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Peripheral blood concentrations of vascular endothelial growth factor and its soluble receptors (R1 and R2) in patients with adrenal cortex tumours treated by surgery

Stężenia naczyniowo-śródbłonkowego czynnika wzrostu i rozpuszczalnych receptorów dla VEGF (R1 i R2) w obwodowej krwi żylnej u chorych z guzami nadnerczy leczonych operacyjnie

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Abstract

Introduction: Neoangiogenesis appears to be an important event in tumour invasion and in the formation of metastases in many endocrine-related human cancers. Vascular endothelial growth factor (VEGF) is a glycoprotein with potent angiogenic, mitogenic and vascular permeability-enhancing activities specific for endothelial cells and acts through VEGF receptors. The aim of the study was to evaluate the plasma blood concentrations of VEGF, sVEGFR1, and sVEGFR2 in patients with benign and malignant adrenal tumours treated by surgery.
Material and methods: We studied the blood before surgery of 41 patients with adrenal cortex tumours and 10 normal subjects without hormonal or CT/USG pathology of the adrenal glands (controls). We studied the blood after adrenalectomy of 16 patients with tumours of the adrenal cortex.
Results: Concentrations of VEGF, sVEGFR1 and sVEGFR2 in blood plasma before as well as 30 days after surgery were evaluated by ELISA. VEGF blood concentrations before surgery did not differ in the patients with the cortical tumours as compared to the controls. After surgery VEGF concentrations decreased among the patients, taken in total, with adrenal cortex tumours and cortical adenomas. Before surgery sVEGFR1 blood concentrations were increased in the patients with Conn's syndrome only in comparison with the controls. After surgery, sVEGFR1 concentrations decreased significantly in the group with cortical adenomas only. Before and after surgery sVEGFR2 blood concentrations did not differ between the groups of patients studied and the controls.
Conclusions: Peripheral blood concentrations of VEGF and its receptors cannot be clinically valuable markers that discriminate between benign and malignant adrenocortical tumours before and after adrenalectomy. **(Pol J Endocrinol 2009; 60 (1): 9–13)**

Key words: *adrenal cortex tumours, adrenalectomy, vascular endothelial growth factor, soluble vascular endothelial growth factor receptor 1, soluble vascular endothelial growth factor receptor 2, angiogenesis, oncogenesis*

Streszczenie

Wstęp: Neoangiogeneza należy do kluczowych mechanizmów patologicznych w przebiegu choroby nowotworowej gruczołów dokrewnych, w tym kory nadnerczy. Naczyniowo-śródbłonkowy czynnik wzrostu (VEGF, *vascular endothelial growth factor*), po aktywacji specyficznych receptorów w komórkach *endothelium*, wykazuje działanie angiogenne, mitogenne i zwiększa przepuszczalność ścian naczyń krwionośnych. Celem pracy było zbadanie stężeń VEGF, sVEGFR1 i sVEGFR2 we krwi obwodowej u chorych z guzami kory nadnerczy o charakterze łagodnym i złośliwym poddanych adrenalektomii.
Materiał i metody: Przed leczeniem operacyjnym zbadano krew u 41 pacjentów z guzami kory nadnerczy oraz u 10 osób zdrowych bez zmian hormonalnych i obrazowych (USG/CT) nadnerczy (grupa kontrolna). Po adrenalektomii zbadano ponownie krew u 16 chorych.
Wyniki: Stężenia VEGF, VEGFR1 i VEGFR2 zbadano w osoczu krwi przed i po 30 dniach od operacji metodą ELISA. Przed operacją stężenia VEGF we krwi nie różniły się pomiędzy całą grupą pacjentów z guzami kory nadnerczy a grupą kontrolną. Po leczeniu chirurgicznym średnie stężenia VEGF zmniejszyły się w całej grupie operowanych chorych i w podgrupie z gruczolakami kory. Stężenia VEGF R1 przed operacją były wyższe tylko w grupie chorych z zespołem Conna, a po adrenalektomii obniżyły się tyko w podgrupie osób z gruczolakami kory. Stężenia VEGFR2 nie różniły się pomiędzy wszystkimi badanymi grupami oraz przed i po operacji.
Wnioski: W praktyce klinicznej oznaczanie stężeń VEGF, VEGFR1 i VEGFR2 we krwi obwodowej u chorych z nowotworami nadnerczy nie pozwala na rozpoznanie guzów kory nadnerczy o charakterze złośliwym. **(Endokrynol Pol 2009; 60 (1): 9–13)**

Słowa kluczowe: *guzy kory nadnerczy, naczyniowo-śródbłonkowy czynnik wzrostu, receptory (1 i 2) dla naczyniowo-śródbłonkowego czynnika wzrostu, angiogeneza, onkogeneza*

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Introduction

The growth of microvessel sprouts from pre-existing vasculature is generally termed angiogenesis [1]. Focal angiogenesis plays a pivotal role in physiological processes such as wound healing, ovulation and placentation [2]. Neoangiogenesis, a multistep process, appears to be an important event in tumour invasion and in the formation of metastases in many human oncogenesis, including adrenal neoplasia [3–5]. Sprouts of new vessels grow mainly by the migration of endothelial cells [6], and vascular endothelial growth factor (VEGF) is one of the most active pro-angiogenic factors. VEGF is a homodimeric 34–42 kDa, heparin-binding glycoprotein with potent angiogenic, mitogenic and vascular permeability-enhancing actions specific for endothelial cells [7]. Elevated levels of VEGF have been reported in the synovial fluid of rheumatoid arthritis patients and in sera from cancer patients [2]. VEGF acts through VEGF receptors (R1, R2, and R3). VEGF and soluble VEGF receptors (sVEGFR1, and sVEGFR2) represent a regulatory system essential for both normal and pathological angiogenesis. The signalling pathway after the binding of VEGF to its receptor includes dimerisation and activation of tyrosine kinases [8]. Administration of recombinant soluble VEGFR1 or VEGFR2 inhibits angiogenesis in the retina, corpus luteum and tumours [9–11].

Neoangiogenesis (evaluated as tumour vessel density) has been recognized as a key process in the development of many types of human neoplasia [2], including breast, bladder, and stomach cancers, and is associated with the development of metastases [12, 13], poor prognosis [14, 15] and reduced survival [16, 17].

The aim of the study was to evaluate the plasma blood concentration of VEGF, sVEGFR1, and sVEGFR2 in patients with benign and malignant adrenocortical tumours treated by surgery and in normal subjects.

Material and methods

Before surgery we studied 26 females (aged from 28 to 72 years; 59.7 ± 4.23) and 15 males (aged from 51 to 76 years; 62.0 ± 10.7) with adrenal cortex tumours [histopathological examination: Conn's syndrome — 7 cases, cortical adenoma — 22, malignant tumours — 6 (cortical carcinoma — 2, metastatic renal carcinoma — 2, metastatic malignant melanoma — 1, metastatic adrenocarcinoma of the colon — 1), myelolipoma — 3, nodular hyperplasia — 2, normotypical adrenal cortex — 1, and 10 apparently "healthy" normal subjects without hormonal or CT/USG pathology of the adrenal glands (controls: 6 females aged from 36 to 59 years, 50.8 ± 8.7 and 4 males aged from 40 to 60 years, 47.5 ± 8.7). After adrenalectomy we studied the blood of 16 patients with adrenal cortex tumours (Conn's syndrome — 4, cortical adenoma — 8, cortical carcinoma — 2, nodular hyperplasia — 1, normotypical adrenal cortex — 1).

Concentrations (pg/ml) of VEGF (sensitivity — 9.0 pg/mL; intra-assay precision — 6.7%), sVEGF R1 (sensitivity — 3.5 pg/mL, intra-assay precision — 2.6%), and sVEGF R2 (sensitivity — 4.6 pg/mL, intra-assay precision — 2.9%) in blood plasma before as well as 30 days after surgery were evaluated by ELISA (R & D Systems, USA). All the subjects gave their informed consent to participation in the study, which was approved by the Ethics Committee of the Medical University of Łódź.

All comparisons were carried out using Statgraphics Centurion XIV software. Statistical verifications were performed using the Kolmogorov-Smirnov normality test. The statistical significance of differences between the groups was determined by ANOVA followed by the least significant difference test (LSD) and Student's paired t-tests. The values are presented as the mean ± SEM. P value ≤ 0.05 or less was considered statistically significant.

Results

Before surgery VEGF blood concentrations (Fig. 1.) did not differ in the patients with cortical tumours, taken as whole, (n = 41; 118.10 ± 17.46, NS), cortical adenomas (n = 22; 120.10 ± 16.92, NS), Conn's syndrome (n = 7; 98.43 ± 30.83, NS) or malignant tumours of the adrenal cortex (n = 6; 179.02 ± 94.84, NS) as compared to the controls (n = 10; 98.78 ± 17.56). After surgery VEGF concentrations (Fig. 1.) decreased significantly in all 16 patients studied with adrenal cortex tumours (43.94 ± 8.12 *vs.* 145.82 ± 24.72; $p < 0.001$) and cortical adenomas (43.43 ± 9.36 *vs.* 144.90 ± 32.48; $p < 0.05$) but showed no significant difference in the cases of Conn's syndrome (30.75 ± 7.38 *vs.* 71.50 ± 26.75, NS) and carcinomas of the adrenal cortex (76.25 ± 62.25 *vs.* 155.95 ± ± 76.25, NS).

Before surgery sVEGF R1 blood concentrations (Fig. 2.) were increased only in the patients with Conn's syndrome (n = 7; 89.60 ± 11.14; $p < 0.05$) and were without significant change in the subjects with cortical tumours of all types (n = 41; 75.97 ± 4.05, NS), cortical adenomas (n = 22; 75.90 ± 5.69, NS) and malignant tumours of the adrenal cortex (n = 6; 65.48 ± 8.11, NS) in comparisons with controls (n = 10; 59.17 ± 3.85). After surgery sVEGFR1 concentrations (Fig. 2.) decreased significantly only in the group with cortical adenomas (54.59 ± 8.86 *vs.* 82.60 ± 12.10; $p < 0.05$) and showed no significant difference for all 16 patients studied (62.85 ± ± 5.02 *vs.* 79.13 ± 8.29, NS), those with Conn's syndrome (73.60 ± 11.36 *vs.* 100.93 ± 15.72, NS) or those with

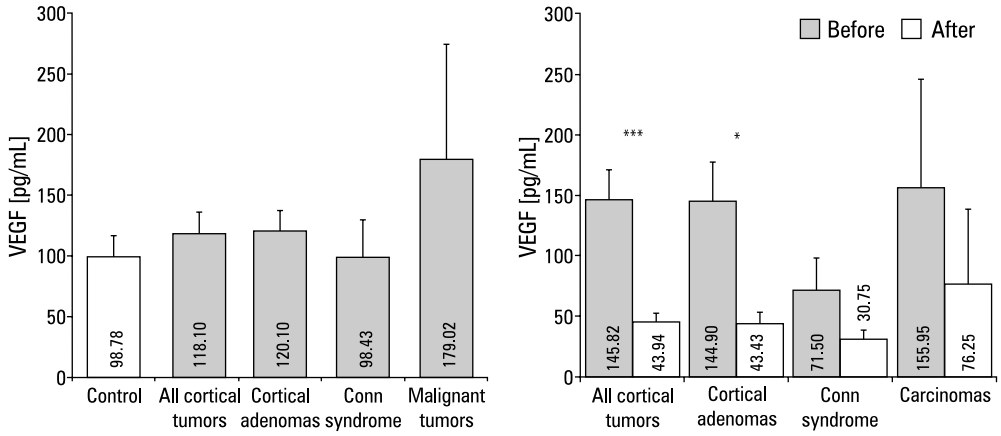

Figure 1. *Peripheral blood concentrations of VEGF in patients with benign and malignant adrenocortical tumours in comparison with controls (left panel) and before and after surgical treatment (right panel) (x ± SEM; *p < 0.05, ***p < 0.001, Control = control group)*

Rycina 1. *Porównanie stężeń VEGF we krwi obwodowej u chorych z łagodnymi i złośliwymi guzami nadnerczy i u osób z grupy kontrolnej (diagram lewy) oraz przed i po leczeniu chirurgicznym (diagram prawy) (x ± SEM; *p < 0,05; ***p < 0,001; Control = grupa kontrolna)*

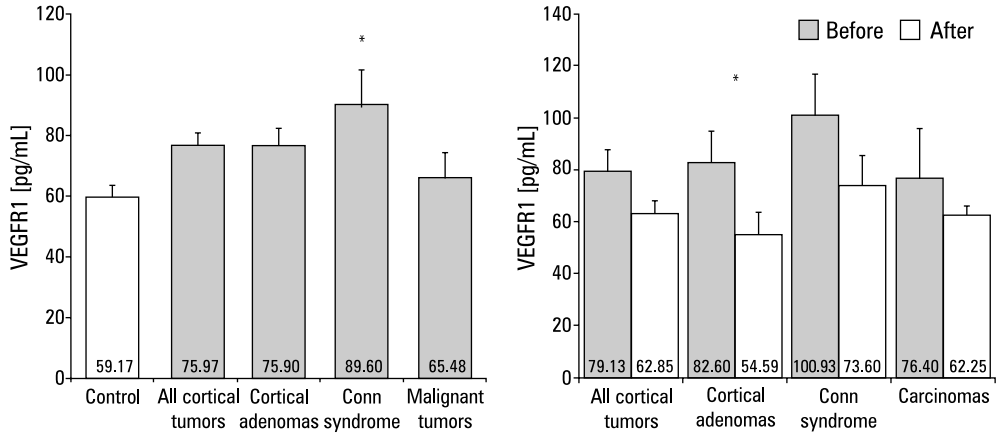

Figure 2. *Peripheral blood concentrations of sVEGF R1 in patients with benign and malignant adrenocortical tumours in comparison with controls (left panel) and before and after surgical treatment [right panel] (x ± SEM; *p < 0.05, Control = control group)*

Rycina 2. *Porównanie stężeń sVEGFR1 we krwi obwodowej u chorych z łagodnymi i złośliwymi guzami nadnerczy i u osób z grupy kontrolnej (diagram lewy) oraz przed i po leczeniu chirurgicznym (diagram prawy) (x ± SEM; *p < 0,05; Control = grupa kontrolna)*

carcinomas of the adrenal cortex (62.25 ± 3.15 *vs.* 76.40 ± ± 19.50, NS).

Before surgery sVEGFR2 blood concentrations did not differ between the patients with all types of cortical tumour (n = 41; 9971.34 ± 340.73, NS), those with cortical adenomas (n = 22; 10437.05 ± 506.87, NS), those with Conn's syndrome (n = 7; 8657.43 ± 686.73, NS) or those with malignant tumours of the adrenal cortex (n = 6; 9796.67 ± 895.44, NS) and the controls (n = 10; 9681.00 ± 524.91). After surgery sVEGFR2 concentrations did not show any significant change in the 16 patients studied in total (11065.28 ± 352.36 *vs.* 10132.61 ± 531.40, NS) and in subjects with adrenal adenomas (11330.63 ± ± 349.62 *vs.* 11376.88 ± 836.42; NS), Conn's syndrome (9761.25 ± 422.36 *vs.* 8194.25 ± 556.15; NS) or carcinomas of the adrenal cortex (10062.50 ± 12.50 *vs.* 9675.00 ± ± 775.00, NS).

Discussion

Adrenal carcinomas are rare, with an estimated prevalence of 4–12 per million population [18]. A recent computed tomography study reported an overall 4.4% prevalence rate of adrenal lesions [19]. Because prognoses for adrenocortical adenomas and adrenal carcinomas are vastly different, it is important to differentiate accurately between the two tumour types. It is generally and widely accepted that angiogenesis, the multistep pro-

cess of new blood vessel formation that is precisely regulated by pro- and anti-angiogenic cytokines, is a critical event for tumour growth and metastasis. VEGF expression has been demonstrated by immunohistochemistry in the normal adrenal medulla of the rat gland [20], but no differences in vascular density between the normal adrenal cortex, adrenal adenomas, and carcinomas were observed in humans using CD34 as the endothelial marker [21]. VEGF mediated through its several receptors is one of the important growth factors in tumour angiogenesis [1, 5]. Peripheral blood VEGF levels may also be abnormal in patients with some endocrine gland neoplasms, namely: normal or high in thyroid cancers [22–24] and increased in pituitary tumours [25]. Hedayati et al. [26] revealed high concentrations of VEGF and low sVEGF receptor levels in the peripheral blood of patients with benign and malignant thyroid tumours as compared to controls. A number of antiangiogenic agents targeting VEGF/VEGF-receptors are therefore currently in pre-clinical and clinical drug trials as part of a combined modality approach to the treatment of cancer [1, 5, 23, 27].

In 2001 Kołomecki et al. [28] reported peripheral blood concentrations of VEGF elevated above the norm in patients with benign and malignant adrenal inicidentalomas before surgery, and VEGF levels were significantly higher in subjects with malignant tumours than in those with benign ones. Zacharieva et al. [29] found the highest plasma levels of VEGF in adrenocortical carcinomas, but VEGF levels were also elevated in patients with Cushing's syndrome, primary hyperaldosteronism and phaeochromocytoma. Moreover, Korzeniowska et al. [30] also demonstrated that patients with adrenocortical carcinoma had blood VEGF levels significantly higher and sVEGFR2 lower than a control group. On the other hand, the mean VEGF concentrations in patients with benign adrenocortical adenoma did not differ from those of the control group, while mean sVEGFR1 and sVEGFR2 levels were lower than the controls. In our present study, we were not able to find any differences in the VEGF peripheral blood concentration of patients with adrenal pathology before surgery in comparison with controls, although higher than normal sVEGRR1 blood concentrations were noted. Others have also found that VEGF levels inside the tumour are significantly lower in benign adrenocortical tumours than in adrenocortical carcinomas [31].

The results of our study show that peripheral plasma blood concentrations of VEGF, sVEGFR1 and sVEGFR2 before and after adrenalectomy cannot discriminate between patients with benign tumours of the adrenal cortex and those with malignant tumours (carcinoma).

Conclusion

Peripheral blood concentrations of VEGF and its receptors cannot be clinically valuable markers that discriminate between benign and malignant adrenocortical tumours before and after adrenalectomy, but may be potentially useful in patients with some types of adrenal tumours who are undergoing surgery.

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