© Med Sci Monit, 2004; 10(5): BR144-150
PMID: 15114263

WWW.MEDSCIMONIT.COM
Basic Research

Received: 2003.04.25
Accepted: 2003.10.24
Published: 2004.05.01

Serum cathepsin B activity during regression of Morris hepatoma 5123 D

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Artur Fedorowski[1,3], Andrzej Steciwko[1], Jerzy Rabczynski[2]

[1] Department of Family Medicine, Wrocław Medical University, Wrocław, Poland
[2] Department of Pathological Anatomy, Wrocław Medical University, Wrocław, Poland
[3] Department of Clinical Medicine, Family Medicine Research Centre, Örebro University, Örebro, Sweden

Source of support: This study was partially supported by grant no. 683 from Wrocław Medical University.

Summary

Background: Serum cathepsin B activity has been considered a potential marker of tumor progression. We previously demonstrated that low-frequency electromagnetic stimulation (LF EMS), called bioresonance therapy (BRT), may both accelerate and inhibit the growth of transplantable hepatoma (Morris type 5123D), including total tumor regression. The aim of this study was to assess serum cathepsin activity during tumor progression and regression.

Material/Methods: Of 60 female rats inoculated with Morris hepatoma cells, 45 were treated with BRT, and the remaining 15 were left without treatment. Fifteen rats without inoculated tumors served as controls. Serum cathepsin B activity was determined, tumor volumes were measured, and histological examinations of the tumor tissues were performed.

Results: Of the 45 BRT-treated rats, tumor regression was observed in 31 rats, and serum cathepsin activity was analyzed in these rats. In all non-treated rats, tumor progression was observed. Serum cathepsin B activity was significantly higher in both the BRT-treated group (27.8±4.1 U/I, $p<0.01$) and the tumor-bearing group (19.9±2.5 U/l, $p<0.05$), as compared to the controls (13.3±3.4U/l).

Conclusions: Cathepsin B may play an important role, not only in tumor expansion, but also during the processes of cancer cell death and resorption. High circulating levels may thus correspond to effective therapeutic response in the course of antitumor treatment.

key words: **neoplasm regression • cancer cell death • apoptosis • necrosis • low-frequency electromagnetic stimulation • bioresonance**

Full-text PDF: http://www.MedSciMonit.com/pub/vol_10/no_5/3647pdf

Word count: 2518
Tables: 2
Figures: 5
References: 30

Author's address: Artur Fedorowski, MD, PhD, Department of Clinical Medicine, Family Medicine Research Centre, AFC S-Huset, Örebro University Hospital, 701 85 Örebro, Sweden, e-mail: artur.fedorowski@orebroll.se

BACKGROUND

Cathepsin B (EC 3.4.22.1) is a lysosomal cysteine protease. Under normal conditions it takes part in the degradation of proteins, which enter the lysosomal system through endocytosis, phagocytosis or autophagia [1,2]. Cathepsin B acts both as an endopeptidase and peptidildipeptidase, thus participating in both early and late stages of protein degradation [3]. The role of cathepsin B in the process of cancer invasion and metastasis has been under discussion for more than ten years [4,5]. Increased cathepsin B expression has been observed in the proliferating blood vessels during glioblastoma and prostate cancer progression, which may indicate that cathepsin B participates in angiogenesis [6,7]. The participation of this enzyme in the process of metastasis seems to be confirmed by the fact that its membrane activity has been detected in metastatic melanoma and colon cancer cells. Inhibition of extracellular cathepsin B activity by oral administration of a soluble selective enzyme inhibitor causes significant reduction in the number and dimension of colon cancer metastases in rat liver lobes [3,8]. It has also been reported that some cancer cells are able to produce the pro-enzyme, procathepsin B [3]. However, there are also several reports in which cathepsin B activity has been found to be unchanged in tumors compared to non-tumor tissues [9]. Furthermore, increased serum cathepsin B activity has been found in patients without neoplastic diseases, for example in patients with liver cirrhosis [10], and some authors emphasize its participation in inflammatory processes, such as pneumonia, pancreatitis, or hepatitis [11,12]. Moreover, there are some published reports in which the correlation between carcinoma progression and cathepsin B is not unequivocal [13,14]. Thus it remains unclear whether increased cathepsin B activity in body tissues and liquids could be used as a biochemical marker of tumor progression.

The aim of our study was to assess cathepsin B activity in different phases of tumor regression, in order to shed more light on cathepsin B as a tumor marker. For this purpose, rats were transplanted with Morris hepatomas, and tumor regression was induced by low-frequency (LF) electromagnetic (EM) stimulation, known as bioresonance therapy (BRT) [15-18].

MATERIAL AND METHODS

Animals: The study was carried out on 83 rats of Buffalo breed, females, age 4 months, body mass 210.0±15.0 g. The animals were kept in plastic cages with a liner of wood shavings. The cages were placed in a ventilated and illuminated room at a temperature of ca. 22°C. The animals were fed with standard laboratory granules and water ad libitum.

The study was focused on Morris tumors (hepatomas, 5123 D), maintained by serial passage in the animal laboratory of the Department of Pathological Anatomy, Wrocław Medical University. Morris hepatoma is a transplantable tumor derived from rat hepatocytes. The 5123 variant is moderately differentiated, grows locally, and gives early metastases to the lungs [19]. According to standard passage procedure, the tumor was excised from the tumor-bearer 3 weeks after implantation. All connective tissue and necrotic foci were removed. A homogenous mass was prepared from the tumor sample in a glass vessel, under sterile conditions.

An electronic device (BICOM, version 4.1, model B 15, manufactured by REGUMED Regulative Medizintechnik Gmbh, Graefelfing, Germany) was used in our experiments. This device is an EM wave modulator designed for the needs of specific EM stimulation, known as bioresonance therapy (BRT). It contains 3 basic elements: (a) input (b) signal processing circuits and (c) output. The input signal (described as *endogenous*) is considered to originate from biological objects that are placed in direct contact with the specially designed electrodes. In this experiment, flat electrodes with gold-plated brass on top were applied, insulated on the bottom by a cork layer, with magnetic foil between the cork and the brass layer. The signal processing circuits are made of the following optionally activated components:

- 1 low- and high-pass filters that enable regulation of the transmitted band in the range 10 Hz – 1 kHz and 1–150 kHz respectively
- 2 phase inverter
- 3 absorption filter (patent-protected molecular filter)
- 4 amplifier/attenuator (range: 64–0.025 times)
- 5 a system enabling sweeping of the whole transmitted frequency band (10 Hz–150 kHz), consisting of (i) wobbulator and (ii) tuned pass band filter characterized by a frequency range covering one octave around the fine-tuning frequency, in a pre-defined cyclic period (3–18 sec).

Output signal (i.e. input signal subjected to modulation and transmitted to the output) is emitted by an identical electrode, and connected to the output lead of the device. Modulation parameters and exposure time can be pre – programmed on the front panel.

Figure 1 presents the general trial profile. Tumor homogenates were intramuscularly implanted in 73 female rats in the form of injections into the right femoral regions (dose volume: 0.3 ml).

We decided to use the following model of EM stimulation: 3 individuals were randomly selected to be placed during experimental exposure in a plastic cage with the input electrode at the bottom, thus serving as a source of input signal for BRT (Group I in Figure 1). 60 females were divided into 4 equal groups (15 individuals each), three experimental (A,B,C) and one control (R). During exposure, the individuals from groups A–C were placed on the output electrode in a plastic cage (max. 3 at a time). The following exposure parameters were applied:

1. Group A – tuned pass band filter on, sweeping time 3 sec, phase inversion on, amplification 64×, exposure time 15 min.
2. Group B – as above, except for amplification 8×.
3. Group C – combined parameters for group A and B, i.e. amplification 64×15min and then amplification 8×15 min (total time: 2×15 min).

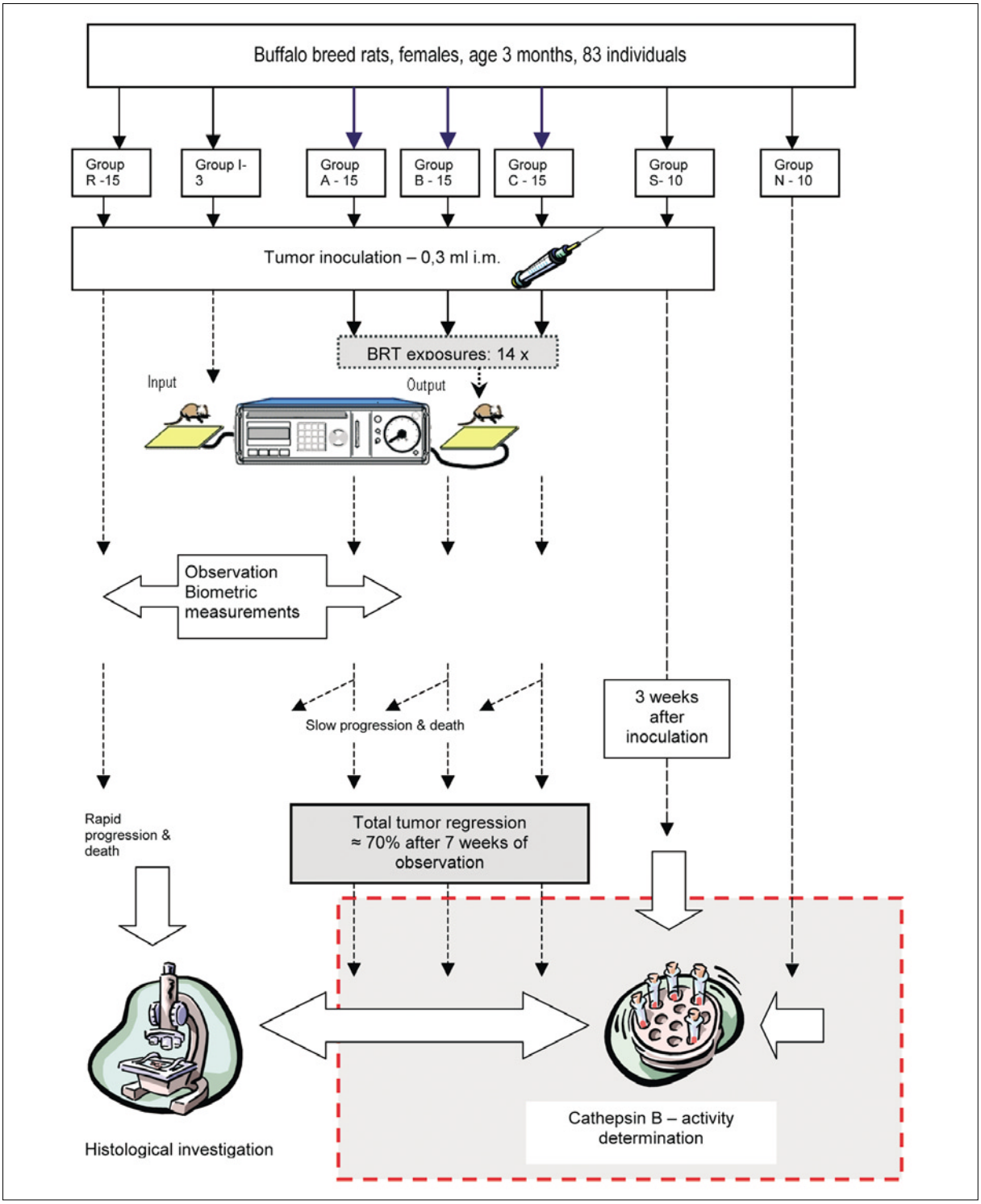

Figure 1. Trial profile. Rats were divided into the following groups: A,B,C – experimental groups (injected initially with Morris tumor, BRT – treated from the 3rd till 18th day, observed for 63 days; survivors were classified as tumor regression and subsequently anaesthetized, blood samples were collected directly from the left ventricle, and histological investigations were performed), I – 'input group' (injected with Morris tumor, placed on the input electrode during all BRT – exposures from the 3rd till 18th day i.e. untreated, no systematic biometric observations were performed, presence of growing tumor was confirmed by palpation), R – first control group (injected with Morris tumor, untreated, observed till natural death – about 4–5 weeks– and only final histological investigations were performed), S – second control group (injected with Morris tumor, untreated, observed till the 21st day after tumor inoculation, anaesthetized, blood samples were collected directly from the left ventricle, and no histological investigations were performed), group N – third control group (not injected, untreated, blood samples collected as above). Blood samples (groups A,B,C – regression only; groups S and N – all animals) were used to determine serum cathepsin B activity by Barett's fluorimetric assay. Histological investigation was carried out using cuttings from the primary tumor locus (1) and the lungs (2).

Table 1. Mean tumor volume (ml) in the experimental groups vs. controls (R) during the first 3 weeks of observation after tumor inoculation.

Day	Group A	Group B	Group C	Group R
11	4.7±1.9*	4.6±2.4*	4.5±1.2*	8.5±3.0
14	5.6±2.0*	6.4±3.1*	6.7±2.0*	13.7±3.8
17	7.0±2.7*	7.4±4.2*	8.0±3.1*	17.0±4.3
21	6.9±3.3*	7.3±4.6*	8.2±3.7*	22.9±5.8

* $p<0.001$

EM stimulation was carried out daily for a total of 14 days, beginning with the day 3 (i.e. days 3–18, excluding Sundays). The animals were observed for 63 days (9 weeks) i.e. double the mean survival time of unexposed tumor-bearing rats. In order to monitor tumor growth in groups A–C and detect tumor regression, a biometric method was applied. As Morris hepatoma is a solid tumor and grows locally, tumor volume can be estimated *in vivo* according to the following formula:

$$V = l \times w^2/2$$

where l = vertical length and w = horizontal width. Measurements were made initially on day 9 and subsequently at 3-day intervals. On the 63rd day, all the animals that had survived the observation period and biometrically demonstrated complete tumor regression (the regression group) were anesthetized with ether. The thorax was opened and a blood sample from the left ventricle was collected. The amputated right hind limbs containing the implantation locus were saved for further histological investigation, which included examination of paraffin cuttings from central and peripheral zones around the implantation locus. The specimens were stained with hematoxylin-eosin (HE). Morphological assessment was carried out using an Jenaval microscope (Carl Zeiss Jena GmbH, Jena, Germany) and the Multi Scan version 5.10 picture analysis system (Computer Scanning System Ltd, Warsaw, Poland).

A group of 10 implanted and unexposed animals (S) formed a separate control group (apart from the control group R) for determination of serum cathepsin B-like activity in tumor-bearers with fully developed hepatoma. Another group, consisting of 10 non-implanted and non-treated individuals (N), served a reference group, used to determine basic enzyme activity in rats. Blood samples were collected on the 21st day after tumor implantation, according to the same procedure used for the regression group (see above).

Serum from the blood samples was used to determine cathepsin B –like activity. Enzymatic activity was estimated according to the fluorimetric assay by Barret in the presence of N – α – carboxybenzoyl – Arg – Arg – 7 – amido – 4 – methylcoumarin (synonym: Z-RR-AMC; MDL number: MFCD00133576, Sigma-Aldrich, USA) as a substrate [20]. The reaction mixture was composed of 0.5 ml of the investigated material (serum diluted 1:10 with a 0.1 % Brij 35 solution) and 0.25 ml of 0.4 M phosphate buffer, pH 6.0, which contained 4 mM Na2EDTA and 8 mM cysteine hydrochloride added ex tempore. After 5 minutes of pre-incubation at 40°C, 0.25 ml of 20 μM substrate was added, followed by 20 min. incubation at the same temperature. The enzymatic reaction was stopped by adding 1.0 ml of interrupting reagent, containing 100 mM natrium monochloroacetate in pH 4.3 buffer. Each actual test was compared to a blind trial, in which the investigated material was added after enzymatic reaction had been interrupted. The fluorescence of the reaction product was measured using an LS 50B Perkin-Elmer Spectrofluorimeter with an activation beam of 370 nm and an emission peak of 460 nm. The results are expressed in U/L, where one unit (U) of activity is defined as the amount of enzyme degrading 1 nmol of substrate per min in the given conditions.

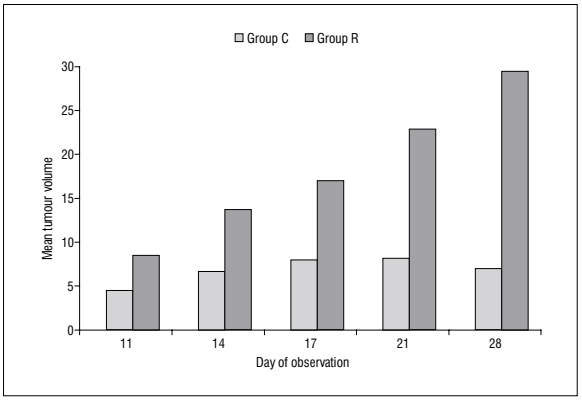

Figure 2. Tumour growth dynamics – group C vs. R.

Statistical analysis

Statistical analysis was performed using Statgraphics Plus 6.0 for DOS software (Manugistics, Inc, Rockville, Maryland, USA). The Hypothesis of Two Means Equality was applied (TWOSAM procedure) with estimation of the significance level (assuming $p<0.05$ as significant). Mean value and standard deviation were calculated for each value.

RESULTS

The tumor growth rate was significantly reduced in the exposed groups (A–C), where two phenomena could be observed in alternation:

1. Successive tumor regression after an initial period of slow progression, with a critical date between day 17 and 21. From this date onwards the regression processes dominated, resulting in a total decay of tumor mass.
2. Tumor growth deceleration, associated with a longer survival time compared to control group (slow-growing tumor).

Table 1 shows the results of biometric observation, which was continued until day 21. After this date, these two processes led to regression of the tumors in many animals; in some cases, there was slow progression with subsequent death. The survival rates were as follows: 9 rats in group A, 10 rats in group B, and 12 rats in group C. Thus, out of 45 BRT-treated rats, we observed total tumor regres-

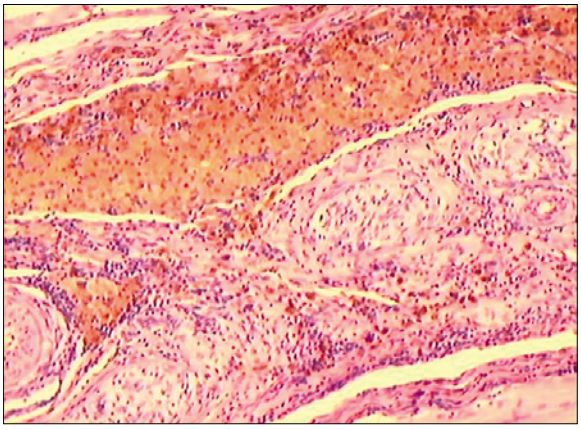

Figure 3. Implantation locus: resorptive granulomas and hemosiderine deposits. No trace of intact hepatoma cells can be found (HE, 120×).

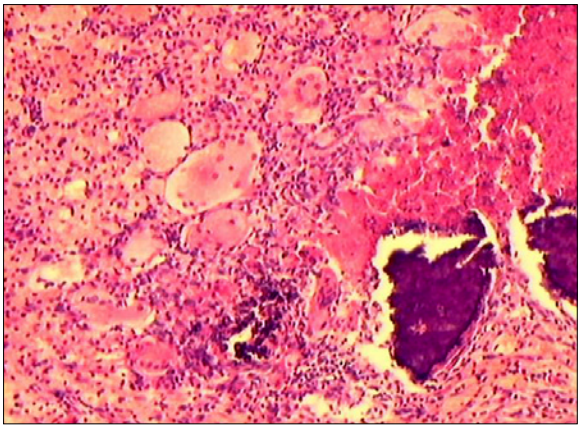

Figure 4. Implantation locus: coagulative necrosis of hepatoma cells with calcification foci and resorptive giant-cell reaction (HE, 120×).

sion in 31 rats (70%). The mean survival time for the rats with no tumor regression was 56.6±7.4 days.

At the same time, the rapid progression of all implanted tumors was observed in the control group (R). The mean survival time in this group was 38.7±6.5 days.

Figure 2 presents the tumor growths in one selected experimental group (C) versus the control group (R) during the four weeks after tumor inoculation, that is, the period of tumor progression and the initial period of tumor regression in the BRT-treated rats.

The histological investigation of the implantation loci in the 'regression group' revealed traces of tumor absorption, in the form of resorptive granulomas associated with hemosiderine deposits and intensive chronic inflammatory process, with macrophages and foam cells dominating the picture (Figure 3). In some cases, nests of entirely or almost entirely necrotic hepatoma cells were observed, surrounded by embankment of macrophages and giant cells accompanied by granulocytic and foam cell infiltration. Even here, both hemosiderine deposits and calcification foci were detected (Figure 4). It was also observed that no lung metastases were present in the lung sections.

Table 2. Cathepsin B activity during tumor regression (A-C) as compared with tumor-bearing animals (S –day 21) and normal rats (N).

Group (number)		Enzymatic activity (U/l)
A	(n=9)	26.4±3.5**
B	(n=10)	26.7±3.8**
C	(n=12)	27.8±4.1**
S	(n=10)	19.9±2.5*
N	(n=10)	13.3±3.4

* p<0.05;
** p<0.01

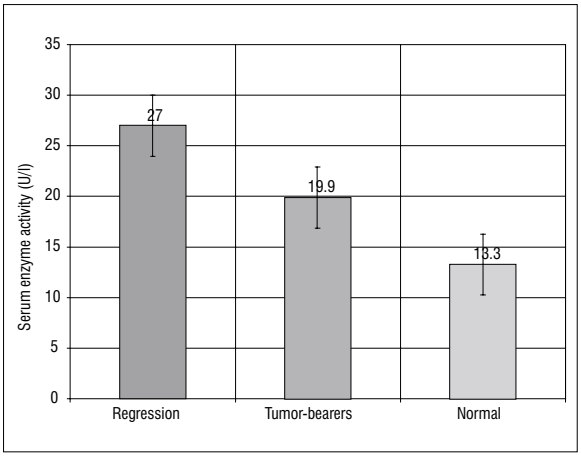

Figure 5. Serum cathepsin B activity in the different groups of Buffalo rats: before tumor implantation, after implantation (during tumor progression), and in the final phase of regression.

As can be seen in Table 2, cathepsin B activity in the regression sub-groups A, B and C, i.e. in those cases where total tumor regression was observed and confirmed by histological investigation, proved to be considerably higher than in the tumor-bearing rats (group S, characterized by the presence of developed tumor and metastatic foci). But in the latter group, cathepsin B activity was in turn significantly elevated when compared to activity in normal, i.e. non – implanted rats (group N). Moreover, no significant difference in cathepsin B activity was observed between the three sub-groups. Figure 5 shows the mean value for these groups (27.0±3.8 U/l) compared to group S (19.9±2.5 U/l, $p<0.05$) and group N (13.3±3.4 U/l, $p<0.01$).

Discussion

Ours is the first study, as far as we know, to examine serum cathepsin B activity, a potential tumor marker, in an experimental model in which both tumor progression and regression could be induced. The main finding was that both tumor progression and regression were associated with increased serum cathepsin B activity. Increased serum cathepsin B activity has been described

not only during tumor progression [4–7,10], but also in non-tumor conditions, such as inflammatory diseases [10–12], which weakens the hypothesis that serum cathepsin B activity can be used as a tumor marker. The mechanisms behind the rather unexpected finding of higher serum cathepsin B activity in rats with tumor regression than in rats with tumor progression will be discussed below.

BRT-induced tumor regression seems to involve two different processes: (i) effective immunological response and/or (ii) tumor cell death. The latter may be due to either apoptosis or necrosis. At the present stage, however, it is not possible to specify which of these alternatives is correct. But this leads us directly to the crucial problem: why does regression influence cathepsin B activity?

Unexposed tumor-bearers demonstrated higher serum activity than non-implanted animals, and this result was of course expected, since elevated enzyme activity has been reported in various rat tumors [21,22]. Cathepsin B's role in tumor expansion is well established, and has been confirmed in such processes as local tumor growth, invasion, migration, and angiogenesis [23,24]. However, the determination of serum cathepsin B activity in the final phase of complete tumor regression brought surprising results: the activity of this enzyme was significantly increased as compared to tumor-bearers (+34.2%, $p<0.05$). What are the possible explanations of this apparent anomaly? We have found two potential answers:

1. Cathepsin B, as a component of macrophage lysosomes [3], takes an active part in the process of tumor resorption. This leads to high circulating levels. In one recent report, increased activity and content of cathepsin B has been described in tumor tissue after implementation of antitumor therapy [25].
2. This enzyme may play a crucial role in tumor cell apoptosis and/or necrosis. Several studies show that TNF–α mediated cell apoptosis (esp. as regards tumor cells) inevitably requires cathepsin B participation, including the final phases of execution and the display of phagocytosis markers [26–28]. TWEAK-induced cell death by necrosis, in which cathepsin B passes from lysosomes to cytosol, has also been seen to be intermitted by cathepsin B inhibitor [29]. According to a quantitative model, a weaker stimulus would trigger a limited release of lysosomal enzymes to the cytoplasm, followed by apoptotic death, whereas a stronger stimulus would lead to a generalized lysosomal rupture and rapid cellular necrosis [30]. Transformed cells demonstrate high protein turnover, which seems to involve increased lysosomal efficacy. Thus cathepsin B would constitute an extremely sensitive element of the system, which when stimulated would act destructively upon malignant cells, leading either to apoptosis or necrosis.

In our study, the histological investigation seems to point to the process of coagulative necrosis followed by intensive tumor resorption. Cathepsin B's role as regards the observed tumor growth stages may therefore be dual: pro-invasive during tumor progression, and pro-apoptotic/necrotic when an external factor (EM stimulation in this case) is applied.

CONCLUSIONS

Cathepsin B may play an important role not only in tumor expansion, but also during processes of cancer cell death and resorption. Its high circulating levels may thus correspond to effective therapeutic response in the course of antitumor treatment.

Acknowledgements

The authors would like to thank the following persons: the staff at the Animal Laboratory, Department of Pathological Anatomy, Wrocław Medical University, for their contribution; the head (Prof. Maria Warwas) and staff of the Department of Pharmaceutical Biochemistry, Wrocław University of Medicine, for providing the laboratory facilities and reagents for the enzymatic tests; and finally, Prof. Peter Engfeldt, Department of Clinical Medicine, Family Medicine Research Centre, Örebro University, for reviewing and helping with the editing of this manuscript.

REFERENCES:

1. Kirschke H, Barrett AJ and Rawlings ND: Proteinases 1: lysosomal cysteine proteinases. Protein Profile 2: 1995; 1587-643
2. Bohley P, Seglen PO: Proteases and proteolysis in the lysosome. Experientia, 1992; 48: 151-57
3. Mort JS, Buttle DJ: Molecules in focus: Cathepsin B. Int J Biochem Cell Biol, 1997; 29(5): 715-20
4. Duffy MJ: The role of proteolytic enzymes in cancer invasion and metastasis. Clin Exp Met, 1992; 10: 145-55
5. Roshy S, Sloane BF, Moin K: Pericellular cathepsin B and malignant progression. Cancer Metastasis Rev, 2003; 22(2-3): 271-86
6. Keppler D, Sameni M et al: Tumor progression and angiogenesis: cathepsin B & Co. Biochem Cell Biol, 1996; 74(6): 799-810
7. Warwas M, Haczyńska H et al: Cathepsin B-like activity as a serum tumor marker in ovarian carcinoma. Eur J Clin Chem Clin Biochem, 1997; 35(4): 301-4
8. Van Noorden CJ, Jonges TG et al: Heterogeneous suppression of experimentally induced colon cancer metastasis in rat liver lobes by inhibition of extra cellular cathepsin B. Clin Exp Metastasis, 1998; 16(2): 159-67
9. Niewczas M, Paczek L, Krawczyk M et al: Enzymatic activity of cathepsin B, cathepsin B and L, plasmin, trypsin and collagenase in hepatocellular carcinoma. Pol Arch Med Wewn, 2002; 108(7): 653-62
10. Tumminello FM, Leto G et al: Cathepsin D, B and L circulating levels as prognostic markers of malignant progression. Anticancer Res, 1996; 16: 2315-20
11. Gerber A, Welte T, Ansorge S, Buhling F: Expression of cathepsins B and L in human lung epithelial cells is regulated by cytokines. Adv Exp Med Biol, 2000; 477: 287-92
12. Reinheckel T, Deussing J, Roth W, Peters C: Towards specific functions of lysosomal cysteine peptidases: phenotypes of mice deficient for cathepsin B or cathepsin L. Biol Chem, 2001; 382(5): 735-41
13. Levicar N, Kos J, Blejec A et al: Comparison of potential biological markers cathepsin B, cathepsin L, stefin A and stefin B with urokinase and plasminogen activator inhibitor-1 and clinicopathological data of breast carcinoma patients. Cancer Detect Prev, 2002; 26(1): 42-49
14. Staack A, Koenig F, Daniltchenko D et al: Cathepsins B, H, and L activities in urine of patients with transitional cell carcinoma of the bladder. Urology, 2002; 59(2): 308-12
15. Fedorowski A, Steciwko A, Rabczynski J: Influence of low-frequency electromagnetic field generated by transformation of endogenous Morris hepatoma field on its growth and metastatic ability (I). Med Sci Monit, 1997; 3(3): 336-41

16. Fedorowski A, Steciwko A, Rabczynski J: Influence of low-frequency electromagnetic field on growth of endogenous Morris hepatoma and its metastatic ability (II). Med Sci Monit; 1998; 4(5): 765-73
17. Brugemann H (ed): Bioresonance and Multiresonance Therapy (BRT). Brussels, Haug International, 1993
18. Lakiza O, Lednyczky G, Zhalko-Tytarenko O: Cytotoxic effect of proper electromagnetic field on melanoma B16 cell culture. BEMS 17th Annual Meeting, Boston, June 18–22, 1995
19. Wepsic HT, Nickel R, Alaimo J: Characterization of growth properties and demonstration of tumor-specific transplantation antigens of Morris hepatomas. Cancer Research, 1976; 36: 246-50
20. Barret AJ, Fluorimetric assays of cathepsin B and cathepsin H with methylcoumarylamide substrates, Biochem J, 1980; 187: 909-19
21. Isidoro C, Demoz M, De Stefanis D et al: High levels of proteolytic enzymes in the ascitic fluid and plasma of rats bearing the Yoshida AH-130 hepatoma. Invasion Metastasis, 1995; 15(3-4): 116-24
22. Satoh Y, Higashi T, Nouso K et al: Cathepsin B in the growth of colorectal cancer: suppressive effect of leupeptin on the growth of DMH-induced rat colon neoplasm. Acta Med Okayama, 1996; 50: 299-303
23. Koblinski JE, Ahram M, Sloane B: Unraveling the role of proteases in cancer. Clin Chim Acta, 2000; 291: 113-35
24. Niedergethmann M, Hildenbrand R, Wolf G et al: Angiogenesis and cathepsin expression are prognostic factors in pancreatic adenocarcinoma after curative resection. Int J Pancreatol, 2000; 28(1): 31-39
25. Korolenko TA, Zhyanaeva SY, Poteryaeva ON et al: Activity and concentration of cathepsin B as prognostic criteria for the development of mouse LS lymphosarcoma and Lewis lung adenocarcinoma. Bull Exp Biol Med, 2002; 133(4): 392-95
26. Foghsgaard L, Wissing D, Mauch D et al: Cathepsin B acts as a dominant execution protease in tumor cell apoptosis induced by tumor necrosis factor. J Cell Biology, 2001; 153 (5): 999-1009
27. Roberts LR, Adjei PN, Gores GJ: Cathepsins as effector proteases in hepatocyte apoptosis. Cell Biochem Biophys, 1999; 30: 71-88
28. Gucciardi ME, Miyoshi H, Bronk SF, Gores GJ: Cathepsin B knockout mice are resistant to tumor necrosis factor – α – mediated hepatocyte apoptosis and liver injury. Am J Pathol, 2001; 159(6): 2045-54
29. Nakayama M, Ishidoh K, Kayagaki N et al: Multiple pathways of TWEAK-induced cell death. J Immunol, 2002; 168(2): 734-43
30. Brunk UT, Dalen H, Roberg K, Hellquist HB: Photo-oxidative disruption of lysosomal membranes causes apoptosis of cultured human fibroblasts. Free Radic Biol Med, 1997; 23: 616-26