Original paper

Activity of cathepsin D and α1-antitrypsin in blood of men with malignant melanoma

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Abstract

Introduction: The aetiopathology of melanoma is (like in other cancers) a result of environmental factors and genetic predispositions. One of the most important environmental factors which has an influence on melanoma aetiology is exposure to UV radiation.
Aim: To estimate the activity of selected parameters in melanoma diagnosis.
Material and methods: In serum of 25 men with melanoma, activity of cathepsin D and α1-antitrypsin (important inhibitors of protease) was estimated. It was a heterogeneous group, because some men were classified according to Clark's classification, and other men were classified by Breslow's classification. Three months after resection of the lesion, only 13 men came back for the next tests.
Results: Activity of α1-antitrypsin in patients before and after removing the affected tissue was similar and related to the control value (0.78 mg of trypsin). Medium activity of cathepsin D in the control group was about 11.88 nM. Activity in patients before surgery was lower: 8.94 nM. After removing the tumour, this activity was 9.31 nM.
Conclusions: It can be supposed that the proteolytic-antiproteolytic balance was disturbed in persons with melanoma, because the activity of cathepsin D in serum of these people was lower than in healthy people, but the activity of α1-antitrypsin was not changed.

Key words: melanoma malignum, cathepsin D, α1-antitrypsin.

Introduction

Factors conducive to malignant melanoma include a fair complexion, freckles, propensity to sunburn, fair hair and birthmarks. These factors are genetically conditioned and the response to UV exposure is a complex process [1–3].

Apart from the conducive factors mentioned above, the progress of malignant melanoma can also be influenced to a great extent by old age, dysplastic nevus syndrome, exposure to ionizing radiation, immunosuppression and genetic defects such as xeroderma pigmentosum. An increased risk was also observed in employees exposed to vinyl chloride, employees of the petroleum industry and people with human papillomavirus [4, 5].

Cathepsin D [E.C.3.4.23.5] is an aspartyl endopeptidase. Similarly to other cathepsins, it is present in cells of all tissues with especially high activity in kidneys, spleen, liver and macrophages. The activity of cathepsin D causes the proteolysis of cellular and extracellular proteins. Considerable intensity of cellular degradation and increased activity of cathepsin D are observed in some inflammations [6]. Increased activity has also been observed in the course of various neoplastic diseases [6, 7].

Inhibition of proteolytic enzyme activity depends for example on the presence of plasma inhibitors, represented e.g. by α1-antitrypsin. It belongs to serine inhibitors with a molecular mass of 55 kD. α1-Antitrypsin is one of the most important protease inhibitors in human blood plasma and a major component of plasma globulin [8]. The activity of protease inhibitors is also one of the biochemical markers in the diagnosis and course of neoplastic diseases.

An important role in maintaining the proteolytic-antiproteolytic balance in a cell is attributed to the balance between the activity of proteases and the activity of their inhibitors.

The activity of selected parameters could be an additional prognostic factor in the course of neoplastic disease and could serve to monitor the treatment.

Aim

In the course of many neoplastic diseases, the imbalance of protease-antiprotease is observed. It was

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decided to establish the usefulness of determining the activity of cathepsin D and α1-antitrypsin in malignant melanoma patients to diagnose and monitor the course of the illness.

Material and methods

Examined group

Twenty-five men, aged from 27 to 79 (average age: 55.5), with diagnosed malignant melanoma, were included in the study. These patients were treated at the Clinic of Surgical Oncology of the Oncological Centre in Bydgoszcz. Malignant melanoma was diagnosed on the basis of histopathology of the resected lesions. Neoplastic tumours were located on the skin of the back, lower and upper limbs and on the auricles. Blood to be used for examinations was taken before removing the tumour and 2–3 months after the surgery. For taking blood after removing the tumour, only 13 of 25 men turned up. They were aged from 31 to 79 (average age: 57.4). There is no information about what happened to the other patients. The control group comprised 14 men aged from 23 to 64 (average age: 32.8). Their blood was taken for the tests once.

Determination of the activity of cathepsin D in blood serum

The activity of cathepsin D was determined using Anson's method. The substrate of the reaction is 2% denatured beef haemoglobin, which is dissolved in 100 ml of 0.1 M citrate-phosphate buffer with pH 3.8. The reaction is started by adding the examined serum to the solution. The next step is incubation at 37°C. This reaction is inhibited with a 0.1 N solution of NaOH. Adding phenol reagent results in blue tinting, the level of which is spectrophotometrically determined with a wavelength of λ = 600 nm. The concentration of this reaction's product is calculated using the curve for tyrosine. The activity of cathepsin D is expressed in nmol tyrosine/mg protein/min.

Determination of α1-antitrypsin activity in blood serum

The activity of α1-antitrypsin was determined using Eriksson's method [9]. The substrate of this reaction is benzoyl-DL-arginine-p-nitroanilide. The absorbance of the test is read with a wavelength of λ = 410 nm. The activity of α1-antitrypsin is expressed in mg of trypsin inhibited by 1 ml of serum.

The activity of chosen enzymes was measured using the SIGMA company reagents. The absorbance of samples was determined using a CECIL CE 2021 spectrophotometer.

Statistical analysis

Statistical analysis was performed using SPSS 12.0 for Windows (SPSS Inc., Chicago, Il, USA). Normal distribution of the results of cathepsin D activity in all of the examined subjects was checked using Kolmogorov-Smirnov's test with Lilliefors' correction. In calculations, a statistical significance level of $p < 0.05$ was adopted.

Results

The activity of cathepsin D in blood serum of all men is presented in Table 1. The average activity of this enzyme in the control group was 11.88 nM. The activity of cathepsin D in patients was lower than in healthy people. Before the surgery, the activity was about 8.94 nM, and after removing the lesion it was 9.31 nM. Differences observed are statistically significant ($p < 0.01$).

In patients (Table 2), the activity of α1-antitrypsin is similar to the control group. There were no statistically significant differences between the levels before and after the surgery and healthy and unhealthy men.

Discussion

The high activity of cathepsin D and low level of inhibitors can facilitate the invasion of neoplastic cells, formation of metastases and neoplasm recurrence. The role of inhibitors is to protect the tissues against damaging the continuity of the basement membrane [10]. In the neoplastic disease, the protease-antiprotease balance is shifted to excessive proteolysis. As a result, the connective tissue stroma near the growing tumour and healthy tissue is damaged.

In the research on malignant melanoma, it has been proved that high activity of cathepsin B or a strong association between cathepsin B and inhibitors of cysteine proteases is closely related to worse prognosis [11, 12].

Table 1. The activity of cathepsin D (10^{-2} nM tyrosine/mg protein/min) in blood serum of men with malignant melanoma before and after the surgery

Parameter	Healthy people (control)	Patients	
		Before surgery	After surgery
Xav ± SD	11.88 ±1.66	8.94 ±2.79	9.31 ±3.91
Number of people	14	25	13
Statistical significance		*$p < 0.03$	*$p < 0.01$

With reference to controls

Table 2. The activity of α1-antitrypsin (mg trypsin/mg serum) in blood serum of men with malignant melanoma before and after the surgery

Parameter	Healthy people (control)	Patients	
		Before surgery	After surgery
Xav ± SD	0.78 ±0.13	0.90 ±0.18	0.86 ±0.20
Number of people	14	25	13
Statistical significance		None	None

Cathepsin D also increases permeability of blood vessels and lymph vessels, thus facilitating the penetration of tumour cells to remote tissues and formation of metastases. Proteolytic enzymes also have the ability to stimulate osteoclasts, which causes metastases in bone tissues, e.g. primary breast cancer [13].

In patients with squamous cell carcinoma of the larynx and attacked lymph nodes, there is a statistically significant positive correlation between the activity of cathepsin D in the cells and a risk of neoplasm recurrence. Therefore, this enzyme could be another prognostic indicator in such patients [14].

In patients with squamous cell carcinoma of the skin and a group of people with remote metastases there is, also statistically significant, higher activity of cathepsin D in all neoplastic tissue. For 5 years from diagnosis to metastases of squamous cell carcinoma of the skin, activity of the enzyme remains high, and does not change. The activity of the enzyme is higher in patients with metastases [15].

The analysis of the activity of cathepsin D in blood serum of people with melanoma was the subject of the research because of its role in acceleration of the disease and formation of remote metastases [16]. High activity of this hydrolase in dysplastic lesions and melanomas was also proved [17].

In this research, the activity of cathepsin D in melanoma patients was lower by about 22% than in healthy people. Tumour resection had no influence on changing the activity of the enzyme. About 3 months after the surgery, the activity of cathepsin D was still lower than in the control group.

Cathepsin D is a recognised diagnostic and prognostic marker in the course of some neoplastic diseases. Based on biochemical analyses it was stated that the activity of procathepsin D in the primary tumour cells is from 4% to 6%. Furthermore, in metastatic cells it is as high as 50%. When the disease intensifies, the activity of this enzyme gets higher. The cells of malignant melanoma and some other skin cancers display high activity of cathepsin B and D in comparison to the activity of preneoplastic states, benign lesions and healthy tissues, from which these cancers begin. In skin cancers, degradation of basement membrane by cathepsin is a phase followed by tumour invasiveness [10, 16, 18].

Normal epidermal cells and melanocytes adhering to the primary malignant melanoma site display low activity of cathepsin B, D, L and H, while benign melanocytic nevi have high proteolytic activity of these enzymes [19].

There are relations between the activity of cathepsin D in the primary melanoma cells and the prognosis of treatment [6]. However, the activity of cathepsin D in blood serum of patients does not always reflect the neoplastic process in the body, as demonstrated by the research of Westhoff *et al.* [20] and results of this research.

The activity of cathepsin D was higher in healthy people than the patients. The lower activity of this enzyme in the melanoma patients both before and after the surgery could be explained by lower synthesis of de novo enzyme, and also its inhibition. The activity of α1-antitrypsin did not change during illness and was the same as in healthy people.

The activity of this hydrolase, estimated by the ELISA test, in people with primary melanoma was comparable to the activity of cathepsin D in the healthy people. In spite of its lower activity, in some of these patients melanoma able to metastasize developed. Results of this research are contradictory to earlier observations. Similar values of the activity of this lysosomal hydrolase in blood serum of the patients and healthy people could be a result of inhibition of the hydrolase activity or lower synthesis.

Lysosomal enzymes have non-specific inhibitors, which mostly belong to serine proteases. They control the *de novo* synthesis and the inhibition reactions. However, these mechanisms are not well known yet. There are several views on how inhibitors of serine proteases exhibit protective action, inter alia, they inhibit tumour growth and stimulate the immunological reaction of the host [21]. According to other authors, serine protease inhibitors facilitate tumour growth and accelerate disease development, including formation of metastases. In such cases, the lysosomal enzymes can be markers of neoplastic progression [22].

One of the better known protease inhibitors is α1-antitrypsin. It maintains the protease-antiprotease balance in the organism, which is the main mechanism protecting tissues against damage as a result of uncontrolled proteolysis, under the influence of high activity of cathepsin [23]. α1-Antitrypsin is also classified as an acute-phase protein, activity of which increases in inflammation, which accompanies the neoplastic processes [24].

According to Henderson, the tumour progress is three times faster with a low level of α1-antitrypsin [25]. This relation can result from overexpression of genes controlling the synthesis of lysosomal enzymes and the simultaneous hydrolysis of protein complexes with α1-antitrypsin in lysosomes [21, 26].

The synthesis of α1-antitrypsin is under genetic control. In recessive homozygotes, the inhibitor content is 10% and in heterozygotes 60% of the total activity. It should be supposed that despite the malignant melanoma, the activity which is similar to healthy people is caused by the genetic background of that small number of patients.

Results of biochemical marking presented in this paper show that the activity of α1-antitrypsin is similar in the patients and healthy people. Based on this, it can be supposed that in the examined group of 25 people with melanoma, the proteolytic-antiproteolytic balance was not disturbed.

Conclusions

It can be supposed that in people with melanoma, the proteolytic-antiproteolytic balance was disturbed because the activity of cathepsin D in blood serum of these people was lower than in healthy people, while the activity of α1-antitrypsin did not change. Determination of selected parameters in blood serum of people with melanoma, apart from other indicators, could be useful in diagnosing and monitoring this disease.

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Conflict of interest

No conflict of interest.

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