GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Expression of Vascular Endothelial Growth Factor during Neoangiogenesis Stimulated by Exposure to High-Intensity Laser Radiation

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Expression of vascular endothelial growth factor was studied during neoangiogenesis stimulated by exposure of ischemic tissues in the limb muscles, liver, and myocardium and normal myocardial tissue to high-intensity laser radiation. Changes in expression were related to cell reactions during the inflammatory and reparative process and did not differ in the studied tissues.

Key Words: *ischemia; neoangiogenesis; high-intensity laser; vascular endothelial growth factor*

Stimulation of neoangiogenesis in ischemic tissues under the influence of high-intensity laser radiation underlies transmyocardial revascularization and correction of ischemia in muscles of the lower limbs and portal hypertension during liver cirrhosis. The connective tissue with a considerable number of new vessels is formed at the site of laser channels. These vessels form anastomoses with vascular network in surrounding tissues, which compensates for insufficient blood supply [7].

Intensive neoangiogenesis in the zone of high-intensity laser irradiation is due to activation of angiogenic growth factors and proteolytic enzymes. Our previous studies showed that basic fibroblast growth factor is a cytokine responsible for proliferation of endotheliocytes and fibroblasts during laser irradiation and possessing histoprotective activity [2]. Experiments performed in the past decade showed that vascular endothelial growth factor (VEGF) plays a major role in neoangiogenesis.

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VEGF increases mitotic activity, stimulates migration of endothelial cells, and coordinates proteolytic changes in the matrix necessary for the growth of new vessels. Hypoxia in cells stimulates the synthesis of VEGF [5]. Hypoxia activates mRNA-binding protein. This hypoxia-induced factor binds to the untranslated region of the VEGF gene by a specific sequence, which accelerates transcription of VEGF-encoding mRNA and increases its stability. Short isoforms of VEGF are released from cells into the surrounding matrix. These isoforms bind to the cell membrane via specific heparin proteoglycans and can be released under the influence of heparinases and proteases during tissue injury and inflammation [4].

VEGF increases vascular permeability and, therefore, can affect distant target cells. The release of plasma components into the extracellular matrix initiates extravascular coagulation and provides activation of plasmin proteases and matrix metalloproteases. Proteolytic reconstruction of the extracellular medium impairs the interaction of integrins from endothelial cells with the matrix and promotes migration of cells during neoangiogenesis [6].

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Here we studied changes in VEGF expression and evaluated correlations between this process and activity of plasminogen activators in ischemic tissues of limb muscles, myocardium, and liver and normal myocardial tissue during healing of a laser channel.

MATERIALS AND METHODS

Experiments were performed on 240 outbred albino rats weighing 180-200 g. An Al'to-300 diode laser (λ=805 nm) served as the source of laser radiation. Energy was supplied via a single-fiber quartz waveguide. Myocardial ischemia in rats was modeled under conditions of chronic stress of hypodynamia and drug treatment (mesatone and obsidan) [3]. Moderate chronic ischemia of anteromedial hindlimb muscles was modeled by ligation of the femoral artery and its branches (including cutaneous arteries) from a level of the inguinal ligament to distal thigh regions. Ischemic cirrhotic damage to the liver was modeled surgically (delayed effect of ischemia-reperfusion).

Laser channel in the myocardium reached the left ventricle cavity. Ischemic tissues of the liver and muscles were characterized by postischemic changes and had 2 channels (5 mm in depth). The animals were killed on days 1, 5, 10, 30, and 90. VEGF expression in cryostat histological sections was studied using specific antibodies against VEGF (Sigma) and Immumark^{tm} detection system (ICN) that included streptavidin-biotin conjugates labeled with alkaline phosphatase. The VEGF expression index was calculated by means of an ocular grid with 100 equidistant points (×400). We analyzed coincidence of the studied index with grid points [1]. The data are expressed in percents of the total number of grid points. Activity of plasminogen activators was measured by zymography of tissue homogenates on agarose gel containing human plasma plasminogen (Sigma) and casein (ICN) and expressed in arbitrary units. The Pierson's linear correlation coefficient was calculated by means of Diamorph Ipso® software to estimate the relationship between VEGF expression and enzyme activity. The relationships were significant at a correlation coefficient of 0.8-1.0.

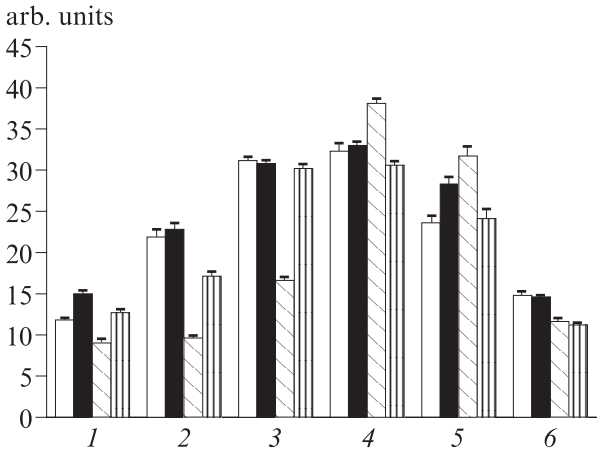

Fig. 1. Expression of vascular endothelial growth factor in tissues exposed to high-intensity laser radiation. Here and in Fig. 2: normal myocardium (light bars), ischemic myocardium (dark bars), muscles (slant shading), and liver (vertical shading). Control (*1*) and days 1 (*2*), 5 (*3*), 10 (*4*), 30 (*5*), and 90 (*6*).

RESULTS

VEGF was microscopically visualized in cells and extracellular matrix of tissues exposed to laser radiation. In most control samples VEGF was localized intracellularly. The degree of VEGF expression was maximum in endothelial cells of blood vessels. The reaction for VEGF was positive in neutrophils and macrophages infiltrating the focus of laser irradiation and loose connective tissue surrounding newly formed vessels.

VEGF expression index increased on day 1 and reached maximum on day 10. The intensity of VEGF expression decreased on day 30 and did not differ from the control on day 90. Changes in VEGF expression induced by laser radiation were similar in ischemic and normal tissues (Fig. 1).

These data indicate that VEGF expression depends on the progression of inflammatory and reparative processes in laser-induced wound. The degree of VEGF expression reached maximum in tissues characterized by most intensive neoangiogenesis.

Activated platelets in vessels surrounding the laser channel form aggregates, secrete granules, and serve as the source of VEGF in the first minutes after high-intensity laser irradiation [7]. In the follow-up period of inflammation VEGF is produced by different cells. At the early stage VEGF is secreted by neutrophils and macrophages; at the late stage this substance is released from proliferating endothelial cells of newly formed vessels and fibroblasts [6,8,9]. The degree of VEGF expression returned to the initial level after complete remodeling of the connective tissue (day 90).

Activity of plasminogen activators was measured in the zone of laser irradiation. Enzyme activity increased on day 1, which correlated with the degree of VEGF expression (Fig. 2). The correlation coefficient in normal myocardium was 0.88. In ischemic tissues of the myocardium, muscles, and liver this coefficient was 0.96, 0.79, and 0.96, respectively. These results confirm the assumption that VEGF indirectly stimulates proteolysis in the extracellular matrix by increasing vascular permeability and activating the system of coagulation and fibrinolysis. Moreover, VEGF can indirectly stimulate this process by initiating the syn-

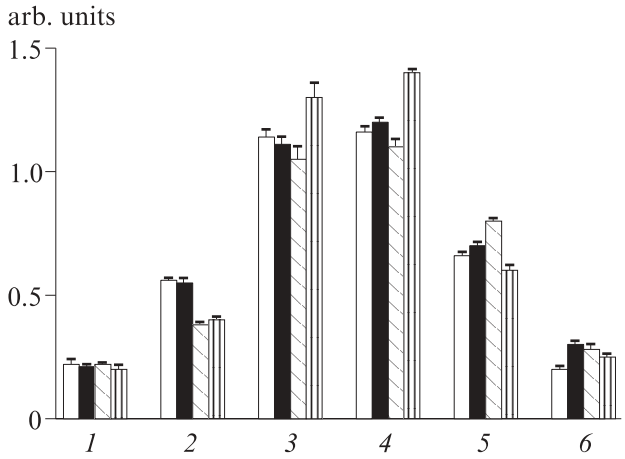

Fig. 2. Activity of plasminogen activators in tissues exposed to high-intensity laser radiation.

thesis of intracellular proteolytic enzymes [8]. Reconstruction of the extracellular matrix contributes to activation of angiogenic cytokines bound to proteoglycans. They include basic fibroblast growth factor, transforming growth factor-β, and VEGF that is localized on cells membranes [10]. It should be emphasized that basic fibroblast growth factor enhances tissue sensitivity to VEGF and stimulates its production in cells.

Our results show that VEGF expression in the focus of laser irradiation was similar in various tissues where new vessels appeared. The content of growth factors and activity of proteolytic enzymes depended on cellular reactions during the inflammatory and reparative process. We concluded that neoangiogenesis initiated by laser irradiation is a natural and standard tissue response to damage in the myocardium, liver, and limb muscles.

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