PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(4):587-592
World Journal of Gastroenterology ISSN 1007-9327

• BRIEF REPORTS •

Effect of vitamin E and human placenta cysteine peptidase inhibitor on expression of cathepsins B and L in implanted hepatoma Morris 5123 tumor model in Wistar rats

Tadeusz Sebzda, Piotr Hanczyc, Yousif Saleh, Bernice F Akinpelumi, Maciej Siewinski, Jerzy Rudnicki

Tadeusz Sebzda, Piotr Hanczyc, Department of Pathophysiology, Medical University of Wroclaw, Poland
Yousif Saleh, Bernice F Akinpelumi, Department of Obstetrics and Gynaecology, Medical University of Wroclaw, Poland
Maciej Siewinski, Faculty of Public Health, Medical University of Wroclaw, Poland
Jerzy Rudnicki, Clinical of Surgery and Oncology, Medical University of Wroclaw, Poland
Correspondence to: Yousif Saleh, PhD, I Department of Obstetrics and Gynaecology, Medical University of Wroclaw, Chalubinskiego street 3; PL-50-368 Wroclaw, Poland. saleh-yousif@mailcity.com
Telephone: +4871-7842413
Received: 2004-02-28 **Accepted:** 2004-05-13

Abstract

AIM: To examine the effectiveness of human placental inhibitors, by injecting vitamin E to rats with transplanted Morris-5123 hepatoma, on the expression of cathepsins B and L in tumor, liver, lung and blood sera after transplantation of Morris 5123 hepatoma.

METHODS: Animals were divided into 10 groups receiving three different concentrations of vitamin E and inhibitors along or in combination and compared with negative control (healthy rats) and positive control (tumor rats). Effectiveness of treatment was evaluated with regard to survival time, tumor response and determination of the activities of proteolytic enzymes and their inhibitors using flurogenic substrates.

RESULTS: Cathepsins B and L activities were elevated by 16-fold in comparison with negative control tissues, and their endogenous inhibitor activity decreased by 1.2-fold before treatment. In several cases, tumors completely disappeared following vitamin E plus human placental cyteine protease inhibitor (CPI) compared with controls. The number of complete tumor responses was higher when 20 m/kg vitamin E plus 400 μg of CPI was used, i. e., 7/10 rats survived more than two mo. Cathepsins B and L were expressed significantly in tumor, liver, lung tissues and sera in parallel to the increasing of the endogenous inhibitor activity compared with the controls after treatment (P<0.0001).

CONCLUSION: The data indicate formation of metastasis significantly reduced in treated rats, which might provide a therapeutic basis for anti-cancer therapy.

Key words: Morris-5123 hepatoma; Vitamin E; Human placenta cysteine peptidase inhibitor; Cathepsin B; Cathepsin L

Sebzda T, Hanczyc P, Saleh Y, Akinpelumi BF, Siewinski M, Rudniki J. Effect of vitamin E and human placenta cysteine peptidase inhibitor on expression of cathepsins B and L in implanted hepatoma Morris 5123 tumor model in Wistar rats. *World J Gastroenterol* 2005; 11(4): 587-592
http://www.wjgnet.com/1007-9327/11/587.asp

INTRODUCTION

Lysosomal cysteine protease (CP) plays an important role in the intracellular protein turnover, proteolytic degradation of endocytosed proteins, maturation cleavage of a number of precursor proteins[1]. Natural CP inhibitors (CPI) include members of the cystatin superfamily (stefins, i.e., family I cystatins, family II cystatins, and kininogens)[2]. Cystatins are distributed in different body fluids, suggesting a regulatory and defensive role against host or exogenous CP. In human blood plasma, CPI activities are represented by α2-macroglobulin, low-molecular mass kininogen and a small amount of cystatin C[3]. Cathepsin B (cat B) is released from lysosomes during tumor necrosis factor-alpha (TNF-α) cytotoxic signaling in hepatocytes and contributes to cell death. These data implicate a sphingosine-cat B interaction inducing lysosomal destabilization during TNF-α cytotoxic signaling[4]. Isidoro *et al*[5] indicate that the lysosomal segregation of cathepsin D was less efficient and its fractional secretion was higher in hepatoma-7777 cells than in hepatocytes; in the second cell types, delivery to lysosomes and processing of procathepsin D were differently sensitive to increases in the vacuolar pH. Plasma and ascitic fluid of rats bearing the Yoshida ascites hepatoma AH-130 were shown to contain high levels of proteolytic enzymes cathepsins B and L, in their latent, acidic-activated forms belonging to different classes active at neutral and acidic pH[6]. Serum from patients with chronic liver diseases (chronic hepatitis, cirrhosis, and hepatomas) showed increased calciferin and acid protease in their serum compared with that from normal subjects[7]. Recent evidence suggests that cathepsin B (cat B) contributes to TNF- α induced apoptosis *in vitro*. The data demonstrate that a cat B-mitochondrial apoptotic pathway plays a pivotal role in TNF-alpha-induced hepatocyte apoptosis and liver injury[8]. Since vitamin E increases the antioxidant status of cells, its influence on cytotoxicity was investigated, this effect was related to the concentration of vitamin E in the cell culture medium. A vitamin E dose-related response was also observed for the decreased toxicity of paracetamol and caffeine[9]. Effects of low corn oil, high corn oil, and high fish oil diets on altered hepatic foci development in female Sprague-Dawley rats were investigated. These results suggest that the type of dietary lipid is a more important determinant for gamma-glutamyl ranspeptidase-positive foci development than the amount of dietary lipid when rats consumed approximately the same amount of calories in all the dietary groups, and the underlying mechanisms may be partially ascribed to the antioxidant/oxidation status and biotransformation/detoxification system of rats[10]. Inhibition

of liver cancer is therefore associated with induction of increased microsomal enzyme activity[11]. It was found that vitamin E could control the cellular formation of *ras* and *myc* oncogenes[12] and raise *in vivo* the level of autogenic cysteine peptidases inhibitors[13]. The information suggests the possibility of its application in adjuvant conventional oncological therapy. Vitamin E could also reduce a cytostatic level in patients after conventional chemotherapy[14]. The combined treatment of transplantable solid mammary carcinoma in Wistar rats using photodynamic therapy and proteinase inhibitor isolated from human placenta was done. The results indicated that in several cases tumors were completely disappeared following treatment HpD-PDT+CPI[15]. The aim of the present study was to investigate the new therapy in the effect of vitamin E and human placenta cysteine peptidase inhibitors on the expression of cathepsins B and L in implanted hepatoma Morris 5123 tumor model in Wistar rats.

MATERIALS AND METHODS

Animals

Male and female Wistar rats weighing 180-200 g (age: approximately 2 mo) were used. The rats were fed with normal diets during the experimental period according to Nirwana *et al*[16]. The food composed of (% w/w): crude protein 20.0, crude fibre 5.0, crude fat 2.5, moisture 13.0, ash 7.0, calcium 0.7-1.4, total phosphorus 0.6-1.2, and nitrogen-free extract 51.0.

Human placenta cysteine protease inhibitor (CPI)

The inhibitors were purified and identified using affinity chromatography on Sepharose 4B - papain, Sephadex G-75 gel chromatography column, and ion exchange chromatography on a DEAE-Sephacel column (Saleh *et al*[17] 2003). A highly purified preparation of cysteine protease inhibitors was obtained with a specific activity of 344.8 mEU/mg protein, and molecular weight of 67 kDa (by SDS-PAGE). The solution was concentrated on centricom (Amicon), lyophilized and stored at -20 ℃ until use. The pure and sterile inhibitors were dissolved in physiological saline and injected subcutaneously (sc.) at the doses of 200 μg and 400 μg per animal (in volume 0.1 mL).

Vitamin E

Vitamin E (400 mg/mL) solution was dissolved in corn oil and injected subcutaneously (sc.) at the doses of 10 and 20 mg/kg per animal (in volume 0.1 mL) obtained from Hasco, (Pharmaceutical Drugs Production, Wroclaw, Poland).

Implantation of tumor

Approximately 140 000 tumor cells of hepatoma Morris 5123 were implanted intramuscularly in the left limb of the Wistar rats. Two wk after implantation, the implanted tumor grew locally early in the course of diseases and eventually invaded the surrounding organs causing ascites and also metastasis in the lungs. Liver microangiography demonstrated that the tumor received blood supply mainly from the hepatic artery. The tumor was detected by histological examinations of these tissues using conventional hematoxylin-eosin (HE) staining.

Experimental design

The experiment was carried out on 100 male and female Wistar rats, which were divided into 10 groups: Group I:10 rats, which were given normal diets without treatment during the experiment as control negative (healthy); group 2 :10 rats with hepatoma Morris 5123, which were given normal diets without treatment during the experiment as control positive (tumor); groups 3 and 4 : tumor rats were injected subcutaneously 10 and 20 mg/kg vitamin E in 0.1 mL corn oil during the experiment groups 5 and 6 : tumor rats were injected subcutaneously 200 and 400 μg human placenta purified cysteine protease inhibitor (CPI) in 0.1 mL physiological solution during the experiment groups 7 and 8 : tumor rats were injected subcutaneously 200 μg CPI plus 10 and 20 mg/kg vitamin E groups 9 and 10 : tumor rats were injected subcutaneously 400 μg CPI plus 10 and 20 mg/kg vitamin E. All animals were injected for 10 d with human placenta purified cysteine protease inhibitors (CPI). From the eleventh day the rats were injected vitamin E for 30 d. After 8 wk, the experiment animals from all groups were narcotized, decapitated and the tumor, lungs, liver, and blood were taken and washed out of the blood with 0.9% NaCl and homogenized in electric Potter's homogenizator according to the methods described by Malicka and Roth[18]. The activities of enzymes and histological examinations were performed.

Determination of cathepsin B and L activities

Cathepsin B activity was measured according to Barrett *et al*[19]. Fluorescence was measured in a luminescence spectrometer, Perkin Elmer LS 50 B at 370 nmol/L excitation and 440 nmol/L emission wavelengths using fluorescent substrate Z-Aeg-Arg-AMC for cathepsin B and Z-Phe-Arg-AMC for cathepsin L. Fluorescence readings of the sample assays were standardized with the reaction product 7-AMC (7-amino-4-methylcoumarin)[20]. One mEU of activity was defined as the quantity releasing 1 nmol/L of 7-AMC.

Inactivation of α-macroglobulin

a-macroglobulin (α-M) was inactivated by incubation with 0.5 mol/L methylamine at 37 ℃ and pH 7.5 for 2 h in samples and activity of CPI^{37} was determined[21]. Fifty uL of sample was preincubated with 50 μL of water and 2.0 mL of 0.5 M methylamine in 0.1 mol/L potassium phosphate pH 7.5 for 2 h at 37 ℃. Fifty μL of 0.1% papain was then added and the samples were incubated for 10 min at 37 ℃. Then 50 μL of 0.66 mmol/L BANA solution was added and the free β-naphtylamine was measured. Inhibitory activity was determined[22].

CPI^{80}, the total activity of cysteine proteinase inhibitors was calculated. The procedure of assay was as follows. Fifty μL of serum was preincubated with 50 μL of 0.03 mol/L HCl at 80 ℃ for 20 min, then with 2.0 mL of 0.5 mol/L methylamine at 37 ℃ for 2 h. Samples were centrifuged and incubated with 0.01% papain solution, then the papain activity was determined and the activity of inhibitors was calculated. CPI: Difference between CPI^{80} and CPI^{37} was calculated, and the results were presented as complex (latent) forms of inhibitors.?$CPI = CPI^{80} - CPI^{37}$. The amount of vitamin E was determined by liquid chromatography, using a HPLC apparatus ("Philips") and a Pye Unicam PU 4020 UV detector. The results were decoded with the use of Peak simple chromatography data system program[23].

Protein concentration

Total protein concentration was determined by the Bradford method[24] using bovine serum albumin as a standard.

Statistical analysis

The levels of variables in the cancer tissues and sera compared with the control were analyzed statistically. The results of cathepsin B and their inhibitor activity assays in the groups under study are given as median and mean±SD. To compare data of tumor and control tissues, Walloon's rank test was used. $P<0.05$ was considered statistically significant. The calculation of survival probability was performed by the method developed by Kaplan and Meier. Checking the significance of a relationship between survival of rats and the levels of

biochemical parameters was based on the Log-rank test. The discrimination levels were calculated by a computer program.

RESULTS

The best results were obtained after combination of 20 mg of vitamin E and a higher dose of CPI were used, i.e., 400 μg, in control groups. In these cases the animals survived for longer than 8 wk. In numerous cases the tumors were completely disappeared following CPI and vitamin E application. Table 1 shows that the number of complete tumor responses was higher when 400 μg of CPI was used plus 20 mg of vitamin E, i.e., 7/10 rats, and survived more than two mo. Whereas application of 10 mg of vitamin E and a lower dose of CPI (200 μg) resulted in only 4/10 total tumor responses, and the rats survived for about 26 d. The difference between these two groups was significant ($P \leqslant 0.0001$). No complete tumor response was achieved in all the other experimental groups. The vitamin E after the last treatment with human placental CPI was injected subcutaneously at the doses of 10 and 20 mg per animal for one month. Cathepsin B and L activity was measured in all animals with tumor homogenates and in untreated animal tissues (control). The mean or median activity of cat B and L was much higher in tumor homogenates in comparison with that in the negative control homogenates and the differences were highly significant ($P \leqslant 0.0001$) before treatment. While no significance was observed in the activity of cathepsins B and L in tumor tissues after the rats were given-high doses of inhibitors plus vitamin E($P \leqslant 0.0005$).

The total cat B and L activity was 88.5 mEU/mg proteins in tumor tissue homogenates compared with 5.8 mEU/mg protein in negative control tissue ($P \leqslant 0.0005$). Cathepsins B and L activities were elevated 16-fold in comparison with negative control tissue. Table 1 shows that the inhibitory activity of CPI was significantly decreased from 10.5 mEU/mg protein in control tissue to 8.5 mEU/mg protein in tumor tissue ($P \leqslant 0.0005$), thus it decreased 1.2-fold in tumor tissues. The complex form △ CPI was also decreased from 14.2 mEU/mg protein in control tissue to 9.0 mEU/mg protein in tumor tissue ($P \leqslant 0.0005$), with a decrease of 1.6-fold in tumor tissues.

Cathepsin B and L activity decreased in all vitamin E groups by 1.0-1.3 fold, while in the rats obtained only CPI, the cathepsin B and L activity decreased by 1.2-1.8 folds. By the results from Table 1 also showed that the activities of cathepsins B and L decreased by 5.8 and 11.8 fold after the rats received 20 mg vitamin E plus 200 and 400 μg CPI in tumor tissues. While the

Table 1 Cathepsins B, L and their inhibitor activities, survival time and the total cure responses of hepatoma morris-5123 in Wistar rats before and after treatment in comparison with negative control (median, mean±SD, range)

	Cathepsins B, L (mEU/mg)	Inhibitor (CPI^{80}) (mEU/mg)	Inhibitor (CPI^{37}) (mEU/mg)	Complex form (△CPI) (mEU/mg)	Survival time (d)	Cure (yes/No)	Value (*P*)
Control negative	5.8	24.7	10.5	14.2			
(healthy)	6.5±1.7	25.4±8.9	11.2±2.8	14.2±6.1	60.0	10/10	0.0005
	(2.4-8.2)	(12.6-40.7)	(2.4-14.5)	(10.2-26.2)			
Control positive	88.5	17.5	8.5	9.0	09–12		
(Tumor)	85.4±12.6	18.8±8.6	9.3±1.2	9.5±7.4	(10.5)	2/10	0.0001
	(10.5-123.9)	(4.6-23.8)	(1.4-12.5)	(3.2-11.3)			
Tumor+ 10 mg	80.5	28.7	16.4	12.3	12-26		
Vit E	81.8±20.6	29.5±12.0	17.0±5.5	12.5±6.5	(19.0)	2/10	0.0005
	(11.0-99.8)	(6.7-35.7)	(3.9-20.3)	(2.8-15.4)			
Tumor+20 mg	78.7	35.6	20.3	15.3	15-30		
Vit E	79.4±20.4	36.6±15.0	21.5±4.6	15.1± 10.4	(22.5)	3/10	0.0005
	(10.7-97.4)	(7.3-40.1)	(4.2-22.0)	(3.1-18.1)			
Tumor+ 200 μg	56.5	46.3	35.8	10.5	16-30		
CPI	57.4±15.1	47.7±20.0	37.0±17.6	10.7±2.4	(23.0)	3/10	0.0001
	(22.1-80.6)	(12.3-52.9)	(9.0-40.0)	(3.3-12.9)			
Tumor+ 400 μg	47.9	67.8	46.8	21.0	18-35		
CPI	48.7±18.6	69.0±25.2	47.3±16.0	21.7±9.2	(21.5)	4/10	0.0001
	(23.2-68.9)	(20.6-88.5)	(14.4-60.1)	(6.2-28.4)			
Tumor+ 10 mg Vit E	41.8	71.8	52.3	18.5	27-38		
+Tumor+ 200 μg CPI	43.0±10.6	73.0±25.4	53.6±22.1	19.4±3.3	(32.5)	4/10	0.0001
	(12.0-46.7)	22.7-82.8	(20.4-63.3)	(2.3-11.5)			
Tumor+ 10 mg Vit E	30.2	82.2	61.0	21.2	30-40		
+Tumor+ 400 μg CPI	31.6±12.0	83.5±29.8	62.4±25.5	21.1±4.3	(35.0)	5/10	0.0001
	(9.8-38.7)	(26.9-93.7)	(24.6-70.2)	(2.3-23.5)			
Tumor+ 20 mg Vit E	15.4	94.4	74.2	10.2	38-60		
+Tumor+ 200 μg CPI	16.5±2.8	95.8±35.4	75.6±33.1	10.2±2.3	(49.0)	6/10	0.0001
	(6.4-20.8)	(37.8-112.7)	(32.3-94.5)	(5.5-18.2)			
Tumor+ 20 mg Vit E	7.5	120.8	105.5	15.3	45-60		
+Tumor+ 400 μg CPI	8.4±1.8	122.0±43.6	107.0±39.2	15.0±4.4	(52.5)	7/10	0.0001
	(2.6-10.2)	(45.8-135.5)	(35.6-118.2)	(10.2-17.3)			

The significance of the differences in Medan values of tumor and control tissues was calculated by Wilcoxon matched pair's signed-rank test.

Table 2 Activity values of cathepsins B and L and their inhibitors in hepatoma Morris 5123 (median, mean±SD, range)

	Before treatment			After treatment		
	Cathepsin B	Cathepsin L	Endogenous (CPI)	Cathepsin B	Cathepsin L	Endogenous (CPI)
	8.2	3.20	12.2			
Control negative(Healthy)	9.4±1.5	3.6±1.3	12.8±2.6		No changes	
	(1.5-12.7)	(1.3-7.4)	(3.4-15.7)			
	67.6	28.4	8.5	7.6	3.4	78.5
Control positive (Tumor)	68.9±23.6	28.8±10.4	9.0±2.3	8.4±2.5	3.8±1.6	79.4±22.5
	(19.4-89.2)	(6.4-35.2)	(3.2-12.5)	(3.4-10.2)	(2.2-5.7)	(23.9-96.5)
	46.6	17.2	4.3	6.3	2.2	63.2
Liver	47.9±20.5	18.8±8.6	5.6±1.1	7.8±2.9	2.7±1.0	64.8±25.7
	(21.3-68.2)	(6.4-25.8)	(1.8-7.7)	(2.9-8.6)	(1.2-5.4)	(27.9-74.3)
	26.7	10.8	3.6	2.6	1.5	45.8
Lung	27.9±18.3	12.0±4.2	4.6±0.9	2.9±0.5	2.0±0.4	47.1±21.5
	(15.8-38.9)	(6.4-19.4)	(1.0-6.8)	(0.8-3.7)	(0.4-3.1)	(23.7-56.0)
	12.6	8.0	1.2	1.5	1.6	114.8
Sera	13.9±4.2	9.3±2.8	1.6±0.1	3.7±0.9	1.7±0.6	115.3±45.4
	(5.6-18.5)	(2.9-11.3)	(0.2-2.3)	(0.04-3.2)	(0.07-3.8)	(44.7-123.9)
P		≤0.0001		NS	NS	≤0.0001

The significance of differences in median values of tumor and control were calculated after and before treatment by Wilcoxon rank test. NS, Not significant.

endogenous inhibitor activity was increased 7.4 and 10.5 folds respectively in the same groups, and the complex form △CPI activity obtained in the same range to negative control group.

The results in Table 2 showed that the mean±SD of cat B and L activities in the tissue homogenates were decreased from 68.9±23.6 mEU/mg protein and 28.8±10.4 mEU/mg protein before treatment to 8.4±2.5 mEU/mg protein and 3.8±1.6 mEU/mg protein, respectively, after the rats received 20 mg vitamin E plus 400 μg CPI. Highly significant differences were observed between the activities of cat B and L in tissue homogenates before and after treatment with 20 mg vitamin E plus 400 μg CPI ($P \leqslant 0.0001$), and this was similar to control tissues. The activities of cat B and L were decreased by 8.2 and 7.6 fold, respectively. The endogenous inhibitors were increased from 9.0±2.3 mEU/mg protein before treatment to 79.4±22.5 mEU/mg protein after the rats were administered 20 mg vitamin E plus 400 μg CPI. This increased by about 9.0-fold. The activities of cat B and L were also decreased significantly in liver and lung tissue homogenates and in sera after treatment ($P \leqslant 0.0001$) in comparison with negative control group. While the endogenous inhibitors also increased significantly in comparison with negative control group ($P \leqslant 0.0001$). The activities of cat B and L were decreased 6.2 fold and 7.0 fold in liver tissues, and 9.3 fold and 6.0 fold in lung tissues, while 8.2-fold and 7.6-fold in sera, respectively after treatment. The endogenous inhibitors were increased 11.6, 10.2-folds, and 72.0-fold in liver, lung tissues and in sera after treatment, respectively. No significance was observed in cat B and L activities in tissue homogenates and in blood sera after the rats obtained high doses of CPI plus vitamin E in comparison to negative control group.

DISCUSSION

Cancer cells are characterized with high motility, loss of function which makes them unite with neighbouring cells, invasiveness, and lack of sensibility to contact inhibition and general change of cell shape. Adhesion and locomotion are the main capabilities of cells in tumor growth and metastasis. Proteases are attractive targets for drug development for therapeutic application. The use of novel inhibitors in clinical practice is dependent not just on their specificity and effectiveness, but mainly on the knowledge of their precise role in mechanisms of the proteolysis system in the development of malignant disease[25]. The combination of vitamin E with other cancer chemopreventive agents appears to be a reasonable procedure[26]. Association of the balance between various proteases and their inhibitors with cancer diseases has been widely studied to understand the molecular mechanisms of development and metastasis of tumors. It is an often presented result in literature that cysteine peptidase activity in various malignant tumor tissues and body fluids of cancer patients was altered compared to physiological levels[27]. It has been widely noted that vitamin E showed numerous beneficial effects through and beyond its antioxidative properties. Consequently, vitamin E is expected to prevent degenerative diseases[28]. In the present study, we investigated the effectiveness of vitamin E in combination with human placental cysteine proteinase inhibitors (CPI). The results showed that the number of complete tumor responses was higher when the rats obtained 400 μg of CPI plus 20 mg of vitamin E, i.e., 7/10 rats. Whereas application of 10 mg vitamin E and a lower dose of CPI (200 μg) resulted in only 4/10 total tumor responses. The present study could be explained in such a way that CPI damaged the cancer cells. The number of total (complete) tumor responses was distinctly higher after application of 400 μg CPI than 200 μg CPI. This indicated that in the same conditions of vitamin E, the efficacy of treatment of CPI was dose-dependent. Saleh *et al*[15] indicated that in several cases tumors completely disappeared following the treatment with mammary carcinomas using HpD-PDT+CPI. Another independent research direction was the therapeutic indications of vitamin E in carcinogenesis processes[29].

The data obtained in this work involving hepatoma morris-5123 carcinoma in model Wistar rats were compared with those in non-cancerous tissues as a negative control before and after treatment. High activity of cathepsins B and L with a noteworthy simultaneous decrease of the activity of endogenous cysteine protease inhibitors was observed before treatment in tumor,

liver, lung tissues and in sera in comparison with negative control tissue homogenates. Cathepsin B and L activities were elevated 16-fold as against negative control tissues before treatment. The endogenous inhibitor activity also decreased 1.2 fold, and the complex form △CPI decreased 1.6-fold in tumor tissues. They provided further evidence for the alteration of the protease/inhibitor balance that occurred during the process of tumor cell invasion. Since the level of proteinases and inhibitors in extracellular fluids may reflect not only their local expression in tumors but also the systemic response to the disease, the mechanisms of the alteration of protease/inhibitor balance are not solved. The highly significant difference was observed between the levels of cathepsins and/or their precursor in malignant tumor tissue before treatment. Tumor tissue activity of cat B and L might be related to the severity of cancer disease and there are some prognostic aspects of such studies since it previously showned-that patients with higher content or increased proteolysis activities of cat B and its precursor in tissue homogenates of breast, ovarian and gastric cancer had significantly higher risk of recurrence or death than the cases with low content of the enzyme[30-32]. After the rats were injected with 20 mg vitamin E plus 400 μg CPI, the cat B and L were expressed significantly in tumor, liver, lung tissues and in sera in parallel to the increasing of the endogenous inhibitor activity ($P \leqslant 0.0001$). The rats survived for a longer period and they returned to normal life without any stress. Nyandieka *et al*[33] suggested that vitamins could inhibit liver cancer by inducing hepatic microsomal enzymes that metabolise aflatoxins to non-carcinogenic products.

Welss *et al*[34] demonstrated that hurpin was an intracellular, differentially spliced member of the serpin superfamily that has been linked to differentiation and apoptosis of human keratinocytes. It can be transiently down regulated by UV light and overexpressed in psoriatic skin lesions. It is a potent and selective inhibitor of the archetypal lysosomal cysteine proteinase cathepsin L.

Sakamoto *et al*[13]. Suggested that vitamin E administration could stimulate macrophages to synthesize interleukin 1 and then, acted on fibroblasts and lymphocytes, thus raising the interleukin 6 levels. As a final effect it could increase T-kininogen level, which is one of the fundamental cysteine peptidase inhibitors, thus suppressing the activity of the enzymes catalyzing processes accompanied neoplastic progress. The results showed that the increased amounts of kininogen might react with cysteine peptidases. Tandon *et al*[29] also supposed that as an effect of vitamin E activity, an inhibition of the "enzymatic metabolism cascade" took place. Moreover, vitamin E removing toxic cytostatics and their metabolites from the organs could facilitate its regeneration after harmful action of the substances. In conclusion, human placental inhibitors used in combination with high doses of vitamin E can significantly reduce the formation of tumors in rats, and provide a therapeutic basis for anti-cancer therapy.

REFERENCES

1 **Siewiñski M.** Autologous cysteine peptidase inhibitors as potential anticancer drugs. *Anticancer Drugs* 1993; **4**: 97-98
2 **Turk B**, Turk D, Turk V. Lysosomal cysteine proteases: more than scavengers. *Biochim Biophys Acta* 2000; **1477**: 98-111
3 **Wiederanders B.** The function of propeptide domains of cysteine proteinases. *Adv Exp Med Biol* 2000; **477**: 261-270
4 **Werneburg NW**, Guicciardi ME, Bronk SF, Gores GJ. Tumor necrosis factor-alpha-associated lysosomal permeabilization is cathepsin B dependent. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G947-G956
5 **Isidoro C**, Demoz M, De Stefanis D, Mainferme F, Wattiaux R, Baccino FM. Altered intracellular processing and enhanced secretion of procathepsin D in a highly deviated rat hepatoma. *Int J Cancer* 1995; **60**: 61-64
6 **Isidoro C**, Demoz M, De Stefanis D, Baccino FM, Bonelli G. High levels of proteolytic enzymes in the ascitic fluid and plasma of rats bearing the Yoshida AH-130 hepatoma. *Invasion Metastasis* 1995; **15:** 116-124
7 **Terayama H**, Shimizu N, Fukuzumi R. Calciferin and cathepsin D-like acid protease in serum in acute and chronic liver injuries in rats and humans. *Clin Chem* 1989; **35**: 2202-2206
8 **Guicciardi ME**, Miyoshi H, Bronk SF, Gores GJ. Cathepsin B knockout mice are resistant to tumor necrosis factor-alpha-mediated hepatocyte apoptosis and liver injury: implications for therapeutic applications. *Am J Pathol* 2001; **159:** 2045-2054
9 **Dierickx PJ**, Smit C, Scheers EM. Cytotoxicity of the MEIC reference chemicals in antioxidant-enriched, rat hepatoma-derived Fa32 cells. *Altern Lab Anim* 2001; **29**: 217-223
10 **Lii CK**, Ou CC, Liu KL, Liu JY, Lin WL, Chen HW. Suppression of altered hepatic foci development by a high fish oil diet compared with a high corn oil diet in rats. *Nutr Cancer* 2000; **38:** 50-59
11 **Nyandieka HS**, Wakhisi J, Kilonzo M. Inhibition of AFB1-induced liver cancer and induction of increased microsomal enzyme activity by dietary constituents. *East Afr Med J* 1989; **66**: 796-803
12 **Prasad KN**, Cohrs RJ, Sharma OK. Decreased expressions of c-myc and H-ras oncogenes in vitamin E succinate induced morphologically differentiated murine B-16 melanoma cells in culture. *Biochem Cell Biol* 1990; **68**: 1250-1255
13 **Sakamoto W**, Yoshikawa K, Shindoh M, Amemiya A, Handa H, Saeki T, Nagasawa S, Koyama J, Ogihara T, Mino M. In vivo effects of vitamin E on peritoneal macrophages and T-kininogen level in rats. *Int J Vitam Nutr Res* 1989; **59:** 131-139
14 **Ells GW**, Chisholm KA, Simmons VA, Horrobin DF. Vitamin E blocks the cytotoxic effect of gamma-linolenic acid when administered as late as the time of onset of cell death-insight into the mechanism of fatty acid induced cytotoxicity. *Cancer Lett* 1996; **98**: 207-211
15 **Saleh Y**, Ziolkowski P, Siewinski M, Milach J, Marszalik P, Rybka J. The combined treatment of transplantable solid mammary carcinoma in Wistar rats by use of photodynamic therapy and cysteine proteinase inhibitor. *In Vivo* 2001; **15**: 351–357
16 **Nirwana I**, Jamaludin M, Khalid BAK, Merican Z, Baharom S. Serium lipids of castrated rats given hormonal replacement and fed diets with added soybean oil or palm oil. *Asia Pacific J Clin Nutr* 1995; **4**: 244-248
17 **Saleh Y**, Siewinski M, Sebzda T, Jelen M, Ziolkowski P, Gutowicz J, Grybos M, Pawelec M. Inhibition of cathepsin B activity in human breast cancer tissue by cysteine peptidase inhibitor isolated from human placenta: immunohistochemical and biochemical studies. *Folia Histochem Cytobiol* 2003; **41**: 161-167
18 Malicka-Blaszkiewicz M, Styczen I, Nowak D, Hanczycowa H, Ponikowski P, Sebzda T. Actin content and polymerization in tumour, liver and serum of the hepatoma Morris 5123 tumour bearing rats. *Mater Med Pol* 1995; **25:** 115-118
19 **Barrett AJ**. Fluorimetric assays for cathepsin B and cathepsin H with methylcoumarylamide substrates. *Biochem J* 1980; **187**: 909–912
20 **Barrett AJ**, Kirschke H. Cathepsin B, Cathepsin H, and cathepsin L. *Methods Enzymol* 1981; **80** Pt C: 535–561
21 **Heidtmann HH**, Salge U, Abrahamson M, Bencina M, Kastelic L, Kopitar-Jerala N, Turk V, Lah TT. Cathepsin B and cysteine proteinase inhibitors in human lung cancer cell lines. *Clin Exp Metastasis* 1997; **15**: 368–381
22 **Siewiñski M,** Krêcicki T, Jarmulowicz J, Berdowska I. Cysteine proteinase inhibitors in serum of patients with head and neck tumors. *Diagn Oncol* 1993; **2**: 323-326
23 **Nilsson B,** Johansson B, Jansson L, Holmberg L. Determination of plasma alpha-tocopherol by high-performance liquid chromatography. *J Chromatogr* 1978; **145**: 169-172
24 **Bradford MM**. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248–

254
25 **Duffy MJ**. The role of proteolytic enzymes in cancer invasion and metastasis. *Clin Exp Metastasis* 1992; **10**: 145-155
26 **Lah TT**, Kos J. Cysteine proteinases in cancer progression and their clinical relevance for prognosis. *Biol Chem* 1998; **379**: 125–130
27 **Lockwood K,** Moesgaard S, Hanioka T, Folkers K. Apparent partial remission of breast cancer in 'high risk' patients supplemented with nutritional antioxidants, essential fatty acids and coenzyme Q10. *Mol Aspects Med* 1994; **15** Suppl: s231-s240
28 **Battisti C**, Formichi P, Tripodi SA, Vindigni C, Roviello F, Federico A. Vitamin E serum levels and gastric cancer: results from a cohort of patients in Tuscany, Italy. *Cancer Lett* 2000; **151**: 15-18
29 **Tandon SK**, Singh S. Influence of vitamin E on preventive or therapeutic effect of MFA and DTPA in cadmium toxicity. *Biomed Environ Sci* 1995; **8**: 59-64
30 **Saleh Y**, Siewinski M, Kielan W, Ziolkowski P, Grybos M, Rybka J. Regulation of cathepsin B and L expression *in vitro* in gastric cancer tissues by egg cystatin. *J Exp Ther Oncol* 2003; **3**: 319-324
31 **Siewinski M**, Saleh Y, Popiela A, Ziolkowski P, Jelen M, Grybos M. Expression of high molecular weight cysteine proteinase inhibitor in ovarian cancer tissues: regulation of cathepsin B expression by placental CPI. *Biol Chem* 2003; **384**: 1103-1107
32 **Saleh Y**, Siewinski M, Sebzda T, Grybos M, Pawelec M, Janocha A. Effects of combined *in vivo* treatment of transplantable solid mammary carcinoma in wistar rats using vitamin E and cysteine peptidase inhibitors from human placenta. *J Exp Ther Oncol* 2003; **3**: 95-102
33 **Nyandieka** HS, Wakhisi J. The impact of vitamins A, C, E, and selenium compound on prevention of liver cancer in rats. *East Afr Med J* 1993; **70**: 151-153
34 **Welss T**, Sun J, Irving JA, Blum R, Smith AI, Whisstock JC, Pike RN, von Mikecz A, Ruzicka T, Bird PI, Abts HF. Hurpin is a selective inhibitor of lysosomal cathepsin L and protects keratinocytes from ultraviolet-induced apoptosis. *Biochemistry* 2003; **42**: 7381-7389

Assistant Editor Guo SY **Edited by** Wang XL **Proofread by** Ma JY