MORPHOLOGY AND PATHOMORPHOLOGY

Vascular Endothelial Growth Factor and Type 2 Receptor for This Factor in Vascular Malformations

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Immunohistochemical study revealed higher level of VEGF expression in vascular malformations compared to that in normal vessels of the adjacent tissue. VEGF expression was slightly higher in the endothelium of arteriovenous angiodysplasias than in venous angiodysplasias. Enzyme immunoassay of tissue extracts showed that the concentrations of VEGF and VEGFR2 differ in arteriovenous and venous malformations. The concentrations of VEGF and VEGFR2 differed more significantly in primary and recurrent forms of arteriovenous angiodysplasias (as compared to venous angiodysplasias).

Key Words: *vascular malformations; endothelium; VEGF; VEGFR2*

Vascular malformations (angiodysplasias) result from impaired angiogenesis during embryogenesis. The incidence of these disorders varies from 0.3 to 5.4% (average incidence 2.6%) in hospitalized patients with peripheral vessel diseases [1,5]. Disturbances in the interaction of proangiogenic and antiangiogenic mediators play an important role in the pathogenesis of vascular malformations [3]. Studying the role of various factors in the pathogenesis and recurrence of various types of vascular malformations will allow us to develop new approaches to noninvasive therapy of these diseases (*e.g.*, target therapy) [2].

Vascular endothelial growth factor (VEGF) is a key positive regulator of angiogenesis. The family of VEGF consists of several secretory glycoproteins, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E. VEGF-A serves as the major regulator of angiogenesis (particularly at the early stage of this process). Hemopoietic islets, endotheliocytes, and primary blood vessels of the embryo do not form in the absence of this factor [8]. Deletion of even one allele in the VEGF-A gene leads to embryonic death [12]. Experiments on mice showed that the inactivation of VEGF-B causes changes in cardiac conduction and increases the number of circulating inflammatory cells, but has no effect on angiogenesis [9]. VEGF-C and VEGF-D are mainly involved in the regulation of lymphangiogenesis.

The effects of all factors of the VEGF family are related to their interaction with specific tyrosine kinase receptors of types 1, 2, and 3. The type 2 VEGF receptor (VEGFR2) is initially expressed by hemangiogenic cells of the lateral mesodermal plate. Specific inactivation of this receptor is followed by abnormal development of hemopoietic islets and embryonic blood vessels, which results in death of the embryo [11]. Activation of VEGFR2 not only increases the proliferation and migration of endotheliocytes, but also improves permeability of blood vessels [4].

Immunohistochemical study and enzyme immunoassay were performed to compare the expression and/

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or concentration of VEGF-A and VEGFR2 in tissues samples of arteriovenous and venous malformations.

MATERIALS AND METHODS

Surgical samples were taken form 13 patients (7 women and 6 men, 22-67 years) with peripheral angiodysplasias. These patients were admitted to the A. V. Vishnevsky Institute of Surgery in 2007-2009. Arteriovenous (7 patients, 4 women and 3 men) and venous malformations (6 patients, 3 women and 3 men) were diagnosed after complex clinical and morphological examination.

Tissue samples were fixed in 10% neutral formalin. Histological study was performed on paraffin sections (5 μ) stained with hematoxylin and eosin. Immunohistochemical study of VEGF expression (DBC, 1:50) involved the immunoperoxidase method after repeated staining with hematoxylin. The results of the reaction were evaluated by a semiquantitative method taking into account the intensity and distribution of staining: (-), negative reaction; (+), mild local reaction; (++), moderate or severe reaction of the membrane or apical intracellular localization; and (+++) severe reaction of the intracellular localization or cytoplasmic distribution.

Tissue samples were stored at -70°C for further EIA. Tissue samples were lysed in a buffer (1:3 ratio) containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, and 1 μg/ml leupeptin. The lysates were centrifuged on an Optima TLX centrifuge (Beckman) at 20,000 rpm and 4°C for 30 min to obtain low-speed cytosol. The concentrations of VEGF-A and VEGFR2 were measured by EIA using standard kits (Quantikine Human VEGF Immunoassay and Quantikine Human VEGFR2 Immunoassay, R&D Systems) according to manufacturer's instructions. The concentration of these markers in tissue samples was expressed in pg per mg protein. Protein content in tissue extracts was estimated by the method of Lowry.

The results were analyzed by Statistica 6.0 software.

RESULTS

Histological study of samples from arteriovenous malformations revealed random clusters of arterial and venous vessels (5-15 μ in diameter; Fig. 1, *a*). Irregular distribution of elastic fibers and local loosening of the inner elastic membrane were found in the majority of arterial vessels. Subintimal sites of hypertrophic smooth muscle cells and signs of focal sclerosis were identified in the wall of venous vessels. Dense fibrous connective tissue was revealed in the peripheral area of angiodysplasias.

Fuzzy cavernous structures of different size and shape were found in histological preparations of venous malformations. Their wall had venous structure (Fig. 1, *b*). The wall of these caverns was characterized by different thickness. The number of muscle fibers was decreased or increased in areas of thinning or thickening, respectively. The regions of dense fibrous connective tissue and focal hemorrhages of different size were found around these structures.

Immunohistochemical study of samples of arteriovenous and venous malformations revealed a moderate increase in the expression of VEGF (as compared to intact vessels of the adjacent tissue). VEGF expression in endotheliocytes of arteriovenous malformations was slightly higher than in venous malformations (strong expression of VEGF in 3 of 7 samples; Fig. 2, *a*, *b*). The reaction with anti-VEGF antibodies was more pronounced in samples from recurrent forms of arteriovenous and venous angiodysplasias (Fig. 2, *c*, *d*).

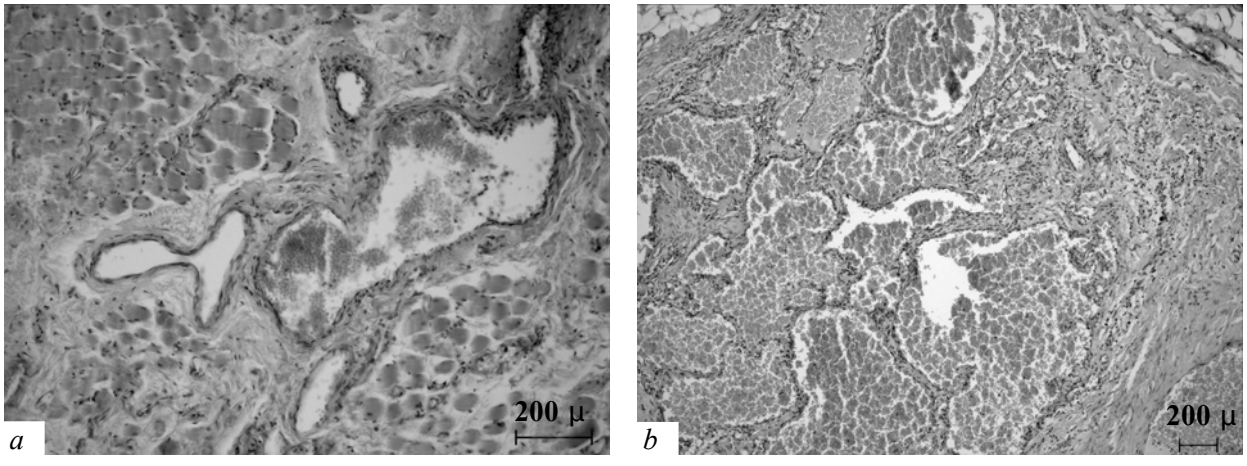

Fig. 1. Histological characteristics of arteriovenous (*a*) and venous malformations (*b*). Hematoxylin and eosin staining.

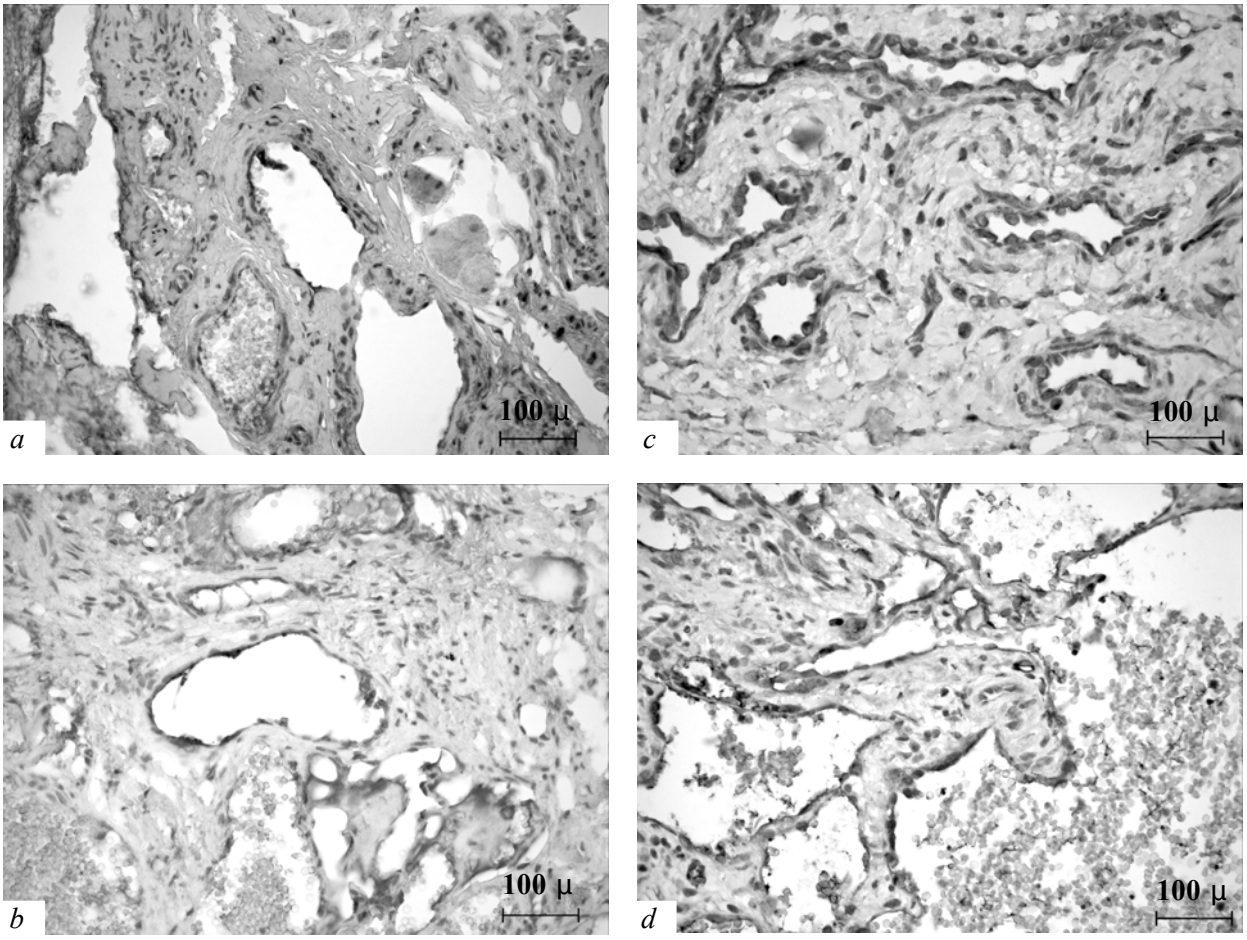

Fig. 2. VEGF expression in primary (*a*, *b*) and recurrent (*c*, *d*) arteriovenous (*a*, *c*) and venous malformations (*b*, *d*). Immunoperoxidase method, ×400.

EIA of angiodysplasia tissue extracts revealed a medium content of VEGF (0.7-21.8 pg/mg protein, average content 10.10±8.54 pg/mg protein) in all samples of primary venous malformations. It should be emphasized that the concentration of VEGF was slightly higher in tissues of recurrent venous malformations (0.6-28.0 pg/mg protein, average content 12.1±14.2 pg/mg protein). However, these differences were statistically insignificant. VEGF concentration was low in all samples of primary arteriovenous malformations (3.8-5.7 pg/mg protein, average content 4.80±1.34 pg/mg protein). The concentration of VEGF was much higher in tissues of recurrent malformations (21.3-21.7 pg/mg protein, average content 21.50±0.27 pg/mg protein; $p<0.05$).

The content of VEGFR2 practically did not differ in tissues of primary venous malformations (50.2-177.0 pg/mg protein, average content 91.1±52.8 pg/mg protein) and recurrent angiodysplasia (38.9-148.0 pg/mg protein, average content 94.0±39.8 pg/mg protein; $p>0.05$). The content of VEGFR2 in tissues of primary arteriovenous malformations (47.0-76.5 pg/mg protein, average content 61.80±20.85 pg/mg protein) was much lower than in recurrent angiodysplasia (232-859 pg/mg protein, average content 545±243 pg/mg protein; $p>0.05$).

Our results show that VEGF and VEGFR2 play an important role in the pathogenesis of vascular malformations, which is consistent with published data. Previous studies demonstrated that the expression of VEGF-A and type 1 receptor for this factor is much higher in endotheliocytes of arteriovenous malformations of the brain (as compared to normal vessels of the brain) [6]. We showed that VEGF expression in angiodysplasias is higher than in intact vessels of the adjacent tissues (immunohistochemical study). It should be emphasized that VEGF expression in the endothelium of arteriovenous angiodysplasias was higher than in venous malformations. EIA of tissue extracts showed that the concentrations of VEGF and VEGFR2 differ in arteriovenous and venous malformations. The more significant differences in the concentrations of VEGF and VEGFR2 in primary and recurrent forms of arteriovenous angiodysplasias (as compared to ve-

nous angiodysplasias) indicate that the VEGF system has an important role in the pathogenesis of this type of malformations. The data on different expression of VEGF and VEGFR2 during malformations can be used for differential diagnostics of these diseases and vascular neoplasms (hemangiomas). Previous observations showed that serum VEGF concentration in children with hemangiomas (particularly proliferating hemangiomas) is higher than in patients with vascular malformations [10,13].

The specific features of expression of VEGF and its receptors in tissues of venous and arteriovenous angiodysplasias probably reflect the pathogenetic differences of these disorders. Our results hold much promise for the development of pathogenetic therapy of these diseases.

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