Expression of Basic Fibroblast Growth Factor in the Course of Neoangiogenesis Stimulated by High-Intensity Laser Irradiation

E. S. Golovneva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 7, pp. 109-111, July, 2002
Original article submitted May 6, 2002

We studied the dynamics of expression of basic fibroblast growth factor during neoangiogenesis in ischemic myocardial, muscle, and liver tissue stimulated by high-intensity laser irradiation. The increase in growth factor expression depended on activity of satellite cell populations involved in healing of laser-damaged tissues.

Key Words: *ischemia; neoangiogenesis; high-intensity laser irradiation; basic fibroblast growth factor*

The formation of new vessels (neoangiogenesis) is observed in both health (ovulation and healing of damaged tissues) and disease (tumor growth, rheumatic diseases, *etc.*). There are two main conditions for neoangiogenesis: 1) the presence of growth factors (specific cytokines stimulating the mitotic, proliferative, and migration activities of endothelial and smooth muscle cells) in tissues and 2) activity of proteolytic enzymes providing conditions for cell migration and formation of new vascular structures.

Basic fibroblast growth factor (bFGF) belongs to a large family of fibroblast growth factors possessing a high angiogenic potential. bFGF is a key regulator of neoangiogenesis acting as an intracellular messenger on the nucleus of the cell where it was produced. A characteristic feature of bFGF is the absence of a signal sequence responsible for its secretion from cells into the extracellular matrix, *i.e.* bFGF can be released only from destroyed cells [3]. bFGF is stored in the extracellular matrix in the form of inert complexes with heparan sulfate proteoglycan and activated when necessary. Target cells for bFGF are endothelial cells, vascular smooth-muscle cells, fibroblasts, macrophages, hepatocytes, and myocytes [7].

Chelyabinsk State Institute of Laser Surgery

Stimulation of neoangiogenesis in ischemic tissues by direct injections of exogenous bFGF or the corresponding DNA in tissues was recently used in clinical experiments [6]. Another way for inducing tissue revascularization is the use of laser technologies, *e.g.* transmyocardial revascularization, correction of ischemia in lower extremities and portal hypertension in liver cirrhosis. Morphological analysis of myocardial, muscle, and liver tissues exposed to laser irradiation revealed the formation of the connective tissue with numerous capillaries at the site of laser channels, some capillaries later differentiate into small arteries and veins. This vascular network forms anastomoses with blood vessels in the adjacent areas and compensates for deficient blood supply [2]. It is obvious that active angiogenesis is stimulated by increased local concentration of endogenous growth factors.

We studied the dynamics of bFGF expression in ischemic myocardial, muscle, and liver tissues exposed to high-intensity laser irradiation.

MATERIALS AND METHODS

Experiments were carried out on 180 random-bred rats (180-200 g). Alto 300 (λ=805 nm) diode laser was used as the source of radiation, the energy was delivered via a monofiber quartz light guide. Two channels 5 mm

0007-4888/02/1341-095$27.00 © 2002 Plenum Publishing Corporation

deep were formed in ischemic liver and muscle tissues and 1 laser channel penetrating the left ventricle was formed in the myocardium. The animals were sacrificed 1-2 min and 1, 5, 10, and 30 days after exposure.

The expression of bFGF was evaluated on cryostat sections by indirect immunofluorescent method with specific antibodies to bFGF (Sigma) and FITC-labeled secondary antibodies (Sigma). The index of bFGF expression was calculated using an ocular grid with 100 equidistant light points (×980, oil immersion) [1]. Fluorescent objects coinciding with the grid points were counted and the result was expressed in percent of the total number of the grid points.

RESULTS

When evaluating the expression of bFGF, we paid special attention to registration and identification of maximally fluorescing cells in histological preparations: mast cells and vascular walls in zones exposed to high-intensity laser irradiation.

Fluorescent granules were seen in the cytoplasm of mast cell and also around degranulated mast cells. Completely destroyed mast cells looking as scattered fluorescent granules were seen in the focus of laser damage at the early terms of the experiment. Mastocyte nuclei did not fluoresce.

Large fluorescent objects protruding into the capillary lumen were seen at the site of laser exposure in the majority of capillaries; electron microscopy showed that these objects are proliferating endothelial cells.

The content of bFGF in organ-specific cells (myocytes, cardiomyocytes, and hepatocytes) was low, but enhanced fluorescence was seen in tissues directly exposed to laser irradiation.

The index of bFGF expression increased starting from day 1 after laser exposure and peaked (more than 5-fold surpassed the control) by day 10 of the experiment. bFGF expression in the myocardium and muscles was more intensive that in the liver. On day 30 bFGF expression decreased and by day 90 returned to normal (Fig. 1).

Comparison of these results with morphological findings showed that the detected increase in the index of bFGF expression correlated with the dynamics of repair processes in the laser wound [2]. bFGF at the initial stages was first produced by laser-activated mast cells with signs of degranulation [5], and then (with progression of the inflammatory reaction) by macrophages, endotheliocytes, and smooth-muscle cells.

Morphological transformation of cells, reorganization of the cytoskeleton, and mitogenic activity of cells induced by bFGF at the site of laser exposure determined early and active proliferation of fibroblasts, endothelial cells, and smooth-muscle cells in vessels and proliferation of ductal epithelial cells and hepatocytes in the liver.

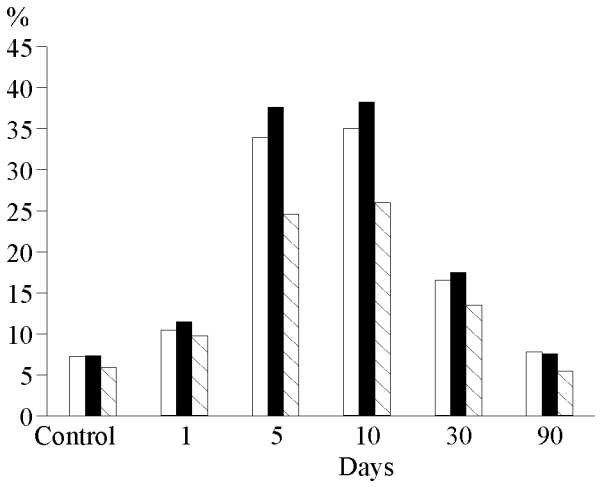

Fig. 1. Dynamics of expression of basic fibroblast growth factor in ischemic tissues after high-intensity laser irradiation. Light bars: myocardium; dark bars: muscles; cross-hatched bars: liver.

It should be noted that function of bFGF is not confined to stimulation of proliferation, mitoses, and cell migration. This cytokine exhibits also a histoprotective effect and improves cell survival under conditions of hypoxia and tissue damage. For example, myocardial infarction zone decreased within the first hours after the start of intravenous infusion of bFGF, when new vessels were not yet formed [7]. This histoprotective effect of bFGF probably minimizes damage and ensures early recovery of specific functions of the adjacent tissues after laser irradiation.

Interesting results were obtained in studies of bFGF interactions with other growth factors and proteolytic enzymes. bFGF induces expression of vascular endothelial growth factor (VEGF) and its cell receptors [4]. VEGF stimulates plasmin-dependent matrix proteolysis and increases collagenase production in cells [7]. Proteolytic enzymes, in turn, activate bFGF fraction stored in the extracellular matrix [3,8]. This determines the peak of bFGF expression on days 5-10 of the experiment. Completion of healing processes in the laser wound by day 90 of the experiment corresponded to normalization of bFGF expression index.

The direct dependence of bFGF expression on activity of cell and enzymatic reactions during inflammation and regeneration in the zone exposed to high-intensity laser irradiation suggests that neoangiogenesis in response to laser irradiation is a stereotypical adaptive reaction of ischemic tissues to damage.

REFERENCES

1. G. G. Avtandilov, *Medical Morphometry* [in Russian], Moscow (1990).
2. E. S. Golovneva, *Lazer Med.*, **5**, No. 3, 29-31 (2001).

3. D. Moscatelli and D. Flaumenhaft, *Ann. N. Y. Acad. Sci.*, **650**, 177-181 (1998).
4. M. S. Pepper, S. Mandriota, M. Jeltsch, *et al.*, *J. Cell. Physiol.*, **177**, 439-452 (1998).
5. Z. Qu, J. M. Liebler, M. R. Rowers, *et al.*, *Am. J. Pathol.*, **147**, 564-573 (1995).
6. F. W. Sellke, R. J. Laham, and E. R. Edelman, *Ann. Thorac. Surg.*, **65**, 1540-1544 (1998).
7. D. R. Senger, *Am. J. Pathol.*, **149**, 1-7 (1996).
8. A. Yanagisava-Miva, Y. Uchida, and F. Nacamura, *Science*, **257**, 1401-1405 (1992).