fluid was found in 60 % of animals in the control group and in 25 % and 45 % of animals in ipNDV and scNDV group respectively. The sc tumor in the place of ip EAT injection was found in 70 % of animals in control group and in 45 % and 60 % of animals in ipNDV and scNDV group respectively.

In scEAT groups most of the metastases were in the abdominal organs, while in lungs and lymph nodes they were rare. The total number of metastases per groups are: control 22, ipNDV 16 and scNDV 32.

Discussion

The *in vivo* tumor therapeutic effect of live NDV has been found to depend on many factors of which the virus dose, the virus strain, the regime of application, and the tumor mass seem most known. The authors have also found that the tumor inhibitory effect was best if the NDV was injected into the tumor.[4]

The mechanism of tumor inhibition by NDV has been studied quite extensively. One of the first ideas was that virus incorporates into the membranes of tumor cells in the process of budding and in this way changes antigenicity of tumor cells.[7] On the other hand, there is no objective evidence, except in tumor-adapted NDV strain,[8] that NDV multiplies in tumor cells. The most argued findings are NDV's effects on the immune system generally through interferon induction,[9] TNF induction and the sensibilization of tumor cells to TNF.[10] Some authors have found a selective cytotoxic effect of NDV on tumor cells *in vitro*.[6]

Usually a few millions of cells are used in the experiments with ipEAT. We used only a few thousands of cells to prolong survival, to allow appearance of more metastases and to obtain a more sensi-

ble model for therapy testing. The regime of NDV application was that proposed for the biological response modifiers (BRM),[11] because viruses are also treated as BRMs.[12]

Our finding is that ip NDV application has stronger tumor inhibitory effects than sc application. Because virus is a diffusible particle, which is absorbed after application by mesothelial pores and endothelial capillary cells into blood system, and the blood supply area of peritoneum is much larger than subcutaneous area, we can expect that in ip application there is much higher concentration of virus in the blood. This is probably why the influence on the involved mechanisms of tumor inhibition is stronger.

The length of survival of experimental groups in ipEAT was longer than in scEAT. This is most probably a consequence of bigger tumor masses in the begining of the virus therapy in scEAT groups. Other authors have found the same effect of tumor mass, using NDV[4] or TNF therapy.[13] It is also the guideline for the use of BRMs that they should not be used for advanced neoplastic diseases.[14]

According to our results, NDV inhibits tumor metastatic rate which is also reflected in the reduced incidence of ascites appearance. Both could be the consequence of the reduced tumor mass on the peritoneal surfaces, as a consequence of NDV influence. It was found *in vitro* that NDV activates peritoneal macrophages which showed a strong cytostatic effect against tumor cells.[7, 15] It was also found that the reduction of ascites appearance is an effect of increased immunologic potency.[16] In the scEAT group we found increased metastatic rate after scNDV. This is probably the result of repeated peritumoral injections where we pricked the tumour cells and introduced them into the vessels.

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Cathepsins and their endogenous inhibitors in clinical oncology

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The invasion and metastasising of tumor cells is closely connected with the disintegration of basement membranes and extracellular matrix. The carriers of these processes are different proteolytic enzymes, among them also cathepsins – a group of ubiquitous lysosome proteinases. A correlation between the changed concentrations and/or activities of cathepsins in the tumor tissue and metastatic potential of tumors was demonstrated on different experimental models in vitro *and* in vivo. *The prognostic relevance of cathepsin D, particularly in breast cancer; and to a lesser extent also of cathepsin B, is nowadays widely studied in clinical oncology. The cells releasing cathepsins also produce their inhibitors. Stefins, cystatins and kininogens are endogenous inhibitors of cathepsin B. Their clinical relevance, either as therapeutic agents or prognostic factors, still remains unknown. For cathepsin D, an endogenous inhibitor has not been found yet.*

Key words: neoplasms; prognosis; cathepsins

Introduction

The behaviour of malignant tumors is typically determined by their ability to invade the surrounding tissues as well as by their potential to form metastases in different parts of the body, at a distance from the primary tumor. Both features are the result of a dynamic and complex process, known as metastatic cascade, i.e. the sequence of interrelated events including numerous interactions between the tumor and its host-organism.[1] In order to be able to form a new metastatic colony, an individual tumor cell or a group of these should successfully pass through each individual stage of the cascade; it should 1) leave the primary tumor, 2) invade the adjoining normal tissues, and 3) enter the blood circulation, which then can take it to the most distant parts of the organism. Once inside the target organ, tumor cell or a group of them must again pass through the vessel wall in order to enter into its new habitat and form a new, secondary colony.[2] In this transition, the cell crosses different tissue compartments which form a mammalian organism. These are separated from one another by two types of extracellular matrix, which pose a few natural tissue barriers to the invading cell, i.e. the basement membranes and the interstitial connective tissue. The basic constituents of these structures are different proteins – particularly collagen, adhesive glycoproteins and proteoglycans.[3]

Disintegration of the extracellular matrix and the ensuing transition of tumor cells through it occur as a result of the activity exerted by different types of proteolytic enzymes which are produced and released onto the surface of cytoplasmic membrane or into its surroundings by the invading tumor cells as well as by host-cells.[4] Proteinases, i.e. the enzymes with endopeptidase activity, which are associated with these disintegration processes, are grouped into four classes with respect to the chemical nature of the groups responsible for the catalytic activity. These are 1) serine proteinases, 2) cysteine or thiol proteinases, 3) aspartic proteinases and 4) metallo-proteinases. The same cells that make up these enzymes also produce their inhibitors (Table 1).[3]

In a normal, non-malignant tissue, the activity of individual enzymes and their endogenous inhibitors is organised in the proteolytic cascade involved in

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Table 1. Major classes of proteinases.

Class	EC number*	Examples	pH range for activity	Examples of protein inhibitor(s)
Serine	3.4.21.-	trypsin chymotrypsin plasmin plasminogen activator thrombin elastase cathepsin G	7-9	PAIs α2-antiplasmin α2-macroglobulin
Cysteine or thiol	3.4.22.-	cathepsin B cathepsin H cathepsin L	3-8	stefins cystatins kininogens
Aspartic	3.4.23.-	pepsin cathepsin D	2-7	pepstatin
Metallo-	3.4.24.-	collagenases gelatinases stromelysins	7-9 neutral neutral	TIMPs

*Based on the Nomenclature Committee of the International Union of Biochemistry (1992).
PAI - plasminogen activator inhibitor; TIMP - tissue inhibitor of metallo-proteinases.

numerous physiological processes such as trophoblastic implantation, embryo morphogenesis, angiogenesis, wound healing, pathologic bacterial and parasitic invasions, etc.[2] The cascade activation is a complex process involving numerous interactions between enzymatically inactive pro-enzymes, active proteinases of different classes, and their inhibitors (Figure 1). Contrary to that, in tumor tissue the regulation of this cascade is altered: it is either

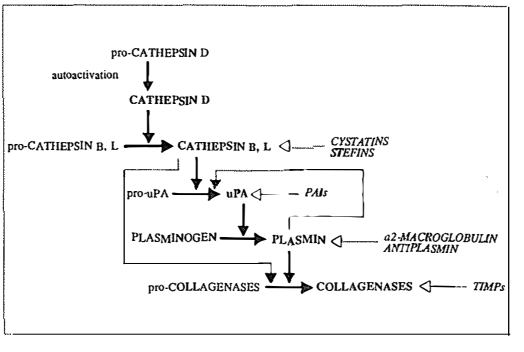

Figure 1. Activation of tumor associated proteinases: a complex pattern of events forming proteolytic cascade which involves enzymatically inactive pro-enzymes and active proteinases. Their action is counter-balanced by specific inhibitors (limited proteolysis).

uPA - urokinase-type plasminogen activator; PAI - plasminogen activator inhibitor; TIMP - tissue inhibitor of metallo-proteinase.

incomplete or wrong. This occurs as a result of the modulation of one or more mechanisms regulating the synthesis, transport and release of the involved enzymes and inhibitors, which further leads to changes in their cell distribution and/or concentrations or activities, ending in the establishment of new, bizarre interrelations between them.[5]

Cathepsins

Cathepsins are ubiquitous lysosome proteolytic enzymes. They were named after the Greek word *"Kathepsin"*, which means "to digest" by Willstätter and Baumann in 1929. Cathepsins are present in all cells of mammalian organisms. Their concentrations varies with respect to individual types of cells and tissues, being particularly high in macrophages and in organs such as the kidney, spleen and liver. As to their chemical composition, these substances are glycoproteins, which – but for few exceptions – all belong to the group of endopeptidases. They take part in numerous physiological processes, such as e.g. intracellular protein turnover and posttranslation processing of some biologically important protein precursors (e.g. insulin and endorphin), as well as in the etiology of several pathological conditions, such as muscular dystrophy, arthritis, emphysema, multiple sclerosis and cancer.[6]

The synthesis of cathepsin precursors occurs on the membrane-bound ribosomes, wherefrom they are transferred cotranslationally into the lumen of endoplasmic reticulum, and proceed into Golgi apparatus where glycosylation takes place. After protein and carbohydrate parts of molecules have been modified, they are transported into lysosomes by means of receptors which recognise mannose-6-phosphate residues present on the precursor of lysosomal enzymes.[6,7]

In cells, these enzymes are present prevailingly inside lysosomes. Beside proteinases, the lysosomes, in their acidic environment with pH ranging between 4.0 – 5.0, also contain a number of other hydrolytic enzymes such as nucleases, glycosidases, lipases, phospholipases, phosphatases and sulphatases. Their major function is to be involved in the controlled degradation of macromolecules, which may be of cellular or foreign origin.[8]

By now, there are 11 different cathepsins known, which differ from each other by their catalytic and molecular properties. They are assigned by letter designation from A to T. Due to the lack of firm evidence, the existence of cathepsins F, I, J, K, M, N, P and R is questionable.[9] The majority of cathepsins are active in acidic pH range whereas in neutral and alkaline pH values they are unstable. The molecular weight of active enzymes amounts to 14-650 kDa (Table 2).[6]

models, both *in vitro* and *in vivo*.[5] On the other hand, the clinical relevance of these enzymes is much less investigated. In terms of their value as prognostic factors in cancer patients, the most thoroughly studied are cathepsins D and B.

Cathepsin D

Cathepsin D is an aspartic endoproteinase with two asparagine groups positioned in its active site. In normal mammalian cells, it is initially synthesised as a precursor 52 kDa protein (pro-cathepsin D), which is transported prevailingly into lysosomes and processed through intermediate 48 kDa form to mature two-chain molecules, each with 34 kDa and 14 kDa respectively. There is only a negligible amount of pro-form accumulated or released from these cells. Inside lysosomes, cathepsin D is involved in the catabolic degradation of numerous intracellular and endocytotically imported proteins.[10] In the ran-

Table 2. Classification of lysosomal cathepsins.

Cathepsin*	EC number	IUB classification	M_r
A	3.4.16.1	Exopeptidase (serine type carboxypeptidase)	100-400.000
B	3.4.22.1	Endopeptidase (cysteine type)	25-29.000
B_2	3.4.18.1	Exopeptidase (cysteine type)	
C	3.4.14.1	Exopeptidase-dipeptidyl	200.000
D	3.4.23.5	Endopeptidase (aspartate type)	48.000
E	3.4.23.34	Endopeptidase (aspartate type)	100.000
F	3.4.99.-	Endopeptidase	50-70.000
G	3.4.21.20	Endopeptidase (serine type)	27-30.000
H	3.4.22.16	Endopeptidase (cysteine type)	26-28.000
L	3.4.22.15	Endopeptidase (cysteine type)	23-29.000
S	3.4.22.27	Endopeptidase (cysteine type)	14-30.000
T	3.4.22.24	Endopeptidase (cysteine type)	34.000

*Cathepsins F, I, J, K, M, N, P and R have not been cassified by the International Union of Biochemistry Committee on Nomenclature (1992). There is no substantional evidence that they exist.

The involvement of cathepsins in the proteolytic processes of extracellular matrix decomposition, as well as an association between changes in their concentrations, activities or distribution and the malignant potential of tumor cells have been confirmed by several studies carried out on different tumor

ge of pH 2.8 – 5.0, cathepsin D can effectively degrade denatured proteins while its activity against native molecules and synthetic low-molecular-weight substrates is limited. It has little or no enzymatic activity at a pH 7.0 or more, and its isoelectric point is between 5.5 – 6.5.[6] Unlike cysteine protei-

nases, cathepsin D is not affected by thiol compounds and thiol blocking reagents; it is effectively inhibited by pepstatin, a potent synthetic inhibitor of aspartic proteinases while endogenous protein inhibitor of cathepsin D in man has not been found yet.[10]

In malignant cells the processing and resulting compartmentization of cathepsin D is delayed and is different than in normal cells. This may be due to the decreased activities of processing proteinase(s) involved in cathepsin D maturation procedure and/or to differences in the structure of pro-cathepsin D: namely, 52 kDa pro-enzyme released from tumor cells contains more acidic isoforms than that from normal cells, despite its almost identical amino acid sequence, and has a more acidic isoelectric point.[11] This could explain cytoplasmatic accumulation of pro- and intermediate enzyme forms by tumor cells as well as markedly increased proportion of secreted pro-enzyme, reaching up to 50 %. On the other hand, the increased secretion could be simply due to the increased cathepsin D gene expression, which saturates the limited number of manosa-6-phosphate receptor sites available, resulting in disruption of pro-enzyme molecule transport into lysosomes.[11,12,13] Generally, breast cancer cells produce 2-30-fold more cathepsin D than normal mammalian cells growing with the same rate.[12]

In human genome, cathepsin D gene is located at the extremity of the short arm of chromosome 11, close to the H-ras oncogene.[14] Its expression in estrogen receptor positive breast cancer cell lines is regulated by estrogens and growth factors.[15] This regulation is tissue-specific: in normal human endometrium, in rat uterus and in the Ishikawa human endometrial cancer cell lines, all of which contain functional estrogen and progesterone receptors in the same way as breast cancer cells, estrogens are unable to stimulate cathepsin D expression.[13,16] In *in vitro* conditions, both pro-enzyme as well as its mature forms stimulate the growth of hormone dependent estrogen-deprived cells of breast carcinoma.[17,18,19] This autocrine mitogenic activity of cathepsin D, however, does not imitate completely the stimulatory effect of estrogen, suggesting that other autocrine growth factors are also required. It can be either due to the direct effects of cathepsin D as a peptide growth factor[18,20] or due to its enzymatic activity. By its proteolytic activity, cathepsin D could play a role in the release of growth factors from precursors or from extracellular matrix and/or activation of their intra- or extracellular receptors, or it could participate in supplying the cells with amino acids available for the formation of new protein molecules.[21] In a similar way, cathepsin D could be involved in the degradation of basement membrane and extracellular matrix components,[22] as well as in the processing and activation of cysteine proteinases, and thereby also in the initiation of proteolytic cascade.[23,24] Auto-activation of the secreted inactive 52 kDa pro-enzyme has only been demonstrated in *in vitro* conditions, at an acidic pH value.[11,12] Since *in vivo*, an acidic microenvironment is more frequently encountered within the cell (i.e. in endosomes and lysosomes) than out of them, it seems that the activation of the secreted pro-cathepsin D is associated with the internalisation of pro-enzyme molecules, together with its substrate, in the process of endo- or phagocytosis. This hypothesis is supported by the finding of large acidic vesicles, containing both mature cathepsin D molecules and endocytosed extracellular matrix, which were present in a much higher concentration in breast cancer cells than in normal breast tissue cells.[25]

With respect to its proteolytic and mitogenic properties, cathepsin D was widely studied as a marker with potential prognostic value. Since then, a number of clinical studies trying to establish a possible correlation between cathepsin D content in tumor tissue and patients survival have been published. The prevailing majority of these are concerned with breast cancer patients and have been carried out - or are under way – independently in several different countries (Table 3).[26-39] Despite the fact that assay types and methodology used for the determination of cathepsin D content in tumor tissue varied from one laboratory to another, the results obtained indicate that high enzyme concentrations are related to poor prognosis. The only exception in this respect is a study by Henry *et al.* which suggests a different, protective role of cathepsin D.[30] Furthermore, the majority of authors state that cathepsin D is a parameter, independent from other prognostic factors (tumor size, steroid receptor status, axillary lymph node involvement, pathohistological grade, S-phase). Cathepsin D was found to be more correlated with metastasising than with cell proliferation or local tumor invasion. Besides, there is also a cathepsin D assay, commercially available on the market, which is easy to perform and reproducible with a satisfactory degree of quality control. This is a solid-phase "sandwich" immunoradiometric assay (IRMA), developed at the University of Montpellier, France (ELSA-CATH-D kit, CIS bio inter-

Table 3. Clinical studies of prognostic significance of cathepsin D in breast cancer patients.

Study	Assay	Group	Number of patients	Median F/U (mo)	Cut-off*	Univariate p-values		Multivariate p-values		Note
						DFS	OS	DFS	OS	
Thorpe *et al.*, 1989 (26)	ELISA	Pre and Peri-MP	242	48	78	0.06	0.3	0.029	n.r.	
		Post-MP	154	67	24	0.039	0.089	0.032	n.r.	
Spyratos *et al.*, 1989 (27)	IRMA	All	122	55	45/70	n.r.	n.r./0.04	0.001/<0.001	n.r.	
		N-	68			n.r.	n.r.	<0.01/0.001	n.r.	
Tandon *et al.*, 1990 (28)	WB	N-	188	64	75	<0.0001	0.0001	0.0003	0.0001	quantifie only 34 kDa form
Romain *et al.*, 1990 (29)	IRMA	All	85	<58	30	0.08	0.02	NS	0.02	
		N+	46			NS	0.02	n.r.	n.r.	
Henry *et al.*, 1990 (30)	IHC	All	94	<60	++/+ *vs.* -	<0.05	<0.1	n.r	n.r	protective role of cathepsin D
		N-	62			NS	NS	n.r	n.r	
		N+	32			<0.025	<0.025	n.r	n.r	
Namer *et al.*, 1991 (31)	IRMA	All	413	84	35	NS	0.03	n.r.	0.02	
		N-	246			NS	NS	n.r	n.r	
		N+	166			0.02	0.008	0.03	0.009	
Granata *et al.*, 1991 (32)	IRMA	N-	199	87	40	NS	NS	NS	NS	
		N- and ER+	148			0.02	0.01	n.r	n.r	
Duffy *et al.*, 1992 (33)	IRMA	All	331	48	40	<0.05	<0.01	n.r	n.r	
		N-	141	51		NS	NS	n.r	n.r	
		N+	149	44		NS	NS	n.r	n.r	
Kute *et al.*, 1992 (34)	IRMA	N-	139	29	63	0.0001	0.0004	0.0263	0.0044	
	EA	N-	138	29	52	0.0031	0.0013	0.22	0.0005	
Pujol *et al.*, 1993 (35)	ELISA	All	123	59	20	0.01	0.03	0.02	NS	
		N-	64			0.07	n.r	n.r	n.r	
		N+	59			0.009	n.r	n.r	n.r	
Isola *et al.*, 1993 (36)	IHC	N-	262	<96	++ *vs.* +/-	<0.0001	<0.0001	<0.0001	<0.01	
Seshadri *et al.*, 1994 (37)	IRMA	All	858	31	25	<0.05	n.r	0.0067	n.r	
		N-	491			NS	n.r	NS	n.r	
		N+	367			0.005	n.r	0.037	n.r	
Gion *et al.*, 1994 (38)	IRMA	All	266	39	31	0.0003	0.0222	<0.001	<0.001	
O'Donoghue *et al.*, 1995 (39)	IHC	Tu cells	103	>60	++ *vs.* +/-	n.r	NS	n.r	n.r	
		Stromal cells				0.0001	0.0086	n.r	n.r	

F/U – follow up; DFS - disease free survival; OS - overall survival; ELISA - enzyme-linked immunossorbent assey; IRMA - immunoradiometric assay; WB – Western blotting; EA -enzymatic activity; IHC - immunohistochemistry; MP - menopausal; N - axillary lymph node; ER - estrogene receptor; NS – non-significant; n.r. - not reported.
*Cut off values for ELISA, IRMA and WB are presented in pmol/mg proteins.

national, GIF-sur-Yvette, France).[40] It quantifies the total enzyme concentration (52kDa, 48kDa and 34 kDa forms) present in cytosols of tissue samples. The latter is also used for the determination of steroid receptor concentrations; this altogether provides a more detailed information on biological properties of tumors. Regarding the above mentioned, cathepsin D already fulfils most of the criteria that should be considered in introducing a new prognostic marker for routine clinical use. However, a number of questions and dilemmas are still open, among them also some which have been posed only recently, by studies just completed: 1) an optimal cut-off value should be selected, which would reliably distinguish between patients with favourable and those with poor prognosis; 2) to find out which form of enzyme is the most potent marker for survival; 3) to establish which type of cells in the tumor is actually overexpressing the enzyme; and 4) to determine its prognostic relevance with respect to menopausal status, steroid receptor status and axillary lymph node involvement. Similarly, it should be investigated how tumors with high cathepsin D concentrations respond to adjuvant therapies: the controversial results reported in the literature, referring to the subpopulation of patients with negative axillary lymph nodes, may be due to different implementation of adjuvant therapies. Only well-controlled randomised clinical studies using an accurately defined and standardised methodology will be able to provide answers to the questions posed. Until then, the routine use of cathepsin D as a prognostic marker with decisive influence on the selection of type or aggressiveness of treatment in individual patients remains unjustified and controversial, despite the promising results obtained so far.

Concentration and/or activity of cathepsin D was also measured in other types of cancer. However, its prognostic significance was generally not studied. A higher concentration of cathepsin D (from 1.5 to 3-fold) was established in laryngeal carcinoma tissue[41] as well as in other types of head and neck tumors[42,43] or their regional metastases,[42] as compared to the adjoining normal tissue of the same patients. None of these studies was able to confirm a correlation between tumor concentrations of the enzyme and the already established clinical and pathohistological prognostic factors. Métayé *et al.* reported a 3-fold higher concentration of cathepsin D measured in 14 samples of thyroid carcinoma tissue than in 7 samples of normal glandular tissue and in 6 samples of benign thyroid nodules. The level of cathepsin D in primary tumors correlated with their size. A similar increase in the enzyme concentration was observed in the tissue of toxic adenomas (8 samples) and in the tissue samples from 7 patients with Grave's disease.[44] Letto *et al.* assessed the level of cathepsin D activity in 67 surgical samples of colorectal carcinoma and in matched paired sets of normal mucosa; the enzyme activity measured in tumor tissue were 1.3-fold higher that in normal mucosa of the same patients.[45] The enzyme activity values found by Tumminello *et al.* in tumor tissue samples from 21 patients with colorectal carcinoma were 1.6-times higher than the respective values measured in normal mucosa of the same patients. A higher activity was observed in the tissue of Dukes' stage A tumors compared to Dukes' B and C, as well as in tumors smaller than 5 cm. There were no differences in cathepsin D concentration between tumor tissue and paired normal mucosa.[46] Cytoplasmic expression of cathepsin D in the cells of gastric adenocarcinoma was studied immunohistochemically in 62 patients by Theodoropoulos *et al.*. Increased expression correlated with early tumor stages (I and II), well- and moderately differentiated carcinomas, positive status of estrogene receptors and a better survival of patients at 36 month.[47] Increased plasma concentrations of cathepsin D assayed in 20 patients with primary hepatocellular carcinoma as well as in 7 patients with liver metastases were reported by Brouillet *et al.*. The values were significantly higher than those established in a group of 56 healthy controls or in 48 breast cancer patients.[48] In a group of 72 patients with primary ovarian carcinoma, Scambia and co-workers reported a worse 3-year progression-free survival for patients with high tumor concentrations of cathepsin D. In 12 patients with metastases in the omentum, the enzyme concentrations measured in the metastatic deposits were 2-fold higher than those found in the primary tumors. Cathepsin D status retained an independent prognostic value for progression when assessed in the multivariate analysis.[49] A correlation between cathepsin D concentration, the grade of tumor differentiation, and the depth of myometrial invasion was also observed in 26 patients with endometrial carcinoma: a significantly higher increase in enzyme level was associated with a higher pathohistological grade and deeper invasion.[50]

Cathepsin B

Cathepsin B belongs to the class of cysteine or thiol proteinases. It has cysteine as essential catalytic group bound to its active site.[50] Human gene

for cathepsin B is localised on the chromosome 8.[52] As all known proteinases, it is first synthesized as a high-molecular-weight inactive precursor with a molecular mass of 37 kDa, which changes into its enzymatically active mature form in the course of posttranslation processing. The molecular mass of the latter ranges between 23-28 kDa; it is found in the cell – depending on species and tissue of origin – as a single- (28 kDa), double- (23 kDa and 5 kDa) or as both single and double chain forms.[51] It is optimally active at a pH of about 6.0 and is poorly active or inactive within the range of neutral and alkaline pH values, depending on the nature of the substrate[51] and the stage of enzyme maturity.[53] As endopeptidase, it exerts an effect on numerous proteinic substrates – also on the components of the extracellular matrix,[54] and has the potential of activating the precursors of some collagenases[55] and urokinase-type plasminogen activator.[56] Thiol reagents and chelators are required for its activation, but it can be activated also by pepsin,[6] cathepsin D[23,24] and metallo-proteinases.[24,57] It has been demonstrated that enzyme activation can also occur as a result of its autocathalytic activity.[58] Cathepsin B is irreversibly inhibited by thiol blocking reagents, and reversibly by leupeptin and other peptide aldehydes, α2-macroglobulin and members of cystatin superfamily (i.e. stefins, cystatins and kininogens). The homology of amino acid sequences suggests its common evolutionary origin with other cysteine proteinase class members.[51]

Unlike in normal cells, where cathepsin B molecules are found prevailingly in lysosomes, in tumor cells a great proportion of the enzyme is found on the cytoplasmic membranes.[59-62] As pro-enzyme, it can also be released into the slightly alkaline surroundings of the extracellular space, and once activated extracellularly, it may be stable in its active form due to the presence of large protein substrates such as extracellular matrix proteins.[53] Correlation between the enhanced activity, mRNA level, the rate of membrane-bound cathepsin B and/or the quantity of high-molecular-weight enzyme forms released into the surroundings on one side, and malignancy of different tumor types of epithelial as well as mesenchymal origin on the other, has been proved in different *in vitro* and *in vivo* experimental models. It seems, however, that this correlation is of qualitative rather than quantitative nature.[63] Furthermore, the reduced inhibitory capacity of human sarcoma derived stefin A, an important intracellular inhibitor of cathepsin B from cystatin superfamily, has been demonstrated too, as a probable result of changes in its structure.[64] This indicates that the activity of cathepsin B in malignant tumors is regulated at many different levels. Its alterations could be attributed to the modulation of synthesis, activation, processing and intracellular transport of enzyme molecules and/or changes in the inhibition by endogenous inhibitors.[51]

An increased concentration and/or activity of cathepsin B molecules was measured in various types of human malignant tumors: carcinomas of the breast,[65-67] colon and/or rectum,[45,68,69] stomach,[70,71] liver,[72] lung,[73-75] uterine cervix,[76] head and neck carcinomas,[43] gliomas,[77] and tumors of the hypophysis.[78] There has been a correlation established between the measured tumor concentration and/or activity of cathepsin B, and individual clinical and pathohistological tumor properties; in some tumors, a correlation with treatment outcome and/or survival was found as well. It should be pointed out that the results of these studies are much less established and conclusive than those referring to cathepsin D and the survival of breast cancer patients.

Lah *et al.* have reported 18.5-times higher cathepsin B activity measured in the breast cancer tissue of 50 patients as compared to the relevant values measured in normal breast tissue of the same patients. There has been no correlation established with pathohistological grade, axillary lymph node involvement, hormone receptor status, and relapse free survival.[67] Similar results of cathepsin B activity measurements in 90 matched pairs of breast carcinoma and normal breast tissue samples were reported by Gabrijelčič *et al.*. Besides the enzyme activity, authors also measured total enzyme concentration in the serum and tissue cytosol: the latter was found to be approximately 3.3-fold the concentration measured in the serum of healthy controls, while the relevant cytosol concentrations were 8.8-fold higher, respectively. Higher enzyme concentrations were found in the tumor tissue cytosols from patients without axillary lymph node involvement and a higher pathohistological grade. In this study, patients' survival was not considered among the parameters observed.[65] A similar negative correlation between cathepsin B concentration in tumor cytosols of 62 breast carcinoma patients and their lymph node status, as well as the status of hormone receptors, was reported by Budihna *et al.*. Besides, patients with cathepsin B tumor concentrations up to 23 mg/g proteins were found to have worse re-

currence-free survival at 54 months than those with higher tumor concentrations. In the multivariate analysis, besides axillary lymph node status, only cathepsin B proved to be an independent prognostic factor.[79] On the contrary, Thomssen *et al.* reported a better 5-year recurrence-free survival in patients with lower cathepsin B concentrations (< 1092 ng/mg proteins). In this study of 167 breast cancer patients tumor concentrations of cathepsin B were 11.3-fold higher than those measured in benign breast tissue, and no correlation to established prognostic factors were found. The relevance of cathepsin B as independent prognostic factor for recurrence-free or overall survival of those patients was not confirmed by the multivariate analysis.[80]

Analysing cathepsin B activity in paired tissue samples from 27 patients with colorectal carcinoma, Sheahan *et al.* registered 1.4-fold higher activity in tumor tissue as compared to the adjoining normal mucosa. The highest enzyme activity was established in a group with Dukes A stage of the disease.[68] Similar findings were reported by Leto *et al.*: cathepsin B activity measured in carcinomatous tissue from 67 patients was 1.4-fold higher than that found in normal mucosa, the increase being evident in patients with Dukes A stage of disease only. There was no correlation with either clinical or pathohistological prognostic factors established.[45] Contrary to that, Campo *et al.* found that the elevated cathepsin B expression correlated with advanced stages of disease. The expression of enzyme was found to be negative in all 15 samples of normal mucosa and 17 samples of benign adenomas. However, in a group of 28 patients with early, non-metastatic tumors (stages I-II), the expression was negative in 6, low in 17 and high in 5 patients. In 41 patients with advanced, metastatic carcinomas (stages III-IV), the expression was negative in 3, low in 17 and high in 21 patients. Lower overall survival at 84 months of follow up correlated with high cathepsin B expression in all cancer patients, whereas after stratification by stages, the correlation was established only for those with advanced disease.[69]

After having compared 33 match pairs of gastric carcinoma and the adjoining normal mucosa, Watanabe *et al.* measured 3-fold higher cathepsin B activity in tumor tissue samples. The enzyme activity was significantly, i.e. 1.9-fold higher in poorly differentiated adenocarcinomas than in well or moderately differentiated tubular adenocarcinomas.[70] Plebani *et al.* reported the results of their cathepsin B measurements in paired tissue samples of 25 patients with gastric cancer. The concentrations found in tumor tissue were twice as high as those measured in normal mucosa. Higher enzyme concentrations were also found in the tissue of patients with regional or hepatic metastases vs. those without metastases, in poorly or moderately differentiated vs. well differentiated tumors, and in diffuse vs. intestinal tumor types. At 27 months, the survival of patients with cathepsin B tumor concentrations below the cut-off value of 265 ng/mg proteins was better than of those with higher enzyme concentrations.[71]

Ebert *et al.* have established a 4.5-fold higher cathepsin B activity in 65 lung tumor tissue samples as compared to the normal lung parenchyma. The activity was found to be insignificantly higher in adenocarcinomas than in other histological tumor types. The highest cathepsin B activity levels were measured in lung metastases. There was no correlation with stage of disease or pathohistological grade established. Elevated activity above the cut-off value of 1674 μU/mg proteins was related to lower survival rates of the patients at 8 months.[75] Higher cathepsin B activity found in the tissue of lung adenocarcinomas as compared to squamous cell carcinomas was reported by Krepela *et al.*[73] and by Lüthgens *et al.* who compared adenocarcinoma cathepsin B activity to the activity measured in squamous cell and small cell carcinomas.[74] In the group of 142 patients with primary lung adenocarcinoma, Inoue *et al.* registered immunohistochemically increased cathepsin B expression in the tumor tissue of cases with stage III and IV of disease as compared to that in cases with stage I, which also correlated with worse overall 5-year survival rates. In a multivariate analysis, cathepsin B expression proved to be an independent prognostic factor associated with death due to disease.[81]

After having compared 53 matched pairs of head and neck carcinoma and adjacent normal tissue, Kos *et al.* found 5.4-fold higher cathepsin B concentration in tumor tissue samples. There was no correlation with clinical and pathohistological prognostic factors established, whereas patients' survival was not included among the parameters observed.[43]

In a study by Hirano *et al.*, serum cathepsin B levels and its urinary excretion were reported to be significantly higher in a group of 7 patients with distant metastases from a variety of cancers than in the control non-cancer patients (11 samples) or in cancer patients without distant metastases (7 sam-

ples). Six weeks after completed radical curative operation the enzyme concentration in the group without distant metastases decreased to the control values. However, in the group of cancer patients with distant metastases after resection of primary tumor, both serum and urine enzyme concentrations were still high – as before surgery. In the group without distant metastases, for all of the resected specimens of cancer tissue, cathepsin B concentrations were significantly, 1.8-times higher than those in normal tissue.[82]

Endogenous cathepsin inhibitors

Endogenous, i.e. physiological inhibitors of proteinases naturally present in tissues, appear always to be proteins. They are involved in the control mechanisms responsible for intra- and extracellular protein breakdown, thus protecting the cell against adverse endo- and exogenous proteolysis. The compilation of new knowledge and information on these substances contributes to better understanding of their role and importance in the process of tumor rise and its consecutive spread. By preserving the delicate balance that exist between tumor cells, extracellular-matrix-bound growth factors and cytokines, and constituents of the matrix, these inhibitors may exert a marked cytotoxic effect on the primary tumor as well as on the existing metastatic lesions. This ability assigns them the role of potential therapeuticals and/or prognostic indicators in all conditions where proteolytic degradation represents the pathophysiological basis for clinical manifestation of disease, thus also in cancer.[83] It seems, however, that it will take long before these substances become a part of the routine therapeutic tools for cancer treatment, considering that all the studies are still carried out at a preclinical, i.e. laboratory level. The same applies to their prognostic value, since until now no reports on this issue could be found in the available literature.

Probably, in view of future clinical use, the most promising of endogenous inhibitors are those which suppress the activity of cathepsins B and D. Considering that an endogenous inhibitor of cathepsin D in man is not known yet, these are – for the time being – restricted only to the inhibitors of cysteine proteinases. Based upon the evolutionary and structural similarities, they constitute a single protein superfamily of cystatins. This is subdivided into three families: stefins (family I), cystatins (family II) and kininogens (family III). There is yet a group of non-inhibitory proteins (family IV) including histidine-rich glycoproteins and α2H-glycoproteins (Table 4). The members of the first three families,

Table 4. Cysteine proteinase inhibitors of cystatin superfamily in human.

Family		Examples	Distribution	M_r
Name	No.			
Stefins	I	stefin A stefin B	intracellular	11.000
Cystatins	II	cystatin C	extracellular	13.000
Kininogens	III	HMW-kininogen	extracellular	120.000
		LMW-kininogen		68.000

HMW – high molecular weight; LMW – low molecular weight.

capable of inhibitory activity, differ from one another with regard to their binding affinity and binding ratio for different cathepsin molecules; in all of them binding is strong though competitive and reversible. Contrary to kininogens and cystatins, which occur at relatively high concentrations in various biological fluids, stefins can be found prevailingly inside the cells. The presence of the molecules of cystatin superfamily inside as well as outside the cells renders them to serve as a "reservoir" for cysteine endopeptidases: they bind the enzymes when released from lysosomes in order to transport and deposit them at other sites in the cell or organism.[84-86]

The measurements of the concentrations and/or activities of cysteine proteinase inhibitors gave controversial results, and the existing literature on these topics is very scarce. Thus, the total activity of inhibitors measured in tumor tissue was found to be either lower,[67] equal[68] or higher[87] than in the adjoining normal tissue. This variability indicates that it is indispensable to determine the contribution of each individual member of cystatin superfamily to their total inhibitory potential. While the role of cystatins and kininogens in the process of the development and spread of malignant tumors has not been extensively studied yet, the involvement of stefins in these processes is more investigated.

In man the stefin family comprises stefin A and stefin B. These are small single-chain non-glycosilated proteins with a molecular weight of about 11 kDa.[84] Genes for family I proteins are located on human chromosome 3[52,88] and do not include secretory signal sequences.[89] This is consistent with the fact that stefins are generally found in the cell cytoplasm,[90-92] although they have also been iso-

lated from the extracellular fluids.[93] They are heat resistant and stable in neutral and alkaline pH range. Although quite similar structurally – 51 % of stefins A and B structure is identical, they differ from one another with respect to their immunological properties which enable immunohistochemical studies of their cellular and tissue distribution.[84] While the presence of stefin B in different tissues is relatively uniform,[84,94,95] stefin A is abundant primarily in various types of epithelial cells and in some cell types of the lymphoid tissue.[96-102] This suggests a possible role of stefin B as the protector of cells against uncontrolled activities of endogenous cysteine proteinases, and the involvement of stefin A in the immunological processes protecting epithelial and lymphoid tissues from invading bacteria and parasites or their (i.e. external) cysteine proteinases[84,92,99] Functional differences between both stefins, which may also be of physiological importance, lye in their inhibitory capacity for individual cathepsins, as well as in their resistance to proteolytic degradation by aspartic proteinase cathepsin D: stefin A is a better inhibitor of cathepsin B than stefin B is, and shows a higher resistance to cathepsin D. Both stefins exert a stronger inhibitory effect on the molecules of cathepsins L and H than of cathepsin B.[84,103,104]

There are several findings implicating stefin A in the process of malignant progression more than any other member of the cystatin superfamily. When determining the total activity of cysteine proteinase inhibitors in 50 matched pairs of breast carcinoma and normal breast tissue, Lah *et al.* found lowered inhibitor activity in carcinoma as compared to normal tissue in two thirds of their patients. In this group, a correlation was established between lower inhibitor activity, higher pathohistological grade and negative hormone receptors, as well as significantly higher relative increase in Cathepsin B and L specific activity between tumor and normal tissue than in the group with unchanged or elevated activity of the inhibitors studied. In the same study, lower mRNA concentrations of stefin A were measured in carcinomatous than in normal breast tissue samples and correlated with the total activity of cysteine proteinase inhibitors.[67] Reduced immunohistochemical staining for stefins A and B in lymphoma and esophageal carcinoma tissue was reported by Järvinen *et al.*;[92] in the latter it could be associated with the dedifferentiation and malignant transformation of epithelial cells. A similar observation applies to squamous cell carcinomas of the human uterine cervix,[105,106] skin,[107] lung,[108] as well as for prostatic adenocarcinoma;[109] there also, stefins A expression was related to cell proliferation and dedifferentiation, suggesting to be an important factor in maintaining cell differentiation. In the case of prostatic adenocarcinoma, the authors even suggest stefin A to be used as a marker in histologic differential diagnosis of malignant and benign lesions, especially in the detection of small carcinomatous foci in the prostate.[109] A high concentration of stefin B along with an unusually low concentration of stefin A – approximately 20-times lower that that found in normal epithelial tissue – was measured in ovarian carcinoma tissue by Kastelic *et al.*. The authors hypothesise that stefin A is down-regulated in malignant ovarian carcinoma.[110] A reduced inhibitory capacity of human-sarcoma-derived stefin A against different cysteine proteinases was reported by Lah *et al.*. It appears to be due to a higher inhibition constant of stefin A for the inhibition of these enzymes, which indicates that endogenous inhibitors of tumor origin exert different inhibitory properties than those originating from normal tissues.[64]

Conclusion

Introducing of new prognostic markers into routine clinical practice enables us to differentiate more precisely between prognostically more and less favourable forms of disease, and thus also influence treatment planning. It is important that patients with favourable prognosis are spared from too aggressive therapy, and vice versa, that those with worse prognosis receive sufficient treatment. The role of cathepsins and their endogenous inhibitors in the development of cancer is indicated particularly from their involvement in the proteolytic processes leading to invasion and dissemination of tumor cells. Their concentrations and/or activities in tumor tissue or body fluids can also be of prognostic value.

By now, it has been generally accepted in clinical oncology that high cathepsin D concentrations in breast cancer tissue should be regarded as indicator of worse prognosis. The prognostic relevance of the enzyme in different subgroups of these patients, as well as in patients with tumors of other sites, is less clear. Likewise, the prognostic value of cathepsin B has also been unclear, while that of other cathepsins has not been extensively studied at all.

The role of endogenous cathepsin inhibitors – cystatins in clinical oncology could be double: they could function as therapeuticals and/or prognostic factors. In view of the fact that most studies in this

field have been carried out on different experimental models only, while clinical trials – rare as they are – involve small series of patients, the question of clinical importance of these inhibitors remains to be solved.

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Langerhans cell histiocytosis. Five new cases and review of the literature

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Five cases of LCH diagnosed and treated in our department the last two years are described. The first case concerns an 8 year old boy with a history of back pain, collapse of the 5th lumbar vertebra and lytic lesions in the skull. The second concerns an 8 month old male with symptoms of chronic otitis media, persisting diaper rash, seborrhoeic dermatitis of the skull and organs' dysfunction. The third case concerns an 8 month old boy with diaper and vesicular rash with remissions and exacerbations of 3 month duration. The fourth case concerns a 3 year old girl with a history of claudication of the left leg and painless nodules over the head. The fifth case concerns a female infant born with necrotic dermatitis and skin nodules. Biopsy established the diagnosis with S-100 and CD1 positive histiocytes in all cases. Treatment ranged from simple observation to systemic therapy including steroids and Vinblastine or Etoposide. The atypical clinical presentation of LCH and the treatment policy in each case are discussed.

Key words: histiocytosis, Langerhans-cell

Introduction

Langerhans cell histiocytosis is a disease which frustrates both clinicians and scientists. Its aetiology is unknown, its pathogenesis is ill understood and the clinical course is unpredictable. LCH can appear at any period of life ranging from birth to old age with a peak between 1–3 years.[1] The incidence in the pediatric range has been estimated at 3–4 per million with males affected twice as commonly as females.[2] The disease has a wide clinical spectrum and prognosis varies accordingly. Five cases of LCH diagnosed and treated in our department the last two years are described.

Case 1

An eight year old boy, the second child of healthy parents was admitted with a history of back pain of a month duration. The X-rays and CT scan revealed collapsed body of 5th lumbar vertebra and lytic lesions in the skull. The physical examination and the laboratory tests did not show other organs to be affected. The diagnosis was established by biopsy of lytic lesion of the skull which revealed, infiltration comprising a mixed population of lymphocytes, occasional eosinophils and large pale cells with a central folded nucleus. Occasional multinucleate cells were present. Immunostaining showed these cells to be S-100, "peanut" agglutinin and CD1 antigen positive. Simple observation and analgesic drugs were the only treatment and at the present time, two years after diagnosis, the patient is in complete remission.

Case 2

An eight month old boy, who was born to healthy unrelated parents, was admitted with symptoms of chronic otitis media, persisting diaper rash and seborrhoeic dermatitis of the skull not responding to multiple local treatment.

The clinical examination revealed hepatosplenomegaly and the laboratory tests liver dysfunction

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and pancytopenia. X-rays showed diffuse mottling of both lungs fields and infiltration of mastoid. Diagnosis was confirmed by biopsy of skin lesion which showed infiltration of histiocytes S-100 protein and CD1 antigen positive. Despite aggressive chemotherapy with methylprednisolone, Vinblastine and Etoposide, the patient succumbed to the disease three months later.

Case 3

An 8 month old male was admitted with persisting diaper rash and reddish brown maculopapular, vesicular rash of the trunk, extremities and the skull, of six months' duration with exacerbations and remissions. The diagnosis was established by biopsy of the skin lesions (Figure 1) which showed infiltration of histiocytes S-100 protein and CD1 antigen positive. There was no anaemia, lymphadenopathy or hepatosplenomegaly and his nutrition was excellent. The bone marrow aspiration showed the presence of 8 % histiocytes. The X-rays did not reveal bone lesions, and the laboratory tests no organs' dysfunction. The patient was treated with methylprednisolone 30 mg/kg for 3 days, following by weekly Vinblastine 6 mg/kg for 24 weeks, according to the LCH I treatment protocol of Histiocyte Society, but also with local application of Nitrogen mustard on the skin lesions. The patient is in remission 15 months after completion of the treatment protocol.

Case 4

This case concerns a 3 year old girl with a history of claudication of the left leg, painless nodules over the head and acute torticollis. X-rays revealed lytic lesions of the skull (Figure 2) and collapse of 8th thoracic vertebra. The diagnosis was confirmed by biopsy of bone lesion which showed infiltration of Langerhans histiocytes on light microscopy and demonstration of CD1 positivity and S-100 protein immunohistochemically. The bone marrow aspiration did not reveal infiltration of the bone marrow. There was no anaemia, lymphadenopathy, hepatosplenomegaly or organs' dysfunction. This patient was also treated according to the LCH I protocol, Arm A, with methylprednisolone and Vinblastine for 24 weeks. During therapy, the number and the size of the skull lesions decreased, but two new soft tissue masses of the skull appeared and later dissappeared. Three months after the treatment protocol was completed, the clinical examination revealed a soft tissue mass of the left jaw and X-rays and MRI showed a lesion of the mandible.

Case 5

The fifth case concerns a female infant born to healthy unrelated parents with necrotic lesions and nodules in the skin. The diagnosis of LCH was made by skin biopsy which showed diffuse infiltration of histiocytes with large pale cytoplasm and central folded nucleus. The demonstration of both CD1 antigen and S-100 protein was positive. There

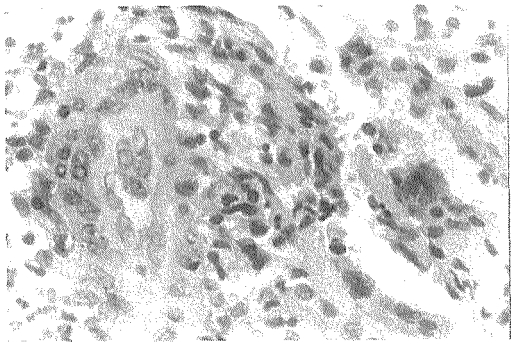

Figure 1. Diffuse proliferation of Langerhans cells in a skin biopsy (Hematoxylin-Eosin x 400).

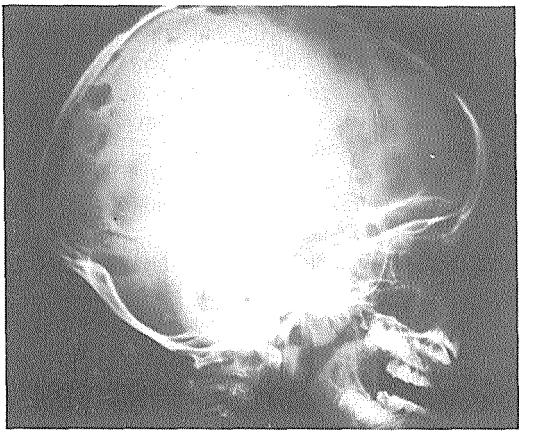

Figure 2. Bone lesions of the skull.

was no anaemia, lymphadenopathy or splenomegaly but the liver was palpable 2 cm. Liver function was normal but the LDH = 350U/l. Skeletal survey showed no lytic lesions but chest X-rays and MRI revealed diffuse mottling (Figure 3) of both lung

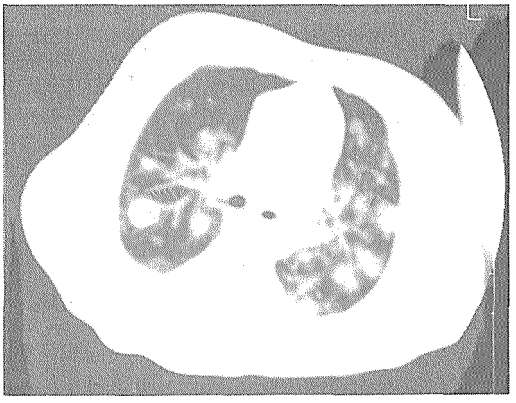

Figure 3. Multiple pulmonary nodules of both lungs fields on chest MRI.

fields. Bone marrow aspiration showed the presence of histiocytes ≥ 6 %. The baby was decided to be treated with prednisolone 1 mg/kg and she responded well with increase of her body weight.

Discussion

Langerhans cell hystiocytosis, previously known as histiocytosis X, is a reactive proliferative disease, characterized by the accumulation of abnormal histiocytes that form infiltrates typical for the disease. The etiology of LCH is unknown and the pathogenesis is not exactly understood.[3-4] For decades the disease has been widely accepted to be a reactive immunologic process rather than a malignancy.[5-7] Recent laboratory studies have demonstrated that the cells in all forms of LCH are clonal exprasions of Langerhans cells or their precursors in the bone marrow and other organs.[8, 9] However clonality does not necessarily indicate a malignant process.[10] LCH includes a wide range of clinical presentations which reflect different facets of the disease. The course of the disease is unpredictable. Patients with localized disease, in general have a good prognosis.[11] Bone is the most common organ affected. Three of the five reported cases here, had bone lesions from skeletal survey while two of them (1st, 4th) had no other organ or affected systems. The first case, a 8 year old boy with collapse of the 5th lumbar vertebra and lytic lesions in the skull, did not receive any treatment except analgesic drugs and simple observationl. The patient is in complete remission two years following inital diagnosis. In older children "single system" disease, usually affecting bones, is a common presentation and may spontaneously regress or require minimal treatment.[12]

Two of the patients described in this report were affected with multisystem disease, and one of them had also organs' dysfunction. This patient, despite aggressive chemotherapy, succumbed to the disease. The other infant has been treated with prednisolone for two months now and is responding well. In very young babies, the most common presentation is that of multisystem disease[13-15] with sometimes organ failure also. Skin rash is particularly common in infants and it is difficult to dinstinguish it from seborrhoeic eczema.[16, 17] The 8 month old male was presented with diaper and vesicular rash with remissions and exacerbations of 3 month duration without other affected organs, except for the presence of 8 % histiocytes in the bone marrow. This patient is in complete remission 15 months following the completion of treatment protocol.[15, 18] His inital presentation points out that persisting diaper rash should be investigated for LCH. The difficulty in diagnosing LCH is more often the result of a failure to consider the diagnosis, rather than a failure to distinguish it from other diseases.

The fourth patient who had multiple bone lesions and soft tissue masses in the skull relapsed three months after the completion of the treatment protocol. Since the patient is in an excellent condition, she remains under observation before any other treatment is decided upon.

In all cases the diagnosis was based upon histological features from the biopsy of the lesion and was confirmed by the typical characteristics of LCH on light microscopy, as well as the additional demonstration of CD1 antigen positivity and S-100 protein immunohistochemically. LCH cells and normal Langerhans cells (LCs) constituvely express a number of phenotypic markers. Most important are class II MCH molecules and CD1a complex. A number of markers are used to identify LCs in tissue specimens but detection of CD1a glycoprotein and identification of Birbeck granules are the two most specific tests. The Birbeck granule can only be identified directly at the ultrastructural level. Surface ATPase is useful in frozen tissue and S-100 in paraffin embedded tissue but neither is specific for LC. Expression of CD1a has been identified by the

Writing Group of the Histiocyte Society (1987) as a feature which establishes a definitive diagnosis in LCH. Three markers, placental alkaline phosphatase (PLAP), peanut agglutinin (PNA) and the interferon gamma receptor, are especially valuable in differentiating normal Langerhans Cells from LCH cells. The Histiocyte Society have also established "confidence levels" for the diagnosis of LCH.[1] Presumptive diagnosis is permitted when examination of conventionally-processed tissue reveals lesions consistent with those defined in the literature. A higher level of diagnostic confidence, referred to as "diagnosis", is justified when these findings are supplemented by the presence of at least two of the following positive stains: S-100 protein, ATPase, a-D-mannosidase or peanut lectin binding. "Definitive diagnosis" requires the demonstration either of Birbeck granules in lesional cells by electron microscopy, or of CD1 a antigenic determinants on the surface of lesional cells.

The outlook for patients with single system disease is excellent with minimal long term sequelae so long as treatment is conservative.[8] For very young infants with bone marrow failure and/or liver dysfunction, mortality of 30–50 % regardless of treatment is reported[11, 12, 14] and this bad prognosis occurred in the 2nd patient, who had organ dysfunction. Most patients have multisystem disease without organ dysfunction and 50 % suffer long term sequelae including small stature, growth hormone deficiency, diabetes insipidus, partial deafness, cerebellar ataxia, loss of dentition, orthopaedic problems, pulmonary fibrosis and biliary cirrhosis.[11, 16, 17, 19]

Although complications of some types of therapies are now well known, the safest and most effective treatment for LCH has not yet been established. The risk: benefit ratio of using chemotherapy and/or radiotherapy, and the manner of their use, need to be weighed carefully.

The continuously changing aspects of the etiology of the disease, the heterogenicity of clinical presentation and the phasma of treatment modalities make LCH a continuous challenge not only to the clinician but also to the scientists.

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Ionization gradient chamber in absolute photon and electron dosimetry

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A variable volume parallel-plate ionization gradient chamber was built to determine the absorbed dose in a polystyrene phantom. The sensitive volume of the gradient chamber is controlled by moving the chamber piston by means of a micrometer mounted to the phantom body. The displacement of the piston is monitored by a calibrated distance travel indicator which is accurate to within 0.01 mm. Irradiations were carried out with cobalt-60 gamma rays, photon beams ranging from 4 MV to 18 MV, and electron beams between 5 MeV to 18 MeV.

With the ionization gradient chamber the calculation of the absolute dose at a given depth in phantom is simple and based on first principles using the slope of the measured ionization as a function of the electrode separation, i.e., the sensitive air volume. The discrepancies between the doses determined with our uncalibrated gradient chamber and those obtained with a calibrated standard chamber are at most 1.08 % and 0.63 % for photon and electron beams, respectively, at all clinical energies, indicating that the gradient ionization chamber can be used as an absolute dosimeter.

Key words: radiation dosage; polystyrene; photons; electrons; absolute dosimetry

Introduction

An accurate determination of the absolute dose rate produced by photon or electron machines is one of the most important components of modern radiotherapy. Radiotherapy clinics most commonly determine the absolute absorbed dose with parallel-plate or cylindrical ionization chambers which are first calibrated at, or trace their calibration factors to, a national standards laboratory. The dose is calculated from the measured ionization in air using the chamber calibration factor and following one of several available protocols (*e.g.,* ICRU,[1] AAPM-TG21;[2] AAPM-TG25;[3] IAEA-WHO;[4] *etc.*) These protocols are based on the standard Bragg-Gray[5,6] or Spencer-Attix[7] cavity theories and incorporate various correction factors, which are used to account for effects of chamber dimensions and wall materials as well as disruptions in the photon and electron fluence caused by the chamber. These correction factors make the dose determination cumbersome and introduce uncertainties in the final result.

The basic Bragg-Gray and Spencer-Attix cavity relationships for the dose D_{med} in medium are:

$$D_{med} = \frac{Q}{m} \overline{W}_{air} \overline{S}_{air}^{med} \qquad (1)$$

and

$$D_{med} = \frac{Q}{m} \overline{W}_{air} \overline{L}_{air}^{med}, \qquad (2)$$

respectively, where Q is the charge collected under saturation conditions in the sensitive chamber air mass m, W_{air} = 33.97 eV[8] is the mean energy re-

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UDC: 539.166.08

quired to produce an ion pair in air, and S_{air}^{med} and L_{air}^{med} are the ratios of unrestricted and restricted collisional stopping powers, respectively, for the medium and air for the electron spectrum at the position of the cavity. Both the Bragg-Gray and the Spencer-Attix formalisms assume that the air cavity within the medium is sufficiently small such that it does not alter the electron fluence in the medium. The Bragg-Gray formalism uses unrestricted stopping powers averaged over the slowing-down spectrum of only the primary electrons, while the Spencer-Attix formalism uses restricted stopping powers averaged over the slowing-down spectrum of all generations of electrons.

It is evident from *Equations (1)* and *(2)* that the dose in medium is proportional to the measured ratio Q/m which in principle should be straight forward to determine. In actuality, Q is easy to measure accurately in clinical beams, however, m is almost impossible to determine with an accuracy of better than 1 % required for clinical use, precluding the direct use of *Equations (1)* and *(2)* in absolute dosimetry. The standard method for obviating this problem is to calibrate the cavity chamber response in a known reference radiation field which has been calibrated previously with a standard free air ionization chamber. This determination of the chamber calibration factor is actually an indirect means of determining the mass of air in the chamber sensitive volume. The chamber calibration factor in conjunction with various troublesome correction factors is then used to determine the dose to the medium.

Investigation of *Equations (1)* and *(2)* has revealed that at least for small m the ratio of Q/m is a constant allowing its replacement with the derivate dQ/dm, resulting in the following modified Bragg-Gray and Spencer-Attix relationships for the dose in medium:

$$D_{med} = \frac{dQ}{dm} \overline{W}_{air} \overline{S}_{air}^{med} \qquad (3)$$

and

$$D_{med} = \frac{dQ}{dm} \overline{W}_{air} \overline{L}_{air}^{med} \qquad (4)$$

The advantage to this approach is that, in contrast to Q/m, dQ/dm is relatively easily measured accurately making the modified Bragg-Gray and Spencer-Attix relationships directly applicable in absolute dosimetry. Similarly to Klevenhagen,[9] we have developed an uncalibrated, variable volume, *ionization gradient chamber (IGC)* capable of measuring the absorbed dose directly in an absolute manner. The chamber developed by Klevenhagen was made of Lucite and required the use of a water tenk for dose measurement; therefore, corrections for the density and fluence differences between Lucite and water had to be considered. Our chamber material is the same as the phantom material (polystyrene); consequently, there is no need for such corrections to the measured signal when determining the absorbed dose in polystyrene. The determination of the absolute absorbed dose for clinical photon and electron beams at a given depth in phantom with the IGC is based on first principles, is simple to evaluate, and agrees well with results obtained with standard calibrated ionization chamber techniques.

Materials and methods

A 7 cm diameter polystyrene piston was fashioned to move inside a cylinder bored along the center of a 30 x 30 x 8 cm^3 polystyrene phantom. Graphite dag was painted on the top surface of the piston, and a 1.5 mm deep and 0.04 mm wide groove was cut through the graphite surface into the piston to form the 2.004 (1 ± 0.001) cm inner diameter measuring electrode and the guard ring of the chamber. The measuring electrode and the guard ring are both connected to ground (the measuring electrode through an electrometer) with electronically shield-

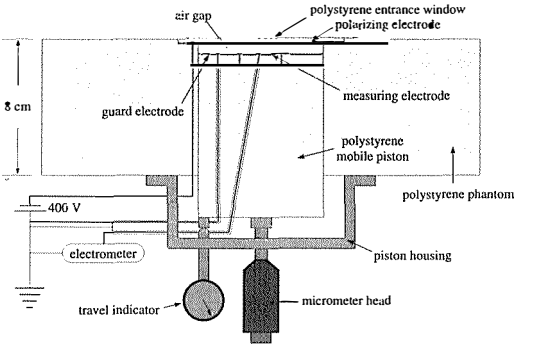

Figure 1. Schematic diagram of the ionization gradient chamber.

ed cables. The polarizing electrode consists of a 0.5 mm thick polystyrene disk painted with graphite dag and fastened to the top of the large phantom. The electronic potential of the polarizing electrode is maintained at ±400 V with respect to the collecting

electrode. The separation between the polarizing and measuring electrodes can vary between 0.5 mm and 10 mm, and is controlled by a micrometer mounted to the phantom body. The movement of the piston (*i.e.,* change in the air sensitive volume) is monitored by a calibrated distance travel indicator which is accurate to within 0.01 mm. In Figure 1 we show a schematic diagram of the IGC. Irradiations of the gradient chamber were performed with a cobalt-60 gamma source, photon beams in the energy range from 4 MV to 18 MV, and electron beams in the nominal energy range from 9 MeV to 18 MeV.

Results and discussion

The specific design of our IGC allows us to determine dQ/dm of *Eq. (4)* with relative ease and a high degree of accuracy. Since dm is directly proportional to the change dz in electrode separation, we can write *Eq. (4)* as follows:

$$D_{med} = \left(\frac{1}{\rho A}\right) \frac{dQ}{dz} W_{air} L_{air}^{poly}, \qquad (5)$$

with ρ the density of air at the ambient temperature and pressure, and A the area of the measuring electrode.

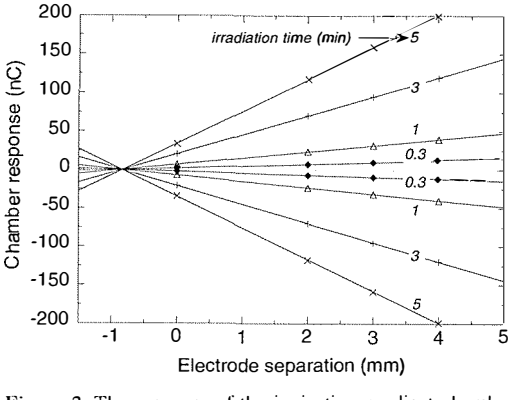

Figure 2. The response of the ionization gradient chamber as a function of electrode separation. The chamber was exposed to cobalt-60 radiation (field-size: 10 x 10 cm^2; source-surface distance: 80 cm; dose rate: 86.7 cGy/min). The buildup region consisted of 3.7 mm of polystyrene.

As shown in Figure 2, the response of our ionization gradient chamber to cobalt-60 radiation varies linearly with electrode separation (correlation coefficient ≥ 0.99995), with dose (irradiation time) a parameter. The chamber response is represented by the measured change Q corrected for the chamber collection efficiency[10, 11] at the polarizing voltage of 400 V and given electrode separation z. All ionization response curves for positive and negative chamber polarities intersect at the same location on the x-axis indicating the true zero electrode separation. We purposely did not calibrate our electrode separation to this intersection point in order to emphasize that there is no need to determine the separation in an absolute manner: only the relative variation in electrode separation is required in *Eq. (5)* The slopes dQ/dz obtained for the given doses in Figure 2 depend linearly on the dose as shown in Figure 3. Similar results were obtained in pulsed

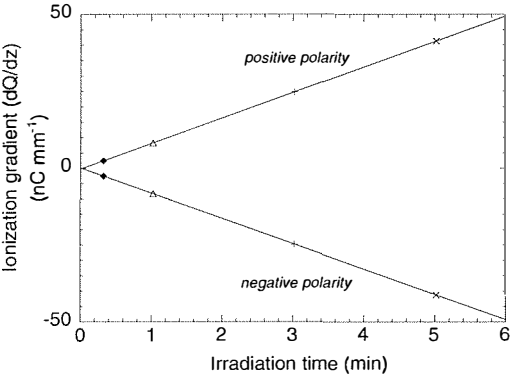

Figure 3. Ionization gradient dQ/dz as a function of the irradiation time. (dQ/dz was determined from data of *Figure 2.*).

photon and electron beams showing that (i) the chamber response is linear with dose and (ii) dQ/dz may be measured with a high degree of precision.

In Tables 1 and 2 we show how the ionization gradient chamber meets its main objective: the absolute dose determination in clinical photon and electron beams, respectively. Doses determined at a given depth in polystyrene with a calibrated Farmer chamber and the AAPM-TG21 protocol for photon beams and AAPM-TG25 protocol for electron beams are compared with doses determined at same depths in phantom with our polystyrene ionization gradient chamber. Tables 1 and 2 also give other relevant parameters used in the absolute dose measurements with the ionization gradient chamber. The discrepancies between doses determined with our uncalibrated gradient chamber and those obtained with the calibrated Farmer chamber are at most 1.08 % and 0.63 % for photon and electron beams, respectively, at all clinical energies indicating that the ionization gradient chamber can be used as an absolute dosimeter.

Table 1. Measurement of photon dose with the ionization gradient chamber.

1	2	3	4	5	6	7
Photon beam	Depth (mm)	$\bar{L}_{air}^{poly}$	dQ/dz ($nCmm^{-1}$)	Dose (IGC) (cGy)	Dose (TG 21) (cGy)	% difference
Co-60	3.7	1.113	8.274	83.41	83.18	+ 0.27
4 MV	10.1	1.108	9.640	97.26	96.79	+ 0.49
6 MV	50.1	1.103	8.313	83.81	84.23	– 0.49
10 MV	50.1	1.094	8.886	88.11	88.36	– 0.29
18 MV	50.1	1.078	9.397	92.56	93.57	– 1.08

(1) photon beam energy; (2) depth d in phantom; (3) ratio of restricted stopping powers[2] (Δ = 10 keV); (4) measured ionization gradient averaged over positive and negative polarities and corrected for charge recombination; (5) dose measured with ionization gradient chamber; (6) dose determined with the AAPM-TG21 protocol[2]; (7) percent difference between (5) and (6).

Table 2. Measurement of electron dose with the ionization gradient chamber.

1	2	3	4	5	6	7	8	9
Electron beam	$\bar{E}_0$ (MeV)	Depth (mm)	$\bar{E}(d)$ (MeV)	$\bar{L}_{air}^{poly}$	dQ/dz ($nCmm^{-1}$)	Dose (IGC) (cGy)	Dose (TG 25) (cGy)	% difference
9 MeV	8.1	15	5.24	1.017	10.469	95.56	96.01	– 0.47
12 MeV	10.8	15	7.96	0.988	10.825	95.83	96.44	– 0.63
15 MeV	13.5	10	11.60	0.964	11.233	97.74	98.21	– 0.49
18 MeV	16.1	10	14.42	0.952	11.484	98.94	98.51	+ 0.44

(1) electron beam nominal energy; (2) mean electron energy at phantom surface; (3) depth d in phantom; (4) mean electron energy at depth d; (5) ratio of restricted stopping powers[2] (Δ = 10 keV) at $\bar{E}$(d), (6) measured ionization gradient averaged over positive and negative polarities and corrected for charge recombination; (7) dose measured with ionization gradient chamber; (8) dose determined with AAPM-TG25 protocol[3]; (9) percent difference between (7) and (8).

Conclusions

Uncalibrated ionization gradient chambers built as part of the phantom in which the dose is measured behave as Bragg-Gray cavities and can be used reliably in the determination of absolute dose. In contrast to the dosimetry with calibrated chambers, the dosimetry with ionization gradient chambers appears simple and requires no troublesome correction factors to account for chamber properties and for the unavailability of high energy photon and electron calibrations at standards laboratories. With our gradient chamber design, no cumbersome apparatus is required to measure the plate separation absolutely in order to determine the absorbed dose in an absolute manner. The charge per unit air mass gradient can be measured accurately (to within 1%) with relative ease in a carefully designed and precisely built gradient chamber. This implies that absolute dose measurements with ionization gradient chambers could be added to the other three currently known absolute dosimetry techniques: calorimetry, chemical (Fricke) dosimetry, and standard free air ionization chamber.

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Book review

Epithelial hyperplastic lesions of the larynx

Vinko Kambič and Nina Gale

Elsevier Science B.V., Amsterdam, 1995. Pages: 265; Illustrations, Tables, Hard cover.
ISBN 0-444-82273-9. Price: 300 DEM

This book is the result of at least 3 decades' continuous clinical work and research at one institution and can be said to present the life experience of an eminent otolaryngologist. Its stated aim – to advance our understanding of laryngeal hyperplastic lesions – has been admirably served in several ways.

The author's own classification of these lesions, introduced in 1971, is similar to the current one of the WHO but clearer in some points with more logical terminology. The introduction of newer techniques, such as histochemistry, imunohistochemistry, morphometry, electron microscopy, flow cytometry, molecular pathology, etc. has made possible a far more exact classification of these lesions. This way, precancerosis can be and is clearly defined as atypical hyperplasia, and is also clearly separated from cancer in situ, while, on the other hand, simple hyperplasia and abnormal hyperplasia are clearly defined as benign lesions.

Own studies of "normal" distribution of ciliary and stratified squamous epithelium of the larynx is presented, showing no common pattern and marked variation. Own studies on distribution of cytokeratins within laryngeal epithelium and on smoking- and alcohol-connected hyperplasia are presented, as is the author's original finding of laryngeal asbestosis.

Strict terminology is advocated throughout, rendering obsolete such favored terms as "hyperkeratosis" and "epidermization" and casting doubt on the term "dysplasia".

Based on this clear classification, the whole gamut of laryngeal epithelial hyperplastic lesion is presented as clinical entities, corroborated with at least 15 years' own experience in each case and this is what, makes this book such a gem: intertwining the deepened understanding of pathology with clinical work in a continuous process through decades and then presenting the results in a comprehensive, orderly, elegant fashion. This clearly would not be possible without closely involving an accomplished pathologist from the very beginning. This book may be taken as an example of how rewarding can be the results of such co-operation.

The illustrations are numerous and high quality throughout, the bibliography comprehensive.

One is really hard put to find an inadequacy in this work, which ought to become a classic in its field. Perhaps, a diagnostic and treatment algorithm, to be used in difficult cases, would be helpful?

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RSNA news

Radiology grant recipients continue careers in research, academic medicine

Chicago – The Research and Education (R&E) Fund of the Radiological Society of North America is proceeding well toward its goal of attracting young researchers to careers in radiological research and academic medicine and helping to fill the gap left by cutbacks in government and other unrestricted funding, according to a survey of grant recipients.

A survey of the 147 researchers awarded grants over the past 10 years through the R&E Fund reports that more than 80 percent of respondents hold a faculty appointment in a medical school and more than 70 percent have continued in research, applying for additional funding.

The R&E Fund was established by the RSNA Board of Directors in November 1984. The first grants were given in 1986. The R&E recipients survey was fielded by the Society in the summer of 1995 to evaluate whether the R&E Fund was meeting its goals and to determine the extent to which past R&E Fund recipients have applied for and received funding to conduct additional research in radiology.

The findings are based on a response rate of 89 percent, or 131 of 147 past RSNA grant recipients. Highlights include:

• More than 80 percent of survey respondents (108 of 131) currently hold a faculty appointment in a medical school.

• 71 percent (93 of 131 respondents) of past RSNA grant recipients have continued their careers as researchers and applied for additional funding. The number of applications submitted per applicant ranged from 5.1 to 2.6.

• Of past RSNA grant recipients applying for funding for additional research, 773 percent (68 of 93) were successful in obtaining funding. The percentage was higher among individuals who had received RSNA grants before 1994; 78 percent successfully obtained additional funds.

• The 68 recipients who obtained funding for additional research since their RSNA grant generated 190 research projects and were the principal investigator in nearly 3 out of every 4 (74 percent) funded projects.

• RSNA grant recipients requested a total of $ 151.4 million for additional research over the 10-year period. More than 34 percent, or $ 52 million, of the requested amount was awarded.

• Between 48 and 84 percent of each category of RSNA grant recipients applied for funding for additional research: nearly 84 percent of scholars; 77 percent of seed grant recipients; 62 percent of fellows; and 48 percent of residents.

The RSNA Research and Education Fund was established with an initial gift of $ 1 million from the RSNA. The RSNA makes a donation equal to the administrative expenses of the fund each year so that every dollar donated supports programs. Today, there are nine grant programs: Scholars Program; Fellows Program, Research Resident Program; Seed Grants; Medical Student/Scholar Assistant Award; Medical Student Award; Roentgen Centennial Fellow; Roentgen Resident/Fellow Research Award; and Outstanding Researcher Award.

The RSNA is an association of 30.000 radiologists and physicists in medicine dedicated to education in the science of radiology. The Society's headquarters are located at 2021 Spring Rd., Suite 600, Oak Brook, Illinois 60521.

[Editor's Note: Individuals interested in donating to or obtaining information about the R&E Fund can contact Jennifer Boylan at the RSNA, 708/571-7868.]

Radiol Oncol 1996; 30: 144-7.

Notices

Notices submitted for publication should contain a mailing address, phone and/or fax number of a contact person or department.

Radiotherapy

September 1-5, 1996.

The ESTRO teaching course "Radiation Physics for Clinical Radiotherapy" will take place in Lueven, Belgium.

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

Esophagology

September 3-7, 1996.

The "5th Polydisciplinary World Congress" organised by O.E.S.O. will be held in Paris, France,

Contact Michele Liegeon, O.E.S.O., 2, boulevard Montparnasse, 75015 Paris; or call +33 1 45 66 91 15; or fax +33 1 45 66 50 72.

Gynaecological Oncology

September 15-21, 1996.

The advanced course will take place in Amsterdam, The Netherlands.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; Fax: +39 2 57 307 143.

Medical Oncology

September 19-21, 1996.

The advanced course will be offered in Milan, Italy.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; Fax: +39 2 57 307 143.

Leukaemia

September 19-21, 1996.

The advanced course "New Trends in the Treatment of Acute Leukaemia" will be offered in Briuni Islands.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; Fax: +39 2 57 307 143.

Medical Physics

September 22-26, 1996.

The "EFOMP (European Federation of Organisations for Medical Physics) Meeting" will be offered *at September, 1996.*

Contact Dr. W.W. Seelentag, Klinik fuer Radio-Oncologie, Kantonsspital, CH-9007 St. Gallen, Switzerland.

Leukaemia

September 23-25, 1996.

The "2nd Educational Forum on Leukaemia"will take place in Bergamo, Italy.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; Fax: +39 2 57 307 143.

Radiotherapy

September 23-26, 1996.

The "15th Annual ESTRO Meeting" will be offered in Vienna, Austria.

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

Radiotherapy

September 23-26, 1996.

The "3th Postgraduate Teaching Course", organised by ERTED (European Radiotherapy Technologist Education Development Group) will be held in Vienna, Austria, at the time of the 15th Annual ESTRO Meeting.

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

As a service to our readers, notices of meetings or courses will be inserted free of charge.

Please sent information to the Editorial office, Radiology and Oncology, Vrazov trg 4, 1105 Ljubljana, Slovenia.

Medical Oncology

September 26-28, 1996.

The advanced course will take place in Milan, Italy.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; Fax: +39 2 57 307 143.

EORTC Studies

September 27, 1996.

The advanced course "One Day Introduction to EORTC Studies" will be organized in collaboration with ESO and will take place in Brussels, Belgium.

Contact EORTC Education and Training Division, Av. E. Mounier 83, 1200 Brussels, Belgium; or call +32 2 772 4621; Fax: +32 2 772 6233.

Paediatric Oncology

September 27-28, 1996.

The ESTRO teaching course "Paediatric Oncology" will take place in Vienna, Austria.

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

Paediatric Oncology

September 29-30, 1996.

The training course will take place in Vienna, Austria.

Contact European School of Oncology, Mrs. D. Gollubics c/o Arztekammer fuer Wien, Fortbildungsreferat, Weihburggasse 10-12, 1010 Vienna, Austria; or call +43 1 515 012 93; Fax: +43 1 515 012 40.

Cancer Clinical Trials

October, 1996.

The advanced course will be organized in collaboration with ESO and will take place in Brussels, Belgium.

Contact EORTC Education and Training Division, Av. E. Mounier 83, 1200 Brussels, Belgium; or call +32 2 772 4621; Fax: +32 2 772 6233.

Lymphomas

October, 1996.

The training course will be offered in Vismir, Greece.

Contact European School of Oncology, c/o Egnatia Epirus Foundation, 9 Hatziyianni St., 115 28 Athens, Greece; or call +30 1 724 3144; Fax: +30 1 724 3145.

Breast Reconstructive Surgery

October 3-5, 1996.

The advanced course will be offered in Milan, Italy.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; Fax: +39 2 57 307 143.

Psycho-Oncology

October 4-6, 1996.

The MSKCC course will be organized in collaboration with ESO and will take place in New York, USA.

Contact Ms. L. Popoff, CME Office New York; or call +1 212 639 6754; Fax: +1 212 772 6233.

Neuro-Oncology

October 6-9, 1996.

The "2nd Congress of the European Association for Neuro-Oncology EANO II" will be held in Wuerzburg, Germany.

Contact Secretariat IMEDEX, Bruistensingel 360 , P.O. Box 3282, 5203 DG's Hertogenbosch, The Netherlands; or call +31 73 6 429 285; or fax +31 73 6 414 766.

Breast Cancer

October 7-9, 1996.

The advanced course "Breast Cancer and Breast Reconstructive surgery will be offered in Milan, Italy.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; Fax: +39 2 57 307 143.

Head and Neck Cancer

October 10-12, 1996.

The training course "Head and Neck Cancer, Including Thyroid Cancer" will take place in Vienna, Austria.

Contact European School of Oncology, Mrs. D. Gollubics c/o Arztekammer fuer Wien, Fortbildungsreferat, Weihburggasse 10-12, 1010 Vienna, Austria; or call +43 1 515 012 93; Fax: +43 1 515 012 40.

Chest Tumours

October 15-18, 1996.

The advanced course will take place in London, U.K.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; Fax: +39 2 57 307 143.

Transplantation

October 18-20, 1996.

The training course "Stem Cell Transplantation and Solid Tumours" will take place in Vienna, Austria.

Contact European School of Oncology, Mrs. D. Gollubics c/o Arztekammer fuer Wien, Fortbildungsreferat, Weihburggasse 10-12, 1010 Vienna, Austria; or call +43 1 515 012 93; Fax: +43 1 515 012 40.

Radiotherapy

October 20-24, 1996.

The ESTRO teaching course "The Role of Radiotherapy in Integrated Cancer Care" will take place in *????*.

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

Gastroenterology

October 22 - November 1, 1996.

The symposium "Gastroenterology Week Freiburg" will take place in Freiburg, Germany.

Contact FF e.V., P.O. Box 6529, D-79041 Freiburg/Br., Germany; or call +49 761 130 3425.

Radiobiology

October 27-31, 1996.

The ESTRO teaching course "Basic Clinical Radiobiology" will take place in Izmir, Turkey.

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

Radiation Oncology

October 28 - November 1, 1996.

The "Annual Meeting of American Society for Therapeutic Radiology and Oncology ASTRO" will take place in Los Angeles, CA, USA.

Contact American Society Therapeutic Radiology/Oncology, 1101 Market Street, 14th Floor, Philadelphia, Pa 19107-2990, USA; or call +1 215 574 3180; or fax +1 215 928 0153

Medical Oncology

November 2-5, 1996.

The "21th ESMO Congress" will be held in Vienna, Austria.

Contact ESMO Secretary, Via Soldino 22, CH-6900 Lugano, Switzerland; or call +41 93 61 31 70; or fax +41 9361 31 72.

MRI and CT

August 1–4, 1996.

The Gold Coast MRI & CT Conference Royal Pines Resort, Queensland, Australia.

Contact: Dr Peter Scally. Mater Hospital, South Brisbane, Australia. 4101. Telephone +61 7 3266 3871; Fax: +61 7 3266 9087.

US in Medicine

September 5–8, 1996.

Australasian Society for Ultrasound in Medicine, 26th Annual Scientific Meeting, Brisbane.

Contact: Dr Peter Scally. Mater Hospital, South Brisbane, Australia. 4101. Telephone +61 7 3266 3871; Fax: +61 7 3266 9087.

Radiology

October 5–11, 1996.

Royal Australasian College of Radiology, 47th Annual General and Scientific Meeting. Burswood Convention Centre, Perth, Western, Australia.

Contact: Dr Peter Scally. Mater Hospital, South Brisbane, Australia. 4101. Telephone +61 7 3266 3871; Fax: +61 7 3266 9087.

Medical Registration

October 28–30, 1996.

2nd International Conference on Medical Registration World Congress Centre, Melbourne, Australia.

Contact: Dr Peter Scally. Mater Hospital, South Brisbane, Australia. 4101. Telephone +61 7 3266 3871; Fax: +61 7 3266 9087.

Competence in Medicine

October 31–November 2, 1996.

Assessment of Clinical Competence in Medicine, a Workshop based Conference, Customs House, Brisbane, Australia.

Contact: Dr Peter Scally. Mater Hospital, South Brisbane, Australia. 4101. Telephone +61 7 3266 3871; Fax: +61 7 3266 9087.

Breast Cancer

November 13–17, 1996.

Third International Leura Breast Cancer Conference, covering all aspects of breast from molecular biology, through

screening, treatment and the management of advanced disease. Fairmont Resort, Leura, New South Wales, Australia.

Contact: Dr Peter Scally. Mater Hospital, South Brisbane, Australia. 4101. Telephone +61 7 3266 3871; Fax: +61 7 3266 9087.

US in Medicine

March 1997.

Australasian Society for Ultrasound in Medicine, 12th Vascular Workshop, Sydney.

Contact: Dr Peter Scally. Mater Hospital, South Brisbane, Australia. 4101. Telephone +61 7 3266 3871; Fax: +61 7 3266 9087.

Radiology

September 11–15, 1997.

Royal Australasian College of Radiology 48th Annual General and Scientific Meeting, Adelaide Convention Centre, South Australia.

Contact: Dr Peter Scally. Mater Hospital, South Brisbane, Australia. 4101. Telephone +61 7 3266 3871; Fax: +61 7 3266 9087.

US in Medicine

September 18–21, 1997.

Australasian Society for Ultrasound in Medicine, 27th Annual Scientific Meeting, Tasmania.

Contact: Dr Peter Scally. Mater Hospital, South Brisbane, Australia. 4101. Telephone +61 7 3266 3871; Fax: +61 7 3266 9087.

Radiology

October 15–19, 1998.

Royal Australasian College of Radiology 49th Annual General and Scientific Meeting. Brisbane Convention Centre, Queensland.

Contact: Dr Peter Scally. Mater Hospital, South Brisbane, Australia. 4101. Telephone +61 7 3266 3871; Fax: +61 7 3266 9087.

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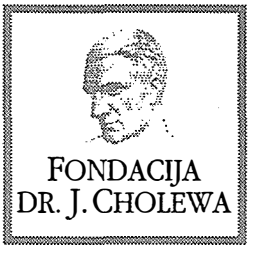

Activity of "Dr. J. Cholewa Foundation" for cancer research and education – report for the first and second quarter of 1996

"Dr. J. Cholewa Foundation" for cancer research and education continues its activity in the first half of 1996 as it was outlined at the meetings of the executive and scientific councils of the Foundation at the end of 1995.

The two researchers that were bestowed research grants in 1995 successfully concluded their basic oncological research studies in reputed cancer research institutions in The United States of America and France.

As mentioned in the previous issue of "Radiology and Oncology", the Foundation invited all the interested individuals to submit their applications for the research grants for scientific work in oncology. Public announcement was made in a major national daily newspaper, and research grants were awarded to four of the applicants, three from Ljubljana and one from Maribor.

The Foundation supported the organisation of a symposium titled "Diagnostic Algoritms in Malignant Disease" that took place in Laško in May, 1996.

At the meeting between the representatives of "Dr. J. Cholewa Foundation" for cancer research and education and "Slovenian Scientific Foundation" (Slovenska Znanstvena Fondacija) the ways of possible future collaboration were explored.

"Dr. J. Cholewa Foundation" for cancer research and education will participate in the sponsorship of the "International Conference on Epithelial Lesions of the Larynx", to be held on October 28–30th, 1996, in Ljubljana, Slovenia.

Collaboration with European School of Oncology from Milan will be extended. Specific points and goals of this collaboration will be discussed with high level officials of the European School of Oncology in the near future.

A decision was taken that the general assembly of "Dr. J. Cholewa Foundation" for cancer research and education will take place in the middle of June, 1996. It is expected the names of new members of the executive council of the Foundation will be proposed on this occasion.

From the past and present activity of the Foundation it is clear that it continues to follow its goals.

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

13th ANNUAL MEETING of the EUROPEAN SOCIETY FOR MAGNETIC RESONANCE IN MEDICINE AND BIOLOGY ESMRMB '96

Conference Dates:	September 12–15, 1996 Prague, Czech Republic
Administrative and Scientific Secretariat:	ESMRMB-Office Neutorgasse 9/2a A - 1010 Vienna/Austria
Telephone #	(+ 43/1) 535 13 06
Fax #	(+ 43/1) 533 40 649
E-mail	milan.hajek@medicon.cz
WWW:	http://www.medicon.cz/esmrmb.html
President (Congress):	Dr. Milan Hájek
President (Society):	Prof. Dr. Patrick J. Cozzone
Estimated Attendance:	1.500

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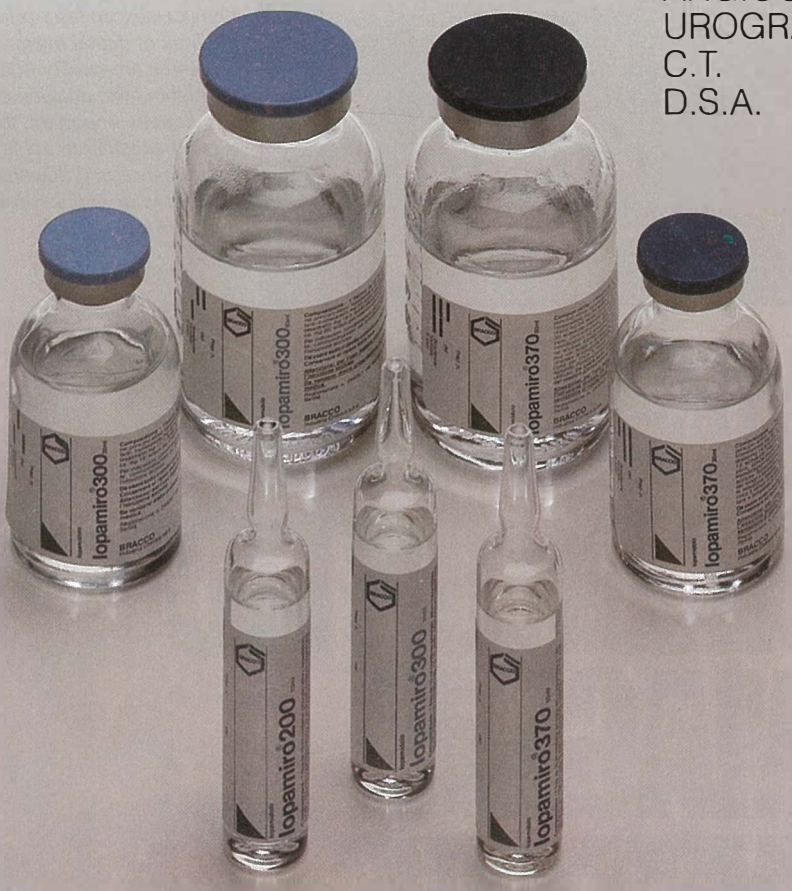

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Fax: (+386 61) 1331 044

Unabridged articles which should be submitted in triplicate to the Organizing Committee by the beginning of the Congress will be published in No. 4/96 of RADIOLOGY AND ONCOLOGY.

AUSTRIA
November 8–9, 1996
Second Announcement

EUROPEAN SOCIETY OF MUSCULOSKELETAL RADIOLOGY (ESSR)

THIRD ANNUAL MEETING

Patronage

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Dr. Josef DECHANT
Mayor of the City of Salzburg

Austrian Röntgen Society (ÖRG)

together with

AUSTRIAN SOCIETY FOR BONE AND MINERAL RESEARCH (ASBMR)

Friday, November 8, 1996, 14.00–17.00
Humboldt-Room

ANNUAL MEETING OF THE AUSTRIAN ASSOCIATION OF RADIOLOGICAL TECHNICIANS

Friday, November 8, 1996, 9.00–12.30
Humboldt-Room

Symposium on

ORGAN SPARING TREATMENT IN ONCOLOGY

Ljubljana, Slovenia
19–21 June 1997

Organized by:

Institute of Oncology, Ljubljana, Slovenia

Under the auspices of:

Alps Adriatic Working Community

Design and Content

The Symposium is designed for medical doctors and other specialists involved in cancer treatment who wish to exchange experiences with oncologists and to contribute to upgrading the knowledge in organ sparing treatment. It will cover the following topics:

- Breast Conserving Therapy
- Bladder Sparing Treatment in Muscle Invasive Bladder Carcinoma
- Organ Sparing Treatment in Head and Neck Carcinoma
- Organ Sparing Treatment in Soft Tissue Tumors
- Quality of Life after Organ Sparing Treatment

Mailing Address

Organizing Committee
Institute of Oncology
Zaloška 2, 1105 Ljubljana
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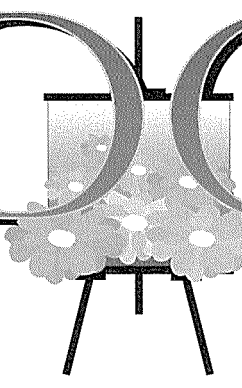

Moč opioidnega analgetika brez opioidnih stranskih učinkov

- centralno delujoči analgetik za lajšanje zmernih in hudih bolečin
- učinkovit ob sorazmerno malo stranskih učinkih

Indikacije: Srednje močne do močne akutne ali kronične bolečine. ***Po tristopenjski shemi Svetovne zdravstvene organizacije za lajšanje bolečin pri bolnikih z rakavim obolenjem tramadol odpravlja srednje hudo bolečino ali bolečino druge stopnje.*** **Kontraindikacije:** Zdravila ne smemo dajati otrokom, mlajšim od 1 leta. Tramadola ne smemo uporabljati pri akutni zastrupitvi z alkoholom, uspavali, analgetiki in drugimi zdravili, ki delujejo na osrednje živčevje. Med nosečnostjo predpišemo tramadol le pri nujni indikaciji. Pri zdravljenju med dojenjem moramo upoštevati, da 0,1 % zdravila prehaja v materino mleko. Pri bolnikih z zvečano občutljivostjo za opiate moramo tramadol uporabljati zelo previdno. Bolnike s krči centralnega izvora moramo med zdravljenjem skrbno nadzorovati. **Interakcije:** Tramadola ne smemo uporabljati skupaj z inhibitorji MA●. Pri sočasni uporabi zdravil, ki delujejo na osrednje živčevje, je možno sinergistično delovanje v obliki povečane sedacije, pa tudi ugodnejšega analgetičnega delovanja. **Opozorila:** Pri predoziranju lahko pride do depresije dihanja. Previdnost je potrebna pri bolnikih, ki so preobčutljivi za opiate, pri starejših osebah, pri miksedemu in hipotiroidizmu. Pri okvari jeter in ledvic je potrebno odmerek zmanjšati. Bolniki med zdravljenjem ne smejo upravljati strojev in motornih vozil. **Doziranje in način uporabe:** *Odrasli in otroci, starejši od 14 let:* Injekcije: 50 do 100 mg i.v.,i.m.,s.c.; intravensko injiciramo počasi ali infundiramo razredčeno v infuzijski raztopini. Kapsule: 1 kapsula z malo tekočine. Kapljice: 20 kapljic z malo tekočine ali na kocki sladkorja; če ni zadovoljivega učinka, dozo ponovimo čez 30 do 60 minut. Svečke: 1 svečka; če ni učinka, dozo ponovimo po 3 do 5 urah. *Otroci od 1 do 14 let:* 1 do 2 mg na kg telesne mase. Dnevna doza pri vseh oblikah ne bi smela biti višja od 400 mg. **Stranski učinki:** Znojenje, vrtoglavica, slabost, bruhanje, suha usta in utrujenost. Redko lahko pride do palpitacij, ortostatske hipotenzije ali kardiovaskularnega kolapsa. Izjemoma se lahko pojavijo konvulzije. **Oprema:** 5 ampul po 1 ml (50 mg/ml), **5 ampul po 2 ml (100 mg/2 ml)**, 10 ml raztopine (100 mg/ml), 20 kapsul po 50 mg, 5 svečk po 100 mg.

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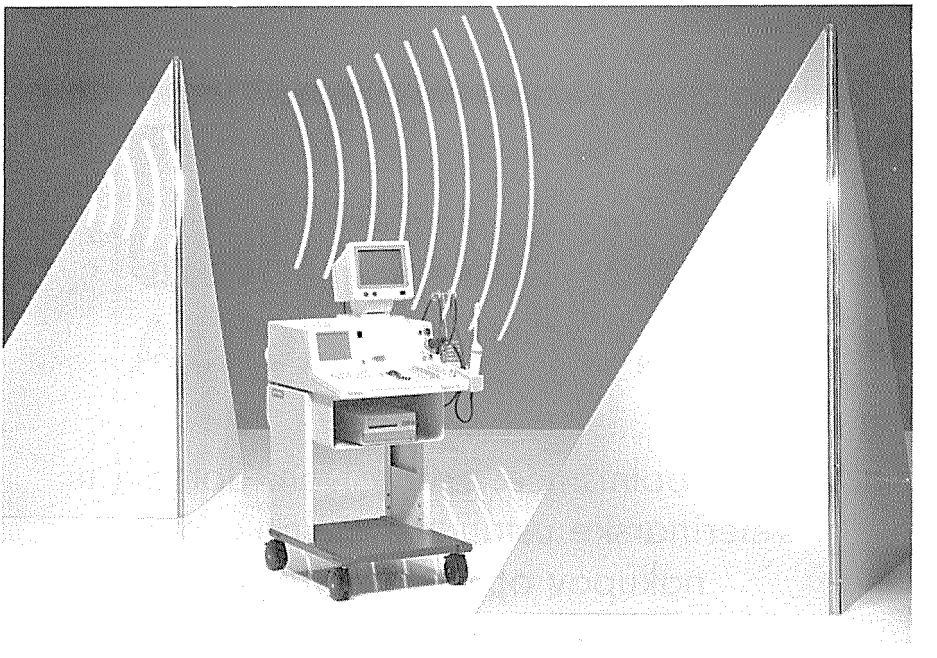

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- **pomembno manjša pogostost alopecije**
- **za kakovostnejše življenje bolnic**

Sestava: 1 steklenička vsebuje 10 mg ali 20 mg pirarubicinovega hidroklorida v obliki liofilizirane snovi. **Oprema:** 10 stekleničk po 10 mg ali 20 mg pirarubicina. **Farmakoterapijska skupina po klasifikaciji ATC:** citotoksični antibiotik/antraciklin. **Indikacije:** zdravilo je indicirano za zdravljenje bolnic z napredovano stopnjo karcinoma dojke v sklopu polikemoterapijskega protokolnega zdravljenja. Pirarubicin uporabljamo pri adjuvantnem in neoadjuvantnem zdravljenju bolnic s karcinomom na dojkah. **Kontraindikacije:** uporaba Piracina je kontraindicirana pri bolnicah z močno okvarjenim delovanjem srca, pri nosečnicah in doječih materah. **Previdnostni ukrepi:** previdnost je potrebna pri dajanju zdravila bolnikom z okvarjenim delovanjem ledvic in jeter (tveganje, da se zdravilo kopiči in okrepi njegovo toksično delovanje). Pred začetkom vsakega novega cikla zdravljenja je treba pregledati celotno krvno sliko in bolničin kardiovaskularni status. **Interakcije:** znana je delna navzkrižna rezistenca tumorskih celic pri zdravljenju z drugimi antraciklinskimi citostatiki. **Doziranje in način uporabe:** pri zdravljenju bolnic s karcinomom na dojki priporočamo dajanje Piracina v odmerku 50 mg/m^2 vsake 3 tedne v sklopu sheme zdravljenja, ki vključuje še ciklofosfamid in 5-fluorouracil (shema FPC). Zdravilo uporabljamo izključno intravensko. **Stranski učinki:** toksični učinek, ki omejuje odmerek, je supresija delovanja kostnega mozga. Pri tretjini bolnic nastane reverzibilna granulocitopenija (do izboljšanja pride v treh do štirih tednih). Pri polovici bolnic se pojavi blažja slabost, ki jo spremlja kratkotrajno bruhanje. Mogoče je kardiotoksično delovanje Piracina, vendar je redkejše in blažje kot pri doksorubicinu. Popolna reverzibilna alopecija se pojavi le pri približno 25 % bolnic. Piracin zelo redko povzroči nastanek stomatitisa in amenoreje.

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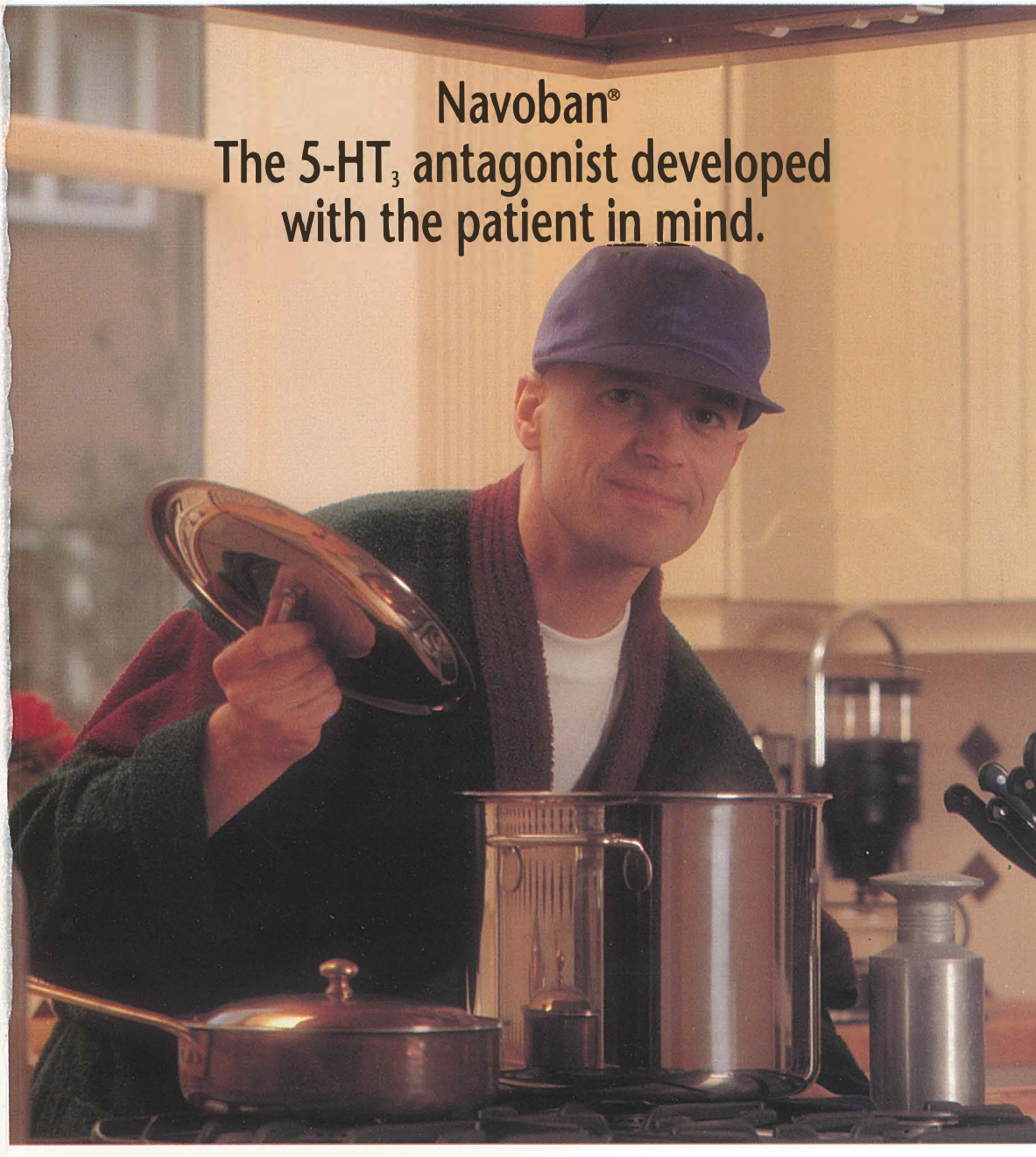
1. Dent RG. Cole P. *In vitro* maturation of monocytes in squamous carcinoma of the lung. *Br J Cancer* 1981, **43:** 486-95.

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

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



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