Serum endostatin levels in patients with lung carcinoma

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Abstract

The purpose of our study was to evaluate the clinical usefulness of serum endostatin levels during chemotherapy of lung cancer in relation to the histopathological type of the tumor, clinical stage and response to therapy

Material and methods: Serum concentrations of endostatin were determined in 37 patiens (24 with non-small cell lung cancer and 13 with small cell lung cancer), 10 healthy subjects constituted controls. To determine endostatin levels (ELISA), venous blood samples were collected from each patient before treatment and after 4-6 courses of chemotherapy.

Results: The serum concentrations of endostatin were found significantly higher in patients in comparison with controls (p=0.003). No statistically significant differences were established between the concentrations of endostatin with regard to such clinical features, as: performance status, clinical stage (III and IV) and histopathological type (non-small cell lung cancer and small cell lung cancer). The concentrations of endostatin did not change after chemotherapy. There was no change of endostatin concentration caused by the response to treatment.

Conclusions: The serum endostatin concentrations were elevated in lung cancer patients.

Key words: endostatin, lung carcinoma.

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Received 11.04.2005 Accepted 14.07.2005

Introduction

Malignant tumor growth and metastasis formation depend on the development of new vessels [1]. This process, called neoangiogenesis is regulated, among the others, by the factors derived from the tumor and caused by the shift of balance between stimulating and inhibiting factors, to the formers' advantage [2-4]. Neovascularization results in the progression through providing oxygen and nutrients necessary to the tumor's growth and facilitates the penetration of tumor cells and their transportation to distant organs [2]. Endostatin is one of natural antiangiogenic factors. It was discovered in the medium of the cell line of mice hemangioblastoma in 1997 year. Endostatin is generated due to the enzymatic digestion of collagen XVIII and it inhibits endothelial cell proliferation and induces the 15-30 – fold increase in their apoptosis coefficient. It prevents from VEGF – induced phosphorylation of VEGFR–1 and VEGFR–2 receptors and inhibits VEGF – induced endothelial cell migration. In animal studies, it exhibited an antiangiogenic effect and inhibited the tumor's growth and metastasis [5,6]. Endostatin has also been found in the sera of healthy volunteers. Its increased levels have been reported in various malignant tumors [6-10]. However, a significance and a role of endostatin in patients with lung cancer are still unknown.

The purpose of our study was to evaluate serum endostatin levels in patients with lung cancer (NSCLC and SCLC) treated with chemotherapy, and their relation to response to therapy.

Material and methods

The study was carried out on 37 patients (31 men and 6 women) aged from 45 to 78 (mean age 60 years), diagnosed with lung cancer and treated by chemotherapy (*Tab. 1*). There were 24 patients with diagnosed non-small cell lung cancer (NSCLC) and 13 patients with small cell lung cancer (SCLC).

In the group examined, the clinical stage was assessed according to TNM classification modified in 1997. Among the

Table 1. **Description of patients**

	Clinical stage	n	Performance status according to Zubrod			F	M	Treatment response			
			0	1	2 and more			CR	PR	NC	P
			n	n	n			n	n	n	n
NSCLC	III A	2	2	0	0	0	2	1	1	0	0
	III B	6	3	2	1	0	6	1	5	1	0
	IV	16	4	4	8	2	6	0	1	10	3
SCLC	III	11	3	1	7	4	3	0	5	4	2
	IV	2	0	1	1	0	2	0	0	2	0

NSCLC – non-small cell lung cancer, SCLC – small cell lung cancer, F – females, M – males, CR – complete response, PR – partial response, NC – stabilization, P – progression of the disease

Table 2. **Comparison of serum endostatin concentration (ng/ml) in patients and controls**

	Patients (n=37)			Controls (n=10)			
	Q_1	Me	Q_3	Q_1	Me	Q_3	p-value*
Endostatin	113.5	147.1	185.1	75.6	102.8	118.1	0.003 #

p-value for Wilcoxon's test; # – statistically significant differences at the significance level of 0.05; Me – median, Q1, Q3 – quartile 1 and 3

examined, there were 19 stage III patients (2 patients – stage III a and 17 – stage III b) the rest of patients – 18 individuals had stage IV. In case of SCLC, stage III corresponded to the limited stage of the disease and stage IV – to the extensive disease (*Tab. 1*). Additionally, performance status of patients was determined according to Zubord's scale. The total of 12 patients had the performance status classified as – 0; 8 patients – 1; and 17 patients – 2 or 3. All patients analysed in the study were treated with chemotherapy. In case of SCLC, they were given 4 – 6 courses of cisplatin + etoposid, and in case of NSCLC, they received 4 – 6 courses of cisplatin + vinorelbin or cisplatin + gemcytabin. In the whole group of patients, 2 patients (with NSCLC) had complete remission, 12 (7 with NSCLC and 5 with SCLC) – partial remission, 17 (11 with NSCLC and 6 with SCLC) – stable disease and 5 (3 with NSCLC and 2 with SCLC) – progressive disease.

The control group included 10 healthy volunteers (3 women and 7 men; mean age 58 years). Blood samples were collected before begining and four weeks after the treatment. Peripheral blood serum was examined. Blood samples of 5 ml each were drawn on clot on empty stomach. After blood clotting and centrifugation at the rotation of 2000 rpm for 10 minutes, the serum was separated and frozen at the temperature of -70°C to determine endostatin.

Endostatin levels were measured in the serum by the use of immunoenzymatic method ELISA with the Quantikine kits of R&D Systems (Minneapolis, USA) according to the producer's instruction. The reader of microplatelets ELx800 of BIOTEK Instruments Inc. was used in our examinations.

Statistical analysis

The correlations between the histopathological tumor type, performance status, clinical stage, tumor size and the concentrations of endostatin were analysed before treatment using non-parametric tests of Wilcoxon and Kruskal-Wallis. The differences between the pairs of groups were examined by means of Dunn's test for multiple comparisons. The hypothesis of normal distribution in particular groups was rejected (Shapiro-Wilk test).

New parameters of x_endostatin were calculated to check the correlation between the response and the therapy:

$$X\_\,\text{endostatin} = \frac{(\text{endostatin after treatment} - \text{endostatin before treatment})}{\text{endostatin before treatment}}$$

It represents relative changes of endostatin values.

The response influence on x_ endostatin was analysed using a variation analysis test with a single classification. The assumptions of variation analysis were checked by means of Shapiro-Wilk's and Bartlett's tests. The differences between the pairs of groups were examined using Bonferroni's test for multiple comparisons. Most of the tests performed were bilateral. Unilateral tests were notified in the description. The significance level was 0.05. The analysis was performed basing on the kit of SAS STAT Release 8.2.

Results

Endostatin was determined in the serum of patients and controls. Endostatin concentration differed significantly between patients and controls (*Tab. 2*). Additionally, the serum concentrations of endostatin obtained in patients were significantly higher (unilateral test) than in controls (p=0.0014).

No statistically significant differences were found between the concentrations of endostatin, and the clinical parameters, such as performance status, clinical stage and histopathologic form defined as small cell and non-small cell lung cancer (*Tab. 3*).

Endostatin concentrations measured after treatment showed no statistically significant changes in comparison with the preliminary levels and did not depend on the response obtained, and remained higher in patients than in controls.

Discussion

Lung cancer still remains the leading cause of mortality in cancer patients in the world. A high percentage of relapses after surgical resection is due to the presence of micrometastases at diagnosis. This high metastatic potential of lung cancer may be enhanced by intensive angiogenesis within the tumor.

Table 3. **Serum endostatin concentration with regard to histopathological pattern and performance status and clinical staging**

	n	Q_1	Me	Q_3	p-value
NSCLC	24	121	146	187	0.75[a]
SCLC	13	114	147	18	
Performance status					0.13[b]
0	12	85	111	166	
1	8	137	149	195	
2 and more	17	133	149	191	
Clinical stage					0.79[a]
III	19	102	147	185	
IV	18	122	145	190	

NSCLC – non-small cell lung cancer; SCLC – small cell lung cancer; a – p-value for Wilcoxon's test; b – p-value for Kruskal-Wallis's test; Me – median, Q1,Q3 – quartile 1 and 3

Figure 1. **Endostatin concentration (ng/ml) in patients and controls**

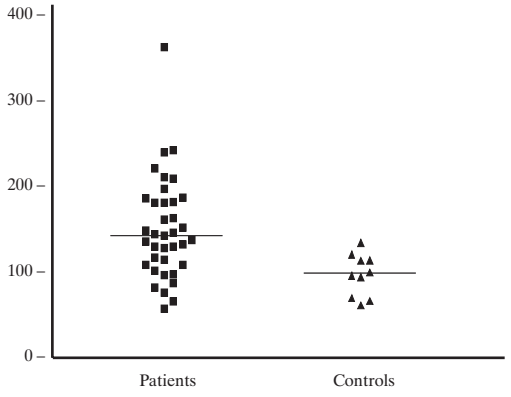

Table 4. **Effect of after – treatment response on relative changes of endostatin concentrations in serum of lung cancer patients**

	Complete and partial remission (n=14) mean +/- of standard deviation	Disease stabilization (n=17) mean +/- of standard deviation	Disease progression (n=5) mean +/- of standard deviation	p-value*
x_endostatin	0.27±0.96	0.21±0.5	0.2±0.72	0.98

* – p – value for the test of variation analysis

In the present study, the concentration of endostatin – a natural angiogenesis inhibitor – was determined in the serum of patients with lung cancer. The concentration of endostatin was significantly higher in patients with lung cancer in comparison with controls. So far, only two studies [8,9] evaluating serum endostatin concentrations in patients with NSCLC, have been published. In these studies the serum endostatin levels were also significantly higher in patients than in healthy controls.

Our study showed no differences of endostatin concentrations between patients with NSCLC and SCLC. To our knowledge, the current study is the first to report serum endostatin levels in SCLC patients.

In our study there were no differences in relation to clinical stage (III vs IV) of lung cancer. Conversely, M. Suzuki et al. [8] found significantly higher endostatin concentrations in patients with the clinical stage higher than Ib disease and in patients with their tumor classified as more than T2 compared to other patients. There were no patients in our study in I and II clinical stage. In our opinion, that is the reason for differences, in the results.

No differences of this inhibitor concentrations were found before and after treatment in the present study. There was no change of endostatin concentration as a response to treatment. After treatment it remained higher when compared to controls. To our knowledge, there were no other published studies evaluating serum endostatin levels in lung cancer patients after chemotherapy.

In a few studies, serum endostatin concentrations were determined in various malignant tumors, such as non-Hodgkin lymphoma, carcinoma of the bladder, acute leukemia, colorectal cancer, soft tissue sarcoma, and they were found higher in the serum of patients than controls [10-14]. In one study, a high concentration of endostatin correlated with the extent stage of disease (10), in another, a high concentration of endostatin was associated with poor prognosis [8,10,14-16]. In most studies, regardless of surgery treatment or chemotherapy, endostatin concentrations remained enhanced in patients with malignant tumors in comparison with controls [12,15]. However, Dhar et al. [17] proved a significantly lower concentration of endostatin in the serum of patients after radical resection of the liver cancer. In Miyashita's et al. [18] and Homer's et al. [19] studies, no differences of endostatin concentrations were found between patients with cancer and healthy volunteers.

These results indicated that elevated levels of endostatin were present in the serum of lung cancer patients, however, the reason for this remains unknown. Further studies are necessary to clarify the source of endostatin production, site of interaction, and mechanism of the activity.

Basing on the results of our study, it may be concluded that the serum endostatin concentrations were elevated in lung cancer patients.

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