Effects of somatostatin on vascular endothelial growth factor (VEGF) secretion from non-functioning pituitary tumoral cells incubated *in vitro*

Hanna Ławnicka [1], Hanna Pisarek [2], Jolanta Kunert-Radek [3] and Marek Pawlikowski [2]

Departments of Immunoendocrinology[1], Neuroendocrinology[2], and Clinical Endocrinology[3], Chair of Endocrinology, Medical University of Łódź, Poland.

Correspondence to: Prof. Dr Marek Pawlikowski, Chair of Endocrinology, Medical University of Łódź, ul.Sterlinga 3, 91-425 Łódź, Poland
e-mail: pawlikowski.m@wp.pl

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Abstract

OBJECTIVES: The aim of the study was to examine the effect of somatostatin (SST) on vascular endothelial growth factor (VEGF) secretion from clinically non-functioning pituitary tumors incubated in vitro.

MATERIAL AND METHODS: Eight pituitary tumors surgically removed were investigated. All of the tumors were diagnosed before surgery as non-functioning. Seven of them were diagnosed after surgery as pituitary adenomas and expressed either gonadotropins or their subunits as detected by immunohistochemistry. Two tumors additionally expressed prolactin and growth hormone. One tumor was immunonegative for pituitary hormones and was diagnosed as haemangiopericytoma. All tumors but one were investigated immunohistochemically to detect the somatostatin receptors and expressed at least 3 of 5 subtypes of somatostatin receptors. The cells isolated from the examined tumors were exposed in vitro to native SST-14. The concentration of VEGF in the culture media was performed by means of ELISA method.

RESULTS: It was found that the exposure on SST-14 resulted in the divergent changes (increase in 4 cases and decrease in 3 cases) in VEGF concentrations in the medium.It seems that the inhibition of VEGF secretion is related to the expression of somatostatin receptor subtypes sst1 and sst2.

CONCLUSIONS: The response of VEGF secretion from pituitary tumoral cells to SST seems to depend on the spectrum of expressed somatostatin receptor subtypes. However, this presumption needs further studies on the larger material.

ORIGINAL ARTICLE

INTRODUCTION

The formation of new blood vessels – neo-angiogenesis – is a process determining the development of solid tumors. To the crucial factors involved in the mechanism of angiogenesis belongs the vascular endothelial growth factor (VEGF). VEGF is a glycoprotein acting as a specific mitogen on endothelial cells. Numerous studies have been shown the increase of VEGF expression in tumors and a correlation between VEGF levels and microvascular density [1,2]. The inhibition of VEGF expression or inhibition of VEGF receptors is suggested as a promising way of the treatment of cancer.The developement of pituitary adenomas, like of the other solid tumors, also depend on neo-angiogenesis. However, a role of VEGF in pituitary tumorigenesis remains unclear. VEGF levels were found to be elevated in patients with pituitary tumors [3,4,5] and administration of anti-angiogenic agents inhibited the growth of experimental estrogen-induced rat prolactinoma [6]. On the other hand, vascularization of pituitary adenomas is poorer than that of normal pituitary and VEGF levels do not correlate with microvascular density in human pituitary adenomas [4].In pituitary adenomas,VEGF receptors were found to be expressed not only in endothelial cells of intratumoral blood vessels but in tumoral cells as well [7]. It has been suggested that VEGF in pituitary adenomas acts not only as stimulator of angiogenesis, but directly promotes growth of tumoral cells. The recent in vitro experiments of Zatelli et al. [8] confirm this hypothesis. Somatostatin analogs are largely used in the medical treatment of pituitary adenomas in man. Their mechanism of action is complex but the effect of somatostatin analogs may be , at least in part, mediated by the inhibition of angiogenesis [9]. The aim of the present study was to investigate the effects of somatostatin on VEGF secretion by cultured pituitary adenoma cells in vitro.

Table I. The data on sex, age and tumor hormone immunopositivity of investigated patients

Initials	Sex	Age	Immunopositivity
WJ	M	71	alphaSU, GH, PRL
DS	M	44	alphaSU, betaLH
RK	F	35	betaLH
JK	F	54	alphaSU, betaFSH, betaLH, PRL,GH
KS	F	44	betaLH
GI	F	69	betaLH
JS	M	64	betaLH
ZT	M	62	immunonegative (hemangiopericytoma)

MATERIAL AND METHODS

Tumors and patients

The tumor tissues were obtained surgically by transphenoidal approach. The study included 8 patients: 4 men and 4 women. All tumors were diagnosed before surgery as hormonally non-functioning After surgery five tumors were diagnosed as pituitary adenomas and one as intrasellar haemangiopericytoma. The detailed data on patients age and tumor phenotype were presented in Table I.

Immunohistochemistry

Each tumor was immunostained using the primary antibodies against the pituitary hormones and alpha-subunit (alpha-SU). To detect the particular subtypes of sst receptors we used the primary antibodies raised against the specific regions of sst receptor proteins, obtained from Gramsch Laboratories (Schwabhausen, Germany). The detailed data on these antibodies were reported elsewhere [10]. The immunostaining was visualized by means of streptavidin-biotin-peroxidase method with 3,3'-diaminobenzidine as chromogen. Intensity of the immunoreaction for sst receptors in particular adenomas was scored semiquantitatively using the following scale: 0 – no reaction, 1 – weak reaction, 2 – moderate reaction, 3 – strong reaction.

Cell cultures

The cells suspensions isolated from tumor tissues were counted in the light microscope and placed into multiwell culture dishes (24 Nunclon Multidishes, Nunc) at a density of 1×10^6 – 3×10^6 cells/well in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (FBS, Biochrom KG, Germany), 100 U/ml penicillin and 100 µg/ml streptomycin (Sigma) and incubated at 37^0C in a humidified atmosphere of 95% air and 5%CO_2.The tumor cell suspensions were incubated for 15 hours (except the sample from patient WJ which was incubated for 18 hours) with the tested substances: somatostatin-14 (SST-14, Sigma) . SST-14 was dissolved in 0.01N acetic acid containing 0.1% FBS. The cultures with solvent alone served as controls. SST-14 was tested in the concentration of 10^{-8}M (n=6–8 wells for each concentration of the tested substances).

Assay of VEGF

VEGF concentrations in the incubation media were measured by ELISA method using the Quantikine human VEGF kit manufactured by RD Systems Inc, Minneapolis, USA.

Table II. Changes of VEGF concentrations in the culture medium (% of controls) after exposure to somatostatin-14 (SST) and the expression of somatostatin receptor subtypes in pituitary tumors

Initials	VEGF	sst1	sst2A	sst2B	sst2A+B	sst3	sst5
JK	+59.8	1	0.5	2	2.5	0.5	2.5
RK	+16.9	not investigated					
GI	+10.1	1	1.5	0.5	2	1	2.5
KS	+8.7	1.5	1	1	2	1	1
ZT	-14.7	0	1.5	0	1.5	1	2
DS	-26.3	1	2	0.5	2.5	3	3
WJ	-65.7	2	1	2	3	0	2

RESULTS

All but one investigated tumor samples secreted the detectable amounts of VEGF into culture media. Only in the sample from one patient JS no measurable amount of VEGF in the culture medium was found, either in control cultures or in cultures exposed to SST. The exposure of these samples to somatostatin resulted in divergent changes of VEGF : an increase in 4 cases and a drop in 3 cases (see table II). As it can be seen in Table II, the drop of VEGF seems to be related to the expression of sst1 and sst2 (summarized expression of sst2A and sst2B)

DISCUSSION

The presence of detectable amounts of VEGF in the incubation media of 4/5 investigated pituitary tumor samples supports the assumption that pituitary tumoral cells express this angiogenic peptide[8]. Similar expression of VEGF by tumoral cells themselves was shown in gliomas [11]. The contribution of vascular cells in secreting VEGF to the culture medium cannot be excluded but should rather be neglected since vascularization of human pituitary adenomas is rather scarce [4,12]. The exposure of the investigated pituitary tumors to SST produced divergent alterations of VEGF secretion (stimulation or inhibition). Lawnicka et al. [13] have found that SST and its analog octreotide suppressed in vitro the proliferation of murine vascular endothelial cells but did not influence the secretion of VEGF to the culture medium. On the other hand, it was shown recently that SST and its analog pasireotide (SOM 230) suppressed both VEGF secretion and cell viability of non-functioning pituitary adenoma cells in the primary culture [8]. The latter authors found the distinct correlation between VEGF inhibition and somatostatin receptor expression: all the adenomas responding to SST or pasireotide with VEGF inhibition equal or greater than 15% expressed sst1 subtype but not expressed sst5 subtype. In turn, in gliomas the inhibition of VEGF secretion seems to depend on sst2 expression [11].Observations reported in the present paper did not reveal such a simple relationship. The inhibitory effect observed by us in 3 cases seems be related with the expression of sst1 subtype, what corroborates with observations by Zatelli et al.[8]. The effect may be also related to summarized expression of sst2 (sst2A+2B) what, in turn, is compatible with data of Mentlein et al.[11]. On the other hand, the inhibitory effect seems not be related to the expression of sst3 and sst5 subtypes. In a half of investigated tumors, an unexpected paradoxical stimulatory effect of SST on VEGF secretion was found. Such stimulatory responses to SST or its analogs were observed earlier in case of other substances tested, for instance chromogranin and alpha-subunit [14]. The nature of such paradoxical responses to SST is unknown. Several possibilities can be suggested. First, some receptor subtypes may constitutionally mediate the opposite (stimulatory) effects. However, we did not reveal any link between expression of the particular subtype of somatostatin receptor and VEGF stimulation. However, such a link cannot be totally excluded because our study was performed on limited number of tumors. The second possibility includes molecular alterations in the receptors themselves or the post-receptor transduction mechanisms connected with pituitary tumorigenesis. Third, because SST may decrease the viability of the investigated tumoral cells [15] it cannot be excluded that in some cases the increase of VEGF concentrations in the culture medium does not reflect its enhanced secretion, but its release from the destroyed cells. To conclude, it seems that the inhibitory response of VEGF to SST depends on the spectrum of expressed somatostatin receptor subtypes. However, this presumption needs further studies on the larger material.

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