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Original article

Evaluation of changes in the activity of proteolytic enzymes and their inhibitors in the processes that accompany the growth of gastric cancer

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Abstract

Background. **Many investigators have observed a correlation between the aggressiveness of malignant tumor growth and the levels of cysteine peptidases and their autogenous inhibitors. Cathepsins B and L are activated by pepsin in an acidic pH. This fact encouraged us to measure the activity of these enzymes in tissue samples of gastric cancer.**

Methods. **We measured the activities of cathepsins B and L, and their precursors and inhibitors, in homogenates of tissue samples obtained from operations for gastric cancer. We compared the results for the homogenates of tissues from three different sites: the tumor center, the zone of cancer invasion (border of the tumor), and healthy tissue.**

Results. **The highest activities of free cysteine peptidases and those in complexes with their inhibitors, as well as their precursors, were observed in the border of the tumor, while the activities in healthy tissue were significantly lower.**

Conclusion. **The local activities of cysteine peptidases and their inhibitors reflect the topographical differences between the center of the tumor, the zone of invasion, and healthy tissues in gastric cancer patients. In addition, the results for the pattern of changes in the activity of cysteine peptidases according to the degree of tissue infiltration were not dependent on the method of measurement (colorimetry vs spectrofluorometry).**

Key words Cysteine peptidases · Cathepsin B · Cathepsin L · Cysteine peptidase inhibitors · Gastric cancer

Introduction

The enzymatic processes that accompany the growth of gastric cancer catalyze the destruction of normal tissues. It has been found that mostly specific proteolytic enzymes participate in such processes, produced either in the cells of the malignant tumor, or in normal cells situated in the zone of tumor invasion [1,2]. The understanding of changes in the activities of these proteolytic enzymes that initiate carcinogenesis has led to the concept of *a cascade of mutual activation of proteolytic enzymes*, which determines the ongoing activation of enzymes that take part in the crucial processes of carcinogenesis [3]. These changes are initiated by the presence of active cathepsin D or pepsin, which in acidic pH, activate precursors of cysteine endopeptidases. During malignant invasion, cathepsin B is initially produced in its precursor form, and the activity of this enzyme is stimulated by cathepsin D or pepsin in a low pH. Both low pH and high pepsin activity are present in the stomach, thus providing optimal conditions for the activation of cysteine peptidase precursors. The precursor enzymes are produced within cancer cells and only after secretion to the surrounding tissues are they activated and catalyze tumor invasion [4,5]. Of all cysteine peptidases (EC 3.4.22) mainly cathepsins B and L are involved in the metabolism of malignant tumors; these subsequently catalyze the conversion of other peptidases into their active forms, which are responsible for the degradation of normal tissues, and also for bringing about tumor invasion and metastases [5,6]. It has been confirmed that active cathepsin B is associated with many key processes in tumor growth; this finding was achieved by measurements of its activity in tissues and serum of people hospitalized for gastric cancer [7,8].

A positive correlation between tumor invasiveness, as well as its metastatic potential, and the secretion of cysteine peptidases (particularly cathepsins B and L) has been well documented in the literature on this subject [3,9]. We postulate that cysteine peptidase activity could be used as a marker of cancer aggressiveness in diagnostic procedures in oncology. The correlation between the progression of early gastric cancer and the activity of cathepsins B and L has been studied by Dohchin et al. [10]. Their results correlated well with immunohistology and it was confirmed that the level of

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these enzymes can be considered a marker of invasion and aggressiveness of gastric cancer.

The aim of our study was to analyze the activity of proteolytic enzymes (cysteine peptidases), and their precursors and autogenous inhibitors, in biopsies of the center of tumor, the zone of invasion, and healthy tissue of the stomach in patients with advanced diffuse type gastric cancer. Additionally, another aim was to compare the above results obtained by means of two different methods of measurement, i.e., colorimetry vs spectrofluorometry.

Patients, materials, and methods

Patients and samples

The study was carried out from January 1998 to March 2000 with the homogenates of samples of resected tissues taken from 29 patients operated on for advanced gastric cancer at the Second Department of General and Oncological Surgery, Wroclaw Medical University. The group included 21 men and 8 women, aged from 28 to 73. In 20 patients, total gastric resection was performed, and subtotal resection was done in 9. All cases were advanced gastric carcinoma, diffuse Lauren type.

After the operation, three tissue samples, about 0.5 g each, were taken from the resected stomach of each patient: (a) from the center of the tumor, (b) from the border of the tumor (zone of invasion), and (c) from visually normal healthy tissues, distant from the tumor. The samples were initially washed and stored frozen in 1.0 ml of normal saline at a temperature of −20°C for about 1 month.

Sample preparation

The following measurements were made in homogenates of these tissue samples: active cysteine peptidases (CP), and their precursors (pCP) and inhibitors, the latter being measured as three forms: active inhibitors of cysteine peptidases (ICP^{37}), the total pool of inhibitors produced by the patient's tissues (ICP^{80}), and the inhibitors bound in enzyme-inhibitor complexes (ΔICP).

For this purpose, thawed tissue samples were homogenized for 20 min in normal saline, in the proportion of 1.0 g tissue in 6.0 ml saline, with a homogenizer MPW-309 (Universal Laboratory Aid, Warsaw, Poland). The homogenates were centrifuged for 30 min at a temperature of 4°C, at a speed of 10 000 *g*, using a Sorvall RC-5B centrifuge (Sorvall, Hamburg, Germany). The supernatant was separated, frozen at −20°C, and then stored at 20°C for not longer than 1 month. When thawed, the clear solutions served for biochemical examinations.

Chemical reagents

The following reagents were used in the study: N-benzoyl-DL-arginyl-β-naphthylamide (BANA), benzoyl-phenyl-arginyl-aminocoumarin (Z-Phe-Arg-AMC), 7-amino-4-methylcoumarin (Mec), inhibitor E-64, and enzymes. Papain and pepsin were manufactured by Sigma (St. Louis, MO, USA); methylamine 40% solution and dimethyl sulfoxide (DMSO) were manufactured by Fluka BioChemika (Birchs, Switzerland).

Measurement of enzyme activities

In the recent literature on the subject the activities of cysteine peptidases have been measured by two equivalent methods: spectrofluorometry and colorimetry. Most of our measurements were made by the colorimetric method, but we also used spectrofluorometry to prove that the results were not dependent on the method used.

Measurement of the activity of cysteine peptidases by spectrofluorometry

The activity of cysteine peptidases was measured with a Perkin Elmer UV/VIS Spectrometer Lambda Bio 20 (Perkin Elmer, Wellesley, MA, USA). One sample contained 100 μl of supernatant from the homogenized tissue, diluted with normal saline as needed according to the concentration of the enzyme; 700 μl 0.4 M phosphate buffer at pH 6.0, including 4 mM ethylenediamine tetraacetic acid (EDTA), 2.5 mM dithiothreitol (DTT), and 200 μl diluted substrate concentrate Z-Phe-Arg-N-Mec, whose concentration was 40 μM.

The sample was incubated at 37°C for 60 min. The reaction of hydrolysis was terminated by adding 2.0 ml of 1.0 mM iodoacetic acid to each sample. Fluorescence of the liberated 7-amino-4-methylcoumarin (Mec) was measured using the wavelength $\lambda = 370$ nm for excitation and $\lambda = 440$ nm for emission. The measurements were made in regard to baseline solution, which differed from the analyzed samples in that 2.0 ml of iodoacetic acid was added before the substrate. The results are given in units of activity of cysteine endopeptidases, calculated for the amount of protein appearing in the sample. One unit of enzyme activity was defined as that amount of the enzyme that would liberate 1 nM of Mec per 1 min, calculated for 1 mg of protein [11].

Measurement of the activity of precursors of cysteine peptidases

To the diluted supernatant samples of tissue homogenates (50 μl each), 150 μl of pepsin, diluted to 0.7 mg/ml in 0.01 M acetate buffer at pH 3.0, was added. The samples were incubated at 37°C for 60 min, then

0.4M phosphate buffer at pH 6.0, containing 4mM EDTA and 200μl of diluted substrate concentrate Z-Phe-Arg-N-Mec, whose concentration was 40μM, were added to make a volume of 1.0ml. The samples were then incubated at 37°C for 60min and the active cysteine endopeptidases were measured as described above. The activity of the precursors was calculated as the difference between enzyme activities measured directly in the homogenate and those measured after preincubation with pepsin [4].

Measurement of the activity of cysteine peptidases by colorimetry

To the samples of homogenate (10μl to 100μl each) 50μl of 0.66mM BANA substrate was added, then 0.01M phosphate buffer at pH 6.0, containing BANA, was added, and after 12h of preincubation at 37°C, free β-naphthylamine was measured by Barrett's method. One unit of enzyme activity of cysteine proteinases was defined as that amount of the enzyme that would liberate 1nM of β-naphthylamine per 1h, calculated for 1mg of protein [12].

Measurement of the activity of cysteine peptidase inhibitors by colorimetry

Before the measurement of the inhibitors of cysteine peptidases, α_2-macroglobulin was inactivated [13]. To measure the active inhibitors of cysteine endopeptidases (ICP^{37}), the analyzed homogenate samples were incubated with papain solution, and after 30min, the amount of liberated β-naphthylamine was measured. The procedure was as follows:

1. Samples of homogenate (50-μl) were incubated in 0.6ml of 0.02M phosphate buffer containing 2mM EDTA and 2mM L-cysteine at pH 6.0. Then 50μl of papain solution in the same buffer was added, the sample was incubated at 37°C for 10min, and liberated β-naphthylamine was calculated from the standard titration curve prepared earlier. One unit of inhibitor activity was defined as that amount of the inhibitor that would inhibit 1 unit of papain activity.
2. The total amount of inhibitors (ICP^{80}) was measured after preincubation of the samples of homogenate supernatant in glycine buffer at pH 2.0, for 20min at 80°C. Then the activity of inhibitors of cysteine peptidases was measured in the presence of BANA substrate, as described above for the measurement of free inhibitors (ICP^{37}).
3. The activity of inhibitors bound in complexes was calculated as the difference between total and free inhibitors: $\Delta ICP = ICP^{80} - ICP^{37}$ [14]. The enzyme activities and the levels of inhibitors were calculated for 1mg of protein [13,14].

The concentration of protein in the analyzed samples was measured by a method using Bradford's reagent, where pure bovine albumin was taken as the standard [15].

Results

The measurements of these enzymatic markers of tumor growth enabled us to compare the individual differences in their activities for each patient. We compared the same parameters between patients, as well as the differences between the sampling sites in each individual patient (Tables 1 and 2, Figs. 1–6). In 17 patients, the highest activities of cysteine peptidases (CP) and their precursors (pCP) and inhibitors in complexes with enzymes (ΔICP) were found in the homogenates of cancer tissue, whereas in the remaining 12 patients, these activities were highest in the homogenates of tissue from the zone of invasion. In the whole series, the lowest mean activities of cysteine peptidases and their precursors and enzyme-inhibitor complexes were found in the homogenates of healthy tissue (Table 1). For the spectrofluorometric measurements, it was noted that the highest activities of cysteine peptidases and their precursors were present in cancer tissue in 22 patients, while in 7 patients they were highest in the tissue from the border of malignant growth (Table 2).

Table 1. Activities of cysteine peptidases, and their precursors and inhibitors, as determined by colorimetry (Col) and spectrofluorometry (F) in gastric cancer tissues

Location of tissue sample		CP (Col)	ICP^{37}	ICP^{80}	ΔICP	CP (F)	pCP (F)
(a) Center of tumor	Mean	0.447	1.118	1.611	0.493	10.170	5.700
	SD	0.139	0.108	0.185	0.076	8.217	3.988
(b) Zone of invasion	Mean	0.397	0.928	1.295	0.368	3.325	4.260
	SD	0.127	0.078	0.039	0.117	2.157	3.507
(c) Healthy tissue	Mean	0.305	0.752	0.861	0.110	1.480	0.825
	SD	0.025	0.399	0.423	0.023	0.863	0.290

CP, cysteine peptidases; pCP, precursors; ICP^{37}, active inhibitors; ICP^{80}, total pool of inhibitors; ΔICP, inhibitors bound in enzyme-inhibitor complexes

Table 2. Individual activities of cysteine peptidases (CP), and their precursors (pCP) and inhibitors (ICP), as determined by colorimetry (Col) and spectrofluorometry (F) in gastric cancer tissues

Patient no.	Tissue sample	CP (Col)	ICP^{37}	ICP^{80}	ΔICP	CP (F)	pCP (F)
I	1 a	0.545	1.195	1.742	0.547	4.36	2.88
	2 b	0.487	0.873	1.323	0.450	1.80	1.78
	3 c	0.322	0.469	0.562	0.093	0.87	1.03
II	4 a	0.687	0.513	1.272	0.760	21.91	32.45
	5 b	0.436	1.697	2.018	0.321	4.09	5.12
	6 c	0.430	0.733	0.847	0.114	0.73	0.98
III	7 a	0.765	1.318	2.294	0.976	8.07	12.67
	8 b	0.809	1.863	2.733	0.870	3.25	9.65
	9 c	0.521	0.245	0.585	0.340	1.15	3.61
IV	10 a	0.876	1.861	2.291	0.430	6.32	2.11
	11 b	0.395	0.634	1.045	0.411	5.69	4.76
	12 c	0.309	0.110	0.331	0.221	0.89	0.76
V	13 a	0.298	0.064	0.601	0.537	0.44	0.69
	14 b	0.365	0.531	1.101	0.570	4.89	1.76
	15 c	0.287	0.321	0.552	0.231	1.32	0.47
VI	16 a	0.890	0.298	0.728	0.430	1.87	3.39
	17 b	0.458	1.953	2.623	0.670	2.08	3.16
	18 c	0.187	0.841	1.165	0.324	0.88	1.01
VII	19 a	0.780	0.991	1.489	0.498	4.37	7.66
	20 b	0.567	0.870	1.224	0.354	2.26	2.63
	21 c	0.320	0.365	0.424	0.059	1.72	1.94
VIII	22 a	0.987	0.361	1.343	0.982	5.98	8.65
	23 b	0.564	0.643	1.399	0.756	1.22	1.38
	24 c	0.234	0.291	0.511	0.220	0.28	0.32
IX	25 a	0.567	1.954	2.386	0.432	4.70	6.97
	26 b	0.432	0.879	1.859	0.980	0.87	1.47
	27 c	0.229	0.194	0.425	0.231	1.14	1.75
X	28 a	0.402	0.980	1.745	0.765	0.72	0.86
	29 b	0.382	0.328	0.868	0.540	8.11	10.96
	30 c	0.287	0.211	0.234	0.023	2.56	3.23
XI	31 a	0.320	0.529	0.876	0.347	1.87	2.40
	32 b	0.469	1.532	2.512	0.980	12.3	1.73
	33 c	0.108	0.851	1.064	0.213	1.60	1.08
XII	34 a	0.980	0.527	1.096	0.569	9.45	13.03
	35 b	0.765	0.265	0.776	0.511	4.64	7.08
	36 c	0.309	0.289	0.609	0.320	2.89	1.06
XIII	37 a	0.387	1.067	1.806	0.739	19.96	15.43
	38 b	0.452	0.829	1.296	0.467	5.80	0.94
	39 c	0.320	0.358	0.708	0.350	3.98	0.64
XIV	40 a	0.321	0.110	0.950	0.840	11.89	9.70
	41 b	0.301	0.321	1.218	0.897	6.98	1.25
	42 c	0.254	0.834	0.849	0.015	2.06	1.85
XV	43 a	0.654	0.231	0.670	0.439	13.76	7.27
	44 b	0.432	0.358	0.738	0.380	4.87	1.56
	45 c	0.322	0.389	0.518	0.129	1.03	0.23
XVI	47 a	0.531	0.320	0.790	0.470	9.85	3.20
	48 b	0.632	0.540	0.851	0. 311	12.54	2.93
	49 c	0.313	0.371	0.477	0.106	2.76	0.97
XVII	50 a	0.431	0.298	1.118	0.820	16.33	6.39
	51 b	0.219	1.012	1.332	0.320	7.83	2.52
	52 c	0.201	0.876	1.263	0.387	3.63	1.64
XVIII	53 a	0.438	0.678	1.135	0.457	7.93	8.47
	54 b	0.576	0.459	1.133	0.674	3.86	1.68
	55 c	0.309	0.769	1.002	0.233	2.64	0.25

Table 2. *Continued*

Patient no.	Tissue sample	CP (Col)	ICP^{37}	ICP^{80}	ΔICP	CP (F)	pCP (F)
XIX	56 a	0.719	0.348	0.695	0.347	12.75	7.11
	57 b	0.812	0.278	0.563	0.285	5.79	1.67
	58 c	0.087	0.304	0.407	0.103	4.98	2.20
XX	59 a	0.689	1.967	2.610	0.643	7.33	3.66
	60 b	0.430	0.876	1.399	0.523	4.76	12.87
	61 c	0.301	0.996	1.253	0.257	2.32	0.88
XXI	62 a	0.597	0.560	1.492	0.932	5.87	2.75
	63 b	0.601	0.398	0.756	0.358	3.97	7.66
	64 c	0.321	0.329	0.562	0.233	0.96	1.02
XXII	65 a	0.760	1.071	1.653	0.582	16.76	5.74
	66 b	0.450	0.894	1.731	0.837	3.76	11.23
	67 c	0.296	0.692	0.923	0.231	9.63	3.53
XXIII	68 a	0.583	0.651	1.218	0.567	12.31	6.87
	69 b	0.210	1.076	1.861	0.785	19.51	7.54
	70 c	0.071	0.873	0.971	0.098	1.98	4.74
XXIV	71 a	0.349	0.349	0.881	0.532	4.75	6.33
	72 b	0.356	0.480	1.391	0.911	3.99	5.35
	73 c	0.207	0.312	0.631	0.319	0.74	2.87
XXV	74 a	0.861	1.073	1.349	0.276	13.22	2.98
	75 b	0.398	0.753	1.589	0.836	7.92	3.59
	76 c	0.200	0.590	0.693	0.103	2.43	2.84
XXVI	76 a	0.471	0.378	0.908	0.530	8.82	3.75
	77 b	0.318	0.210	0.972	0.762	7.43	5.87
	78 c	0.197	0.386	0.707	0.321	6.86	3.85
XXVII	79 a	0.542	0.476	1.147	0.671	13.25	3.22
	80 b	0.498	0.531	0.878	0.347	11.37	4.96
	81 c	0.295	0.379	0.598	0.219	1.24	1.07
XXVIII	76 a	0.431	0.396	0.915	0.519	13.25	3.01
	77 b	0.397	0.365	0.862	0.497	11.37	6.16
	78 c	0.254	0.254	0.600	0.346	6.21	0.22
XXIX	79 a	0.349	1.042	1.481	0.439	15.98	8.52
	80 b	0.307	0.983	1.268	0.285	4.85	6.74
	81 c	0.287	1.034	1.160	0.126	2.09	0.62

(a) center of tumor, (b) zone of invasion, and (c) healthy tissue

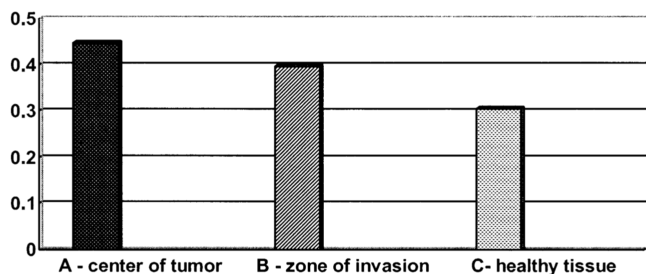

Fig. 1. Mean activities of cysteine peptidases (CP) in three different types of tissue samples in gastric cancer, as determined by colorimetry (Col)

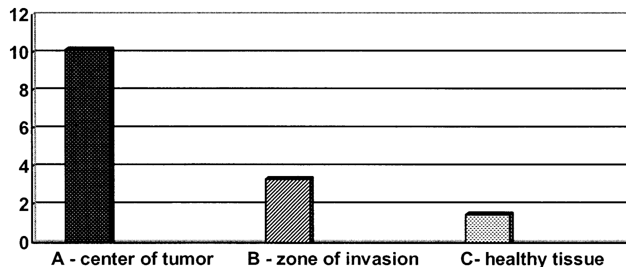

Fig. 2. Mean activities of cysteine peptidases (CP) in three different types of tissue samples in gastric cancer, as determined by spectrofluorometry (F)

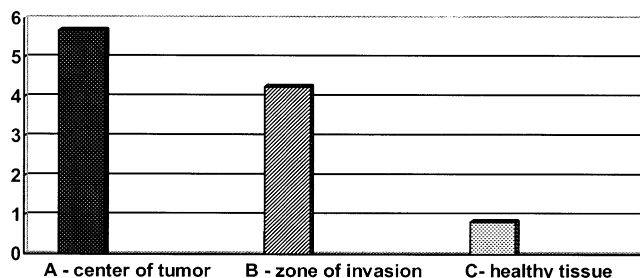

Fig. 3. Mean activities of precursors of cysteine peptidases (pCP) in three different types of tissue samples in gastric cancer

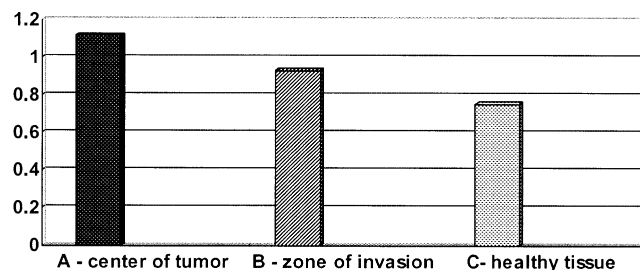

Fig. 4. Mean activities of active inhibitors of cysteine peptidases (ICP^{37}) in three different types of tissue samples in gastric cancer

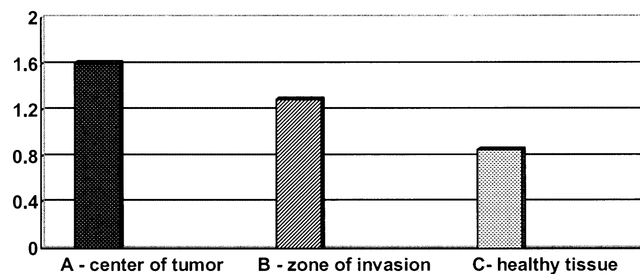

Fig. 5. Mean activities of total pool of cysteine peptidase inhibitors (ICP^{80}) in three different types of tissue samples in gastric cancer

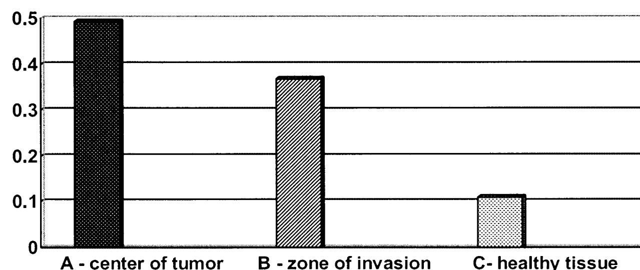

Fig. 6. Mean activities of cysteine peptidase inhibitors bound in enzyme-inhibitor complexes (ΔICP) in three different types of tissue samples in gastric cancer

Discussion

The reported relationships between selected proteolytic enzymes and the aggressiveness of malignant tumors indicated possible changes in the activities of cysteine peptidases and their autogenous inhibitors in the homogenates of tissues obtained from patients operated on for gastric cancer. To date, cathepsins B and L have usually been measured by enzyme-linked immunosorbent assay (ELISA) in the tissues and serum of patients with gastrointestinal cancers [16]. Such assessments of cathepsins B and L in tissues resected during operations for gastric cancer had shown maximum concentration in the tumor mass, while in the surrounding healthy tissue these enzymes were less abundant. This confirmed the previous assumptions that cysteine peptidases may play a key role in the processes accompanying malignant growth. Our study was intended to provide supplementary information on changes in enzymatic activity in tumor growth. The measurements of precursors of cathepsins B and L provided information about the potential of malignant tumor expansion. Activation of these precursors is an indicator of enhanced potential of tumor growth. On the other hand, the activity of inhibitors of these enzymes indicates the organism's defense ability against cancer. In the samples analyzed in this study, we measured three forms of specific inhibitors of cysteine peptidases: active inhibitors, the total pool of inhibitors produced, and the inhibitors bound in enzyme-inhibitor complexes. The active inhibitors were characterized by the ability to inhibit papain, which is a cysteine peptidase.

The inhibitors complexed with cysteine peptidases, which are secreted by tumor cells, we considered one of the markers of malignancy, and we referred to them as ΔICP [9]. Gastric cancer was intentionally selected as the subject of our study, considering the acidic pH and the presence of pepsin in the stomach, which can activate the precursors of cysteine peptidases to the active form [4]. We surmise that the observed differences between the tissues sampled from the tumor mass, the zone of invasion, and the normal tissue within the same patient with gastric cancer result from the action of pepsin at low pH, which directly affects the tissues in which the malignant tumor is growing, thus catalyzing the transformation of cathepsin B and L precursors to the active enzymes, which in turn, enhances the tumor's invasiveness. Our results for the measured activities of CP, pCP, and ΔICP suggest that these parameters may prove to be a useful diagnostic tool for evaluating the dynamics of malignant growth within the tissues of gastric cancer. In the future, these parameters may play some role in the designing of the extent of the operation. To elucidate this we are planning a similar study

with respect to bioptates taken endoscopically before the operation. Other important information implied by previous studies is that there may be some possibility of treating gastric cancer with specific inhibitors of cysteine peptidases, isolated from egg white. So far we have been able to demonstrate the inhibition of cysteine peptidases which initiate the growth of gastric cancer only in vitro, in homogenates of tissues sampled from resected material after operation [17]. The information that cysteine peptidase inhibitors isolated from egg whites are able to inhibit the elevated activity of cysteine endopeptidases in gastric cancer cells suggests that these inhibitors might be used as a component of new-generation drugs in so-called "inhibitor therapy" [18]. These inhibitors have been proved nontoxic and may be administered orally. In addition, there is a possibility of labelling them with fluorescent agents approved for medical use, which might be helpful in UV evaluation of the extent of malignant infiltration [19]. There are also prospects for introducing a new method of therapy that would consist of cysteine proteinase inhibitor therapy combined with photodynamic therapy [20].

Moreover, antibodies against these inhibitors may be used for detecting the activity of cysteine peptidases in preoperative endoscopic bioptates, which could be helpful in planning the extent of the operation [21]. In 2002 we found that, in tissue cultures of malignant tumors, cysteine peptidase inhibitors labelled with radioactive iodine reacted selectively, only with cancer cells [22]. So far these investigations have been limited to in vitro experiments, but at the moment we are preparing a series of experiments which might allow further studies in vivo, at first on animals; we hope that in the future, such studies could be carried out in gastric cancer patients.

Conclusions

1. Local activities of cysteine peptidases and their inhibitors reflect the topographical differences between the center of the tumor, the zone of invasion, and healthy tissues in gastric cancer patients.
2. The findings of the pattern of changes in the activity of cysteine peptidases according to the degree of tissue infiltration are not dependent on the method of measurement (colorimetry vs spectrofluorometry).

References

1. Plebani M, Herszenyi L, Cardin R, Roveroni G, Carraro P, Paoli MD, et al. Cysteine and serine proteases in gastric cancer. Cancer 1995;76:367–75.
2. Herszényi L, Farinati F, Plebani M, Carraro P, Roveroni G, De Paoli M, et al. Prognostic role of cysteine and serine proteases in gastric cancer. Orv Hetil 1996;137:1637–41.
3. Schmitt M, Janicke F, Graeff H. Tumor-associated proteases. Fibrynolysis 1992;6:3–26.
4. Pagano M, Capony F, Rochefort H. Pro-cathepsin D can activate in vitro procathepsin B secreted by ovarian cancers. C R Acad Sci III 1989;309:7–12.
5. Maciewicz RA, Wardale RJ, Wotton SF, Duance VC, Etherington DJ. Mode of activation of the precursor to cathepsin L: implication for matrix degradation in arthritis. Biol Chem Hoppe Seyler 1990;371:223–30.
6. Mion K, Cao L, Day NA, Koblinski JE, Sloane BF. Tumor cell membrane cathepsin B. Biol Chem 1998;379:1093–9.
7. Laszlo A, Sohar I, Karacsonyi-S, Petri A, Trojan I. Activities of serum cathepsin (B, H and L) and metalloproteinase (MMP7-ase) in patients with gastrointestinal and bronchial malignant tumours. Acta Med Hung 1990;47:107–9.
8. Sheahan K, Shuja S, Murnane MJ. Cysteine protease activities and tumor development in human colorectal carcinoma. Cancer Res 1989;49:3809–14.
9. Siewinski M, Gutowicz J, Mikulewicz W, Zarzycki A. Participation of cysteine endopeptidases in the invasion and metastasis of tumor and in neoplastic transformation. Cancer Biotherapy Pharmaceuticals 1996;11:321–31.
10. Dohchin A, Suzuki J, Seki H, Masutani M, Shiroto H, Kawakami Y. Immunostained cathepsins B and L correlate with depth of invasion and different metastatic pathways in early stage gastric carcinoma. Cancer 2000;89:482–7.
11. Barrett AJ. Fluorimetric assays for cathepsin B and cathepsin H with methylcoumarylamide substrates. Biochem J 1980;187:909–12.
12. Krecicki T, Siewinski M. Serum cathepsin B-like activity as a potential marker of laryngeal carcinoma. Eur Arch Otorhinolaryngol 1992;249:293–5.
13. Minakata K, Asano M, Sato T, Harada N. Assay of a cysteine inhibitor in serum or plasma. Hoppe Seyler's Z Physiol Chem 1982;363:493–8.
14. Siewinski M, Krecicki T, Berdowska I, Jarmulowicz J. Cysteine proteinase inhibitors in serum of patients with head and neck tumors. Diagn Oncol 1993;2:323–6.
15. Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principal of protein-dye binding. Anal Biochem 1976;72:248–54.
16. Farinati F, Herszényi L, Plebani M, Carraro P, De Paoli M, Cardin R, et al. Increased levels of cathepsin B and L, urokinase-type plasminogen activator and its inhibitor type-1 as an early event in gastric carcinogenesis. Carcinogenesis 1996;17:2581–7.
17. Saleh Y, Siewinski M, Kielan W. Inhibition in vitro of cysteine endopeptidases-like activity in stomach carcinoma tissues. Cancer Detect Prev 2002;26:1291.
18. Lah TT, Kos J. Cysteine proteinases in cancer progression and their clinical relevance for prognosis. Biol Chem 1998;379:125–30.
19. Gutowicz J, Michalak K, Pola A, Berdowska I, Siewinski M. Fluorescence labelling of thiol proteinase inhibitors excreted from urine of colorectal cancer patients. Current Topics in Biophysics 1996;21:238–45.
20. Saleh Y, Ziólkowski P, Siewinski M, Milach J, Marszalik P, Rybka J. The combined therapy of transplantable solid mammary carcinoma in Wistar rats by use of photodynamic therapy and cysteine proteinase inhibitors. In Vivo 2001;15:351–8.
21. Siewinski M, Saleh Y, Popiela A, Ziólkowski P, Jelen M, Grybos M. Expression of high molecular weight cysteine proteinase inhibitor in ovarian cancer tissues: regulation of cathepsin B expression by placental CPL. Biol Chem 2003;384:1103–7.
22. Czecior E, Szymaniec S, Siewinski M, Fortuna W, Miedzybrodzki W. A potential use of cysteine endopeptidases inhibitors of upper respiratory tract tumors. Otolaryngol Pol 2002;56:573–6.