The effect of combined therapy on activity of cathepsin D and alpha-1-antitrypsin in the blood serum of women with cervical cancer

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Summary

Purpose of investigation: The aim of the study was to determine the activity of cathepsin D (CTSD) and alpha-1-antitrypsin (AAT) in the blood serum of women with cervical carcinoma treated with different modes of therapy. *Methods:* The study was conducted on 68 women suffering from carcinoma of the uterine cervix, that were irradiated intracavitarily by a Selectron LDR brachytherapy unit. Additionally, all patients were treated with different therapy methods according to clinical stage. *Results:* In women with cervical cancer, CTSD activity was higher while AAT activity was lower both before and after brachytherapy sessions as compared to controls. Six months after the end of therapy, the activity of CTSD and AAT reverted back to the values characteristic for healthy women. *Conclusion:* The estimation of cathepsin D and alpha-1-antitrypsin activity during the course of cervical cancer management may be useful in early detection of potential recurrence and/or widespread metastasis formation.

Key words: Cathepsin D; Alpha-1-antitrypsin; Cervical cancer; Therapy.

Introduction

Cervical cancer is the most commonly occurring neoplasm in women. The recommended management of this carcinoma is primal surgery and adjuvant or neoadjuvant radiotherapy as external beam radiotherapy and/or intracavitary brachytherapy. There is no treatment of choice for early-stage cervical carcinoma in terms of overall or disease-free survival, but the combination of surgery and radiotherapy has the worst morbidity [1]. In advanced stages the results of radiotherapy are not satisfactory and more recently concurrent chemoradiation, particularly with cisplatin-based regiments are successfully applied [2]. With the aim of yielding the best cure with minimum complications, the mode of treatment or combination of different treatment methods should depend not only on the neoplastic process grade, but also on the age, any concomitant illnesses, histological type, cervical diameter and menopausal status of patients [3].

For the early diagnosis of uterine cervical carcinoma, cytological, histological and biochemical tests are of great importance [4]. Although potential cervical cancer markers are considered to be of prognostic value, they in fact have no significant role in the clinical management of cervical cancer. Squamous cell carcinoma antigen (SCC) together with other assays, seems to be a useful tool in the determination of response to chemotherapy [5], but yet large trials are needed to validate it. The invasive and metastatic potential of malignant cells results from complex interactions of numerous factors. An important role in the occurrence and development of neoplasms, and also in the formation of widespread metastasis is attributed to lysosomal enzymes [6].

It is suggested that novel approaches for the selection of specific prognostic factors that would be valuable as indications for administration of different management are needed. The aim of the paper was to determine the activity of lysosomal protease - cathepsin D (CTSD) and activity of alpha-1-antitrypsin (AAT) - one of the protease inhibitors in the blood serum of women with cervical cancer before and after the combined therapy.

Patients and Methods

The study was performed on 68 women (an average age 50) being treated for carcinoma of the uterine cervix in the Regional Center of Oncology in Bydgoszcz. The patients were divided into three groups as regards the management, which was planned individually, depending on the degree of clinical advancement of the neoplasm according to the FIGO scale and the general state of patients' health. Intracavitary brachytherapy (Selectron LDR unit) was used in all of the patients. The 1st group consisted of 37 patients in clinical Stage I and early Stage II. In those women neoadjuvant brachytherapy was used (45-50 Gy, Cs^{137}). After four or six weeks, the patients underwent surgery (the Werthein-Neigs method). Twenty-one patients in clinical Stage II and III were included in the 2nd group. They were given combined treatment as neoadjuvant brachytherapy (50-60 Gy, Cs^{137}) prior to external telecobalt therapy (45 Gy, cobalt 60) and chemotherapy (cisplatin + 5-fluorouracil). Ten

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women from the 3rd group were given brachytherapy and adjuvant external teletherapy. The given doses were the same as in the 2nd group.

Blood samples were taken before the brachytherapy, after two brachytherapy sessions and about six months later, during a check-up. A set of control blood samples was obtained from 25 healthy women without any known disease (average age 56). Venous blood was taken, alongside other diagnostic tests and was placed in dry sterile tubes to obtain blood serum. The serum was then frozen and kept at the temperature of -20°C until the activity of CTSD and AAT was evaluated.

Cathepsin D activity was determined according to the Anson method [7], while alpha-1-antitrypsin activity was determined using the method of Eriksson [8]. CTSD activity was expressed in 10^{-2} nM of tyrosine released during hemoglobin hydrolysis per mg of protein per min and AAT concentration was expressed in mg inhibited activity of trypsin in 1 ml of blood serum.

All the data were statistically analyzed by means of a one-way ANOVA test. Correlation coefficients of examined parameters were also calculated. The statistically significant level of $p < 0.05$ was accepted.

Results

The activity of CTSD (Table 1) in the blood serum of patients from all three groups both before therapy and after two brachytherapy sessions was about three-fold higher than in healthy women ($p < 0.05$). During the period of treatment the activity of the enzyme did not alter in a statistically significant way. After six months of therapy CTSD activity in all three groups of patients decreased and reverted back to the values observed to those in healthy women. At this time a significant improvement in the clinical state of patients was also observed. When comparing patients treated with different modalities, there were no statistically significant differences in CTSD activity among the 1st, 2nd and 3rd groups.

In women suffering from cervical cancer the activity of AAT (Table 1) was lower compared to the controls ($p < 0.05$). As a result of six-month treatment, an increase in AAT activity occurs and then there were no statistically significant differences in comparison to the control group. The activity of the protease inhibitor was found to be unaltered after two brachytherapy sessions as compared to the value before the start of therapy. No statistically significant differences in AAT activity among patients from the three groups were found.

Moreover, comparing the activity of CTSD with AAT activity, we found that in the blood serum of women suffering from cervical cancer both before and after the treatment, high enzyme activity is accompanied by low inhibitor activity ($r = –0.80$; $p < 0.05$).

Table 1. — *Activity of cathepsin D (CTSD) and alpha-1-antitrypsin (AAT) in the blood serum of women with cervical cancer treated with combined methods and the control group (values are given as means ± SD).*

Control Group (healthy women)		PATIENTS before the treatment	after two therapy sessions	six months after the end of therapy
	CTSD (10^{-2} nM tyrosine/mg protein/min)			
2.64 ± 0.45	1st group	7.26 ± 2.28[a]	7.72 ±2.62[a]	2.91 ± 1.02[bc]
	2nd group	7.87 ± 3.29[a]	7.88±5.70[a]	2.46 ± 0.78[bc]
	3rd group	8.03 ± 4.35[a]	10.06 ± 4.63[a]	3.01 ±1.05[bc]
	AAT (mg impeded trypsin/ml serum)			
1.84 ± 0.38	1st group	0.63 ± 0.33[a]	0.60 ± 0.30[a]	1.80 ± 0.52[bc]
	2nd group	0.77 ± 0.44[a]	0.78 ± 0.33[a]	1.88 ± 0.52[bc]
	3rd group	0.85 ± 0.45[a]	0.59 ± 0.44[a]	1.81 ± 0.71[bc]

1st group – brachytherapy prior to surgery, 2nd group – teletherapy before brachytherapy + chemotherapy, 3rd group – external beam radiation adjuvant to brachytherapy.
– statistically significant differences in comparison with the control group: [a]$p < 0.05$;
– statistically significant differences in comparison with patients before therapy: [b]$p < 0.05$;
– statistically significant differences in comparison with patients after two therapy sessions: [c]$p < 0.05$.

Discussion

Cathepsin D plays the main proteolytic role within the cells, but the quantity and activity of this enzyme depends on the type of cell and its metabolism [9]. The high CTSD activity in fibroblasts of tumor tissue is known to facilitate the hydrolysis of intercellular structures, detachment of neoplastic cells from the primary tumor followed by their migration, and then invasion of other tissues [10]. Cathepsins are probably responsible for proteolytic degradation of the extracellular matrix which may be the reason for cancer cell spreading and metastasis formation [11]. Lysosomes in neoplastic cells possess very labile membranes which cause the easy release of enzymes into the cytoplasm and consequently the dynamics of the lysosomal enzyme activity in blood serum is closely connected with the enzymatic activity within the tumor tissue [12]. Higher cathepsin D activity observed in different carcinomas as compared to healthy controls is supposed to be related to a shorter disease-free interval and overall survival of cancer patients [13]. Nevertheless, in the literature there are very few data concerning CTSD activity in cervical cancer patients. In the present study we revealed higher cathepsin D activity in the blood serum of women with cancer of the uterine cervix both before the therapy and after two brachytherapy sessions in comparison to the control group. The increased activity of other lysosomal enzymes such as cathepsin B, alkaline phosphatase and acid phosphatase in women suffering from cervical cancer was previously reported by some authors [14-16]. Utrera-Barillas *et al.* [17] demonstrated that the complex interaction between increased cathepsin B activity and expression of some genes associated with invasiveness of a neoplasm in cervical cancer patients may have an effect on the clinical behavior of the tumor.

Protease inhibitors fulfill an important role in maintaining the proteolytic balance of the organism, thus intensified proteolysis in tumor tissue as well as in surrounding tissues may be related to the disturbed inhibition of lysosomal proteases in cancer cells. In this paper we revealed a high negative correlation between CTSD and its inhibitor activity ($r = –0.80$; $p < 0.05$) in women with cervical cancer. The level of alfa-1-antitrypsin is thought to have a direct relationship with cervical cancer conditions [18]. In this paper we showed the decreased AAT activ-

ity in the blood serum of cervical cancer patients before the start of therapy, and after two brachytherapy sessions it was still lower than in the control group. The defective inhibition of the proteases of cervical carcinoma cells was discussed concerning its in vivo significance for invasion of neoplasm [19]. It seems that measurement of CTSD activity together with AAT activity in blood serum may be significant in predicting recurrence in cervical cancer patients.

Biochemical tests that play a significant role as the designation of so-called neoplastic markers, do not always reflect the management efficacy as well as the response of patient organisms to the administration of different treatment methods. In the present study, no statistically significant alterations were found among the three groups of patients with the differently applied management. It may prove the fact that the mode of therapy has no effect on the recovery of lysosomal enzymes in those women. However, treating cervical cancer patients with surgery, radiotherapy and chemotherapy improves the clinical state of patients and it seems that the proteolytic balance plays an important role in this phenomenon. In this study we demonstrated that the activity of cathepsin D as well as alpha-1-antitrypsin in the blood serum of women with cervical carcinoma had been brought back to normal after six months of combined therapy, which may testify to the treatment efficacy. As multiple lysosomal enzymes are involved in invasion and metastasis, the most useful information may be obtained by the combined measurement.

Conclusion

Determination of the activity of cathepsin D along with alfa-1-antitrypsin activity during the course of cervical cancer may not be useful in monitoring the treatment process itself, but it may help to estimate the efficacy of applied management with the aim of early detection of potential recurrence and/or widespread metastasis formation.

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