ONCOLOGY

Serum Angiogenic Factors in Patients with Neuroendocrine Tumors of Abdominal Organs

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 148, No. 10, pp. 438-441, October, 2009
Original article submitted July 28, 2009

The initial (before treatment) levels of VEGF, endostatin, and tumor necrosis factor-β (TNF-β) were measured in the sera of 20 patients with malignant and benign neuroendocrine tumors of the abdominal organs and 25 healthy controls. The initial levels of VEGF, endostatin, and TNF-β in the total group of patients with neuroendocrine tumors of abdominal organs did not differ from the control. A significant difference was detected between the mean serum concentrations of endostatin in patients with benign and malignant neuroendocrine tumors: 64.1±14.7 and 107.8±14.1 ng/ml, respectively (p=0.043). The content of VEGF, endostatin, and TNF-β did not correlate with patients' gender and age. A direct correlation between endostatin, TNF-β concentrations and maximum size of the primary tumor was detected in patients with malignant neuroendocrine tumors. Direct correlations between the initial levels of VEGF and endostatin and an inverse correlation between VEGF and TNF-β concentrations were detected in patients with benign neuroendocrine tumors. Relapse-free survival was significantly lower in patients with serum endostatin concentration >100 ng/ml and TNF-β <30 pg/ml.

Key Words: *vascular endothelial growth factor (VEGF); endostatin; tumor necrosis factor-β (TNF-β); neuroendocrine tumor*

The formation of new microvessels on the basis of the vascular network existing in tissue, or neoangiogenesis, is an obligatory condition for tumor growth and metastasizing [7]. Numerous capillaries in the tumor promote its rapid proliferation due to constant delivery of nutrients and oxygen with blood and promote attenuation of apoptotic activity of tumor cells.

Angiogenesis in the tumor is regulated by many inductors and inhibitors. A special role among them is played by vascular endothelial growth factor (VEGF), the key activator of neoangiogenesis, involved in the mechanisms of tumor growth and metastasizing [2,8]. It is proven that VEGF increases permeability of blood vessels and stimulates migration of endothelial cell to adjacent tissues with the formation of tubular structures. Endostatin is an inhibitor of angiogenesis. This protein realizes its antiangiogenic effect via stimulating endothelial cell apoptosis and reducing the concentrations of apoptosis inhibitors [6]. Some authors presented data on the relationship between endostatin production and tumor prognosis (specifically, breast cancer, lung cancer) and treatment efficiency [5,9]. Opinions on the role of tumor necrosis factor-β (TNF-β) in the mechanisms of angiogenesis stimulation and inhibition in the tumors vary [4,9].

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In addition, despite elevated levels of VEGF, endostatin, and TNF-β detected in the sera of the majority of patients with solid tumors [5,8-10], clinical significance of these factors and their role in the mechanisms of neuroendocrine tumor (NET) growth and progress remains unknown [1,3]. Some authors noted a pronounced positive correlation between serum levels of endostatin and VEGF in patients with pituitary adenoma [12] and hepatoma [11].

We measured the initial (pretreatment) serum levels of VEGF, endostatin, and TNF-β in patients with malignant and benign NET of the abdominal organs with different main clinical characteristics of the disease and evaluated the contribution of these parameters to disease prognosis.

MATERIALS AND METHODS

Twenty patients (10 men and 10 women aging 21-78 years, mean age 46.9±6.6 years) with malignant (n=12) and benign (n=8) abdominal NET were examined. The tumors were located in the stomach (n=5), small intestine (n=5), colon (n=3), pancreas (n=3), liver (n=3), and appendix vermiformix (n=1). The patients were examined and treated at N. N. Blokhin Cancer Research Center in 1999-2003. Clinical diagnosis of abdominal NET was made in all patients for the first time and verified by the results of histological study.

Serum concentrations of VEGF, TNF-β, and endostatin were measured after overnignt fasting before therapy by enzyme immunoassay using R&D reagents for VEGF and endostatin and Bender MedSystems reagents for TNF-β.

The results were statistically processed using Excel and SAS (version 11.0) software. Survival curves were analyzed by the Kaplan—Meier method and compared by the log-rank method.

RESULTS

In healthy individuals (control group), the mean concentration of VEGF was 150.9±43.6 pg/ml (20.6-461.9 pg/ml), endostatin 99.9±9.7 ng/ml (62.2-133.8 ng/ml), and TNF-β 29.1±8.8 pg/ml (12.5-61.7 pg/ml). No correlations between serum levels of VEGF, endostatin, TNF-β, patients sex and age were detected in the control group.

A trend to an increase (p=0.17) in the initial serum levels of VEGF (269.0±86.5 pg/ml) and TNF-β (40.7±17.5 pg/ml) in comparison with the control (150.9± 43.6 and 29.1±8.8 pg/ml, respectively) was noted in patients with abdominal NET.

No significant correlations between patients' age, maximum size of the primary tumor, and initial serum levels of VEGF, endostatin, and TNF-β and between the concentrations of these markers were detected in the total group of patients. Patients aged under 50 years (n=10) were an exception: the mean serum concentration of VEGF in them varied within a wide range (35.5-742.1 pg/ml), the mean level being 302.5±144.9 pg/ml. A significant positive correlation between patients' age and initial serum VEGF concentration (r= 0.47; p<0.05) was detected in this group.

The concentrations of VEGF, endostatin, and TNF-β did not depend on patients' sex. The mean concentrations in men were: 222.5±107.3 pg/ml for VEGF, 102.2±18.3 ng/ml for endostatin, and 28.4±12.8 pg/ml for TNF-β. In women the values were as follows: 315.4±129.3 pg/ml for VEGF, 82.9±17.3 ng/ml for endostatin, and 51.9±30.3 pg/ml for TNF-β.

In 12 patients with malignant abdominal NET, the mean serum levels of serum VEGF, endostatin, and TNF-β did not differ significantly (p=0.25) from those in the control group and were 257.1±115.4 pg/ml, 107.8±14.1 ng/ml, and 33.7±14.4 pg/ml, respectively.

Similarly as in the total group of patients, no correlations between the initial serum levels of VEGF, endostatin, and TNF-β and patients' age and between the concentrations of these markers were detected in patients with malignant endocrine cell tumors of the abdominal organs. However, significant positive correlations between the maximum size of the primary tumor and endostatin (r=0.45; p<0.05) and maximum size of the tumor and serum TNF-β (r=0.53; p<0.05) were detected for malignant NET. The initial VEGF level and maximum size of NET were in an inverse negative correlation (r=-0.41; p>0.05).

The mean concentrations of angiogenesis factors in patients with benign abdominal NET were 293.1± 176.2 pg/ml for VEGF (44.9-655.7 pg/ml), 64.1±14.7 ng/ml for endostatin (43.6-85.6 ng/ml), and 43.8±31.8 pg/ml for TNF-β (12.5-113.4 pg/ml). No statistically significant differences between the initial concentrations of VEGF, endostatin, and TNF-β were detected in patients with benign endocrine cell tumors and in the controls. No differences between VEGF and TNF-β concentrations were detected in patients with benign and malignant abdominal NET (p=0.27). On the other hand, a significant difference between the mean serum concentrations of endostatin in patients with benign and malignant NET was detected (64.1±14.7 and 107.8±14.1 ng/ml, respectively, p=0.043). In addition, a significant positive correlation between the initial serum concentrations of VEGF and endostatin (r=0.53) and a significant negative correlation between VEGF and TNF-β levels (r=-0.53; p<0.05) were detected in patients with benign NET.

Hence, simultaneous elevation of serum levels of VEGF and endostatin and reduction of TNF-β level are characteristic of patients with benign abdominal NET.

In order to evaluate the relationship between the levels of the studied markers and survival of patients with NET, the mean concentrations of molecular biological markers in the control were taken as the reference values; the patients were divided into groups with VEGF, endostatin, and TNF-β levels above and below the mean level in the control.

Distant metastases were detected in 4 of 6 patients with initial serum VEGF concentration below the mean control level, in 3 (75%) of these patients they were located in the peripheral lymph nodes and the small intestinal mesenteric lymph nodes. Of 14 patients with initial VEGF concentration above the mean level in the control, metastases were detected in 8 (57.1%) and local relapse in 1 (7.1%) patient. All metastases were located in the viscera: in the liver in 7 (87.5%) and in the lungs in 1 (12.5%).

Analysis of 3- and 5-year overall survival of patients with endocrine cell tumors with different initial VEGF concentration (below or above the mean control value) revealed no statistically significant differences in these groups: 80.0±17.9 and 100%, 80.0±17.9 and 100%, respectively (p=0.2). The 3-year relapse-free survival values were 22.2±19.5 and 37.5±14.1%, respectively. The median of relapse-free survival virtually did not differ in the groups of patients with the initial VEGF concentration below and above the mean control level and was 4.6 and 8.6 months, respectively (p=0.35).

Distant metastases and one local relapse were detected in 5 of 12 patients (41.7%) with endostatin concentration below the mean control level; in 3 patients the metastases were located in the liver. In patients with endostatin concentration above the mean control level the metastases were 2-fold more incident: in 7 of 8 (87.5%). In 1 of these patients metastases were combined with local relapse; metastatic involvement of the liver was noted in 5 (71.4%) cases.

Patients with endostatin concentration above the mean control value predominated in the group with malignant abdominal NET. All 7 patients with benign NET had serum endostatin concentrations below the mean control level.

Analysis of 3- and 5-year overall survival of patients with endocrine cell tumors with different initial endostatin concentration (below or above the mean control value) showed statistically the same values in these groups: 100 and 80.0±17.9%; 100 and 80.0±17.9%, respectively (p=0.2). The 3-year relapse-free survival differed significantly in these groups: 21.4±18.2 and 0%, respectively (p=0.02).

Distant metastases were detected in 7 of 12 (58.3%) patients with the initial concentration of TNF-β below the mean control level and local relapses were detected in 2 patients; in 6 cases they were located in the liver and lungs. Four of eight (50%) patients with TNF-β concentration above the mean control level developed metastases; liver involvement was detected in 2 of these. A relapse of the tumor was detected in the group of patients with TNF-β concentration below the mean control level.

Analysis of 3- and 5-year overall survival of patients with endocrine cell tumors with different concentrations of TNF-β (above and below the mean control level) showed no statistically significant differences in these groups: 100 and 85.7±13.2%; 100 and 85.7±13.2%, respectively (p=0.3). Three-year relapse-free survival differed significantly in these groups: 13.6±12.3 and 21.8±19.2%, respectively, the medians of relapse-free survival in the two groups being 1.0 and 19.2 months, respectively (p=0.01).

A positive correlation between serum endostatin concentration and maximum size of the tumor was detected in the total group of patients and in groups with malignant and benign tumors separately.

Hence, the mean concentrations of VEGF, endostatin, and TNF-β in patients with abdominal NET do not differ significantly from those in the control group. Serum concentrations of these neoangiogenesis markers did not correlate with patients' age. However, serum concentrations of endostatin and TNF-β increased significantly with enlargement of malignant NET. In benign NET, the increase of VEGF concentration was paralleled by a statistically significant elevation of endostatin level and reduction of TNF-β level. Presumably, synchronous elevation of endostatin and VEGF concentrations attenuated the proangiogenic effect of VEGF in patients with benign abdominal NET. The mean concentration of endostatin in patients with malignant NET was higher than in benign tumors; the initial serum endostatin concentration >100 ng/ml and of TNF-β <30 pg/ml were associated with an earlier relapse of the disease. Hence, their initial measurements in the serum can be used for predicting early relapse and, presumably, for determining indications for target drug therapy, while measurement of endostatin concentration as an additional test for characterization of the malignancy potential of abdominal NET.

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