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Short Communication

A possible application of cathepsin B activity determination for estimating the spread of the cervix uteri carcinoma

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The value of cathepsin B activity determination for evaluation of the extent of disease was investigated in 98 patients with the cervix uteri carcinoma and 25 women with cervix uteri dysplasia. The measurements were performed before treatment. Cathepsin B activity was estimated in serum using Z-Phe-Arg-NMec, and in tumor tissue using Z-Arg-Arg-pNA · HCl as substrates. The mean activity of the enzyme increased both in serum and tumor tissue with progression of neoplastic disease and was dependent on the clinical stage of cervical carcinoma. It should be stressed, however, that among the patients with the clinically observed early stage of the disease, higher cathepsin B activity was observed in those in whom metastases to pelvic lymph nodes were detected than in those in whom the disease was limited to cervix uteri.

The extent of disease is one of the most important factors in determining the policy of cervical carcinoma treatment. Gynaecological examination has been regarded as an essential method of evaluating the local spread of the disease. However, it is well known that clinical examination is often inaccurate and errors in FIGO staging have been made [1]. On the other hand, the occurrence of metastases in regional lymph nodes is recognized as an important prognostic factor for cervical carcinoma patients and metastases have been found in patients with early non-bulky disease [2]. In view of the difficulties in defining the exact clinical status of the cervical carcinoma patients, it seems that introduction of an additional diagnostic test could be helpful.

Cathepsin B, a cysteine proteinase, has been implicated in tumorigenesis and has been found to associate with plasma membranes of malignant cell lines [3]. Several investigations undertaken to identify tumor-associated degradative enzymes showed that cathepsin B is probably implicated in the degradation of host tissue barriers during the invasive process [4, 5]. Sloane [6] has also found a correlation between metastatic capacity of experimental melanoma B16 cells and production of cathepsin B by these cells. Data reflecting the clinical significance of a cathepsin B lysosomal proteinase in cervix carcinoma are, however, scarce [7].

The aim of this study was to estimate the applicability of cathepsin B activity measure-

Abbreviations: Z-Arg-Arg-NNap, carbobenzoxy-arginyl-arginyl-2-naphthylamide; Z-Phe-Arg-NMec, carbobenzoxy-phenylalanyl-arginine-7-amido-4-methylcoumarin.

ment for determining the extent of disease in patients suffering from cervical carcinoma.

MATERIALS AND METHODS

The study was performed on 98 women with the cervix uteri carcinoma and, as a negative control, on a group of 25 patients with dysplasia of the cervix uteri. The extent of the disease was staged using the classification of FIGO. All patients had histologically proven cervical carcinoma. In 38 patients the spread of the disease was re-evaluated at the time of the surgery by surgical findings and by histological examination. The age of patients with cervix uteri carcinoma ranged from 30 to 75 years (median 51.2 years) and in those with dysplasia from 23 to 48 years (median 35.4 years).

The measurements of cathepsin B activity were performed before treatment. The blood samples (10 ml) were drawn from the arm vein without anticoagulants into plastic tubes, and left for clotting at laboratory temperature for 1 h, then the samples were centrifuged at 3000 r.p.m. The tumor speciments dissected for biochemical analysis were homogenized in 250 mM sucrose with 5 mM EDTA, pH 6.5, at 4°C, then centrifuged at 900 r.p.m. for 5 min and the pellets containing nuclei, tissue fragments and cell debris were discarded. The blood sera and tissue homogenates were stored at –20°C until being analyzed, but not longer than 1 week.

Enzymatic activity in sera was measured against Z-Phe-Arg-NMec (Biochem., Switzerland) as a substrate by a direct fluorimetric method according to Barrett [8]. With this substrate combined hydrolysing activities of cathepsins B and L were evaluated. In tissues homogenates Z-Arg-Arg-NNap (Calbiochem, Switzerland) was used as a substrate to measure cathepsin B activity [9]. Protein determinations were made by the phenol method of Lowry *et al.* [9] using bovine serum albumin as a standard. As the upper normal limit (cut-off) of cathepsin B activity we took the control average plus one standard deviation (SD). Statistical analysis was performed using analysis of variance and Student's *t*-test. Probability values of 0.05 or less were considered to be significant.

RESULTS AND DISCUSSION

The results are presented in Table 1.

In serum, the combined enzymic activities in patients with the stage Ia were practically the same as in women with dysplasia of the cervix uteri. In patients with stage Ib and IIa inter-

Table 1

Enzymic activities (mean ± SD) in sera and tissue homogenates derived from patients with cervical carcinoma and with dysplasia of cervix uteri (control)

Clinical stage	Number of patients	Z-Phe-Arg-NMec hydrolysing activity in sera nkat/liter of serum	Cathepsin B activity in homogenates of cervix uteri tissue nkat/mg of protein
Dysplasia	25	0.21 ± 0.28	8.90 ± 6.81
Cervical carcinoma			
Ia	8	0.19 ± 0.30	27.1 ± 19.2
Ib	23	0.40 ± 0.88	64.2 ± 27.4
nodes (–)	14	0.20 ± 0.11	22.5 ± 12.2
nodes (+)	9	1.22 ± 0.49	70.7 ± 18.4
IIa	15	0.44 ± 0.69	66.0 ± 28.4
nodes (–)	8	0.22 ± 0.15	21.4 ± 10.0
nodes (+)	7	1.19 ± 0.39	71.7 ± 20.1
IIb	17	2.01 ± 2.40	70.5 ± 28.4
III	25	3.10 ± 2.48	82.5 ± 43.5
IV	10	5.75 ± 3.25	81.9 ± 17.5

mediate levels of the measured activity were observed. It should be stressed, however, that after reevaluation of the stage of disease at the time of surgery it appeared that the patients with metastases to pelvic lymph nodes had higher enzymic activities, both in serum and in tumor tissue, than the patients with the disease limited to the cervix uteri ($P < 0.001$, t-test). The enzymic activities distinctly increased with the severity of the disease and were the highest in the stage IV patients. Analysis of variance showed that the relationship between the cathepsin B activity in serum and the clinical stage was significant ($P < 0.01$).

The obtained mean values of cathepsin B activities in tumor tissue also were increased at more advanced stage of the disease, from 27.1 nkat/mg of protein in stage Ia up to about 82.5 nkat/mg of protein in stage III.

Numerous investigations have demonstrated that cathepsin B is widely distributed in tissues and that many experimental and human neoplasm tumors exhibit an increased activity of this enzyme as compared to normal tissues [3, 11–13]. The elevated cathepsin B-like activity is probably due to its increased production and secretion by tumor cells, or to a disorder of the enzyme elimination from circulation [4, 14–16]. However, very few clinical studies have demonstrated an association or correlation between tumor or serum concentration of this proteinase and the clinical advance of neoplastic disease. Pietras *et al.* [17] demonstrated enhanced serum activity of cathepsin B in women exposed prior to childbirth to diethylstilbestrol (DES). Mean serum cathepsin B activities were analyzed by them in relation to severity of vaginal pathology as compared to levels of cathepsin B in healthy women. The results revealed a marked elevation in cathepsin B activity in serum of patients with vaginal adenosis (10 fold) and adenosis with concomitant dysplasia (26 fold). The most dramatic elevation was evident in patients with carcinoma (46 fold). The results were expressed as a proportional increase in relation to the levels of cathepsin B in healthy women. Benitez-Bribesca *et al.* [14] reported a significant increase ($P < 0.001$) of cathepsin B in serum and vaginal fluid of all patients with the cervix uteri carcinoma including patients with clinical stage 0 (carcinoma *in situ*). According to them, cathepsin B elevation has a direct and linear correlation with the clinical stage of cancer, reaching its highest level at the most advanced stages. They also emphasized, that after treatment the serum level of cathepsin B showed a marked decline if a regression of disease was achieved. Haczyńska & Warwas, [18] tested the diagnostic value of serum cathepsin B activity in patients with ovarian carcinoma. The level of this enzyme in women with recurrent or stage III-IV disease was higher as related to stage I-II ($P < 0.001$).

We examined the relative merits of measured cathepsins activity particularly in the assessment of the extent of cervical carcinoma. It seems of importance that there was a striking correlation between serum or tumor cathepsins activities and the advance of the disease. In contrast to Benitez-Bribesca *et al.* [7, 16], we have not found increased activity of cathepsins at early stages of the disease limited to the cervix uteri. It should be stressed, however, that in the early cervical carcinoma patients with metastases to pelvic lymph nodes, the level of measured cathepsins was markedly increased. Since such metastases are considered to be a major prognostic factor in cervical carcinoma [2] the high cathepsin B concentration in serum and tumor tissue in patients with early stages of the disease could serve in clinical practice as a biochemical marker of the neoplasm invasion. Our results are also in agreement with the hypothesis that an increased level of cathepsin B is associated with invasion of surrounding tissue by cancer cells [6].

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