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Reaction of Mast Cells in the Zone of Polypropylene Mesh Implantation

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Reaction of mast cells of adult male Wistar rats (n=15) in the zone of polypropylene mesh fixation was studied by histochemical, immunohistochemical, and traditional morphological methods on days 1, 5, 10, and 30 after implantation. Immediately after the intervention, mast cells stimulated the processes aimed at wound healing. Secretion of mast cells was clearly regulatory. These cells migrated to the zone of injury for subsequent activation of their function. The number of cNOS+ mast cells near the polypropylene mesh was maximum on day 1 and the number of iNOS+ mast cells peaked on day 5 of the experiment, which probably represented a compensatory reaction. Presumably, stimulation of fibrillogenesis was largely due to the activatory effect of mast cells on the fibroblast function, but not to collagen production by these mast cells.

Key Words: *mast cells; polypropylene mesh; experiment*

Polypropylene mesh implants are used in reconstructive surgery for strengthening of myoaponeurotic formations [3,13,14]. The mesh is resistant to mechanical factors, is not degraded by enzymes, and retains tenacity and integrity throughout patient's life. The implant is well integrated into the adjacent tissues causing no discomfort, rejection, or allergic reactions [2,9,14]. However, implantation of foreign material of any kind triggers the inflammatory reparative reaction of the adjacent tissues. Mast cells (MC) play an important role in this process. However, their reaction in the zone of polypropylene endoprosthesis fixation has been never studied in detail.

We studied the reaction of MC in the zone of polypropylene mesh fixation at different terms after implantation to experimental animals.

MATERIALS AND METHODS

The study was carried out on adult male Wistar rats (n=15; 300±50 g) in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the requirements of the World Society for Protection of Animals (WSPA).

Surgipro-SPMM-149 polypropylene mesh (1× 1 cm) was implanted to the posterolateral surface of the thorax on the right (12 rats). Specimens of tissues adjacent to the implant and from the symmetrical contralateral region were studied on days 1, 5, 10, and 30 after surgery. Material from 3 rats kept under the same conditions served as the control. The animals were decapitated under thiopental narcosis; specimens for the study were fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4; 4°C, 4 h), impregnated in cold 30% sucrose solution in 0.1 M PBS, and cryostat sections (30-40 μ) were sliced.

Cell composition and activities of catecholamines and constitutive (cNOS) and inducible NO synthase (iNOS) in MC were studied on sections embedded in polystyrene. Some sections were stained with hematoxylin-eosin and methylene blue, others were studied by fluorescent histochemical method to detect catecholamines (Fumess, Costa, 1975) or stained for NADPH diaphorase (Hope, Vincent, 1989) to detect cNOS; iNOS was detected by im-

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munocytochemical method (Webbetal, 2001) using antiserum to iNOS [15].

The preparations were examined under a Leica DM fluorescent microscope (λ=486 nm) and a Carl Zeiss Jena microscope (λ=486 nm).

The density (cell count per mm^2) and area of MC were evaluated using AllegroMC automated image analysis system [8]. Coefficient of MC degranulation was calculated by the formula: $A=C/B$, where C was the count of degranulating cells and Bis total count of MC. The significance of differences between the means was evaluated by Student's t test.

The results were processed using Microsoft Excel 2010 and Biostat (Biostatistics version 4.03) software.

RESULTS

In controls, MC were located along the blood vessels or in the intervascular regions on the right and on the left in the connective tissue layer of the thoracic posterolateral surface. Methylene blue staining detected few spindle-shaped and spherical MC, with a profile area of 200-300 μ^2 and cytoplasm filled with raspberry-colored metachromatic granules. Fluorescent microscopy showed bright green fluorescing cells in the same regions; NADPH diaphorase was detected in solitary MC stained dark blue; no iNOS$^+$ MC were detected in control animals.

Degranulation is a morphological sign of functional activity of MC irrespective of the method of their detection. By the degree of this process, the cells were classified as non-degranulating, poorly degranulating, and actively degranulating cells with massive release of granules [5]. In control rats, non-degranulating or poorly degranulating MC with solitary granules released from the cell predominated.

The morphological picture of specimens collected at the site of polypropylene mesh fixation on day 1 after its implantation was characterized by exudative reaction of aseptic serous inflammation type. The bulk of the cells were granulocytes, with solitary polynuclear leukocytes and negligible number of lymphocytes (Fig. 1, *a*). The adjacent vessels of the microcirculatory bed were plethoric, with stasis. The tissues adjacent to the implant were edematous. The count of MC increased significantly during this period (Fig. 2, *a*); cells with signs of moderate and active degranulation appeared (Fig. 1, *b*, *c*).

By the end of day 1 the concentration of fluorescent MC increased significantly (Fig. 1, *d*). In addition to cells with bright green fluorescence (82% cells; $p<0.05$), MC with yellow-green (12%; $p<0.05$) and yellow fluorescence (6%; $p<0.05$) appeared. According to previous data [7], bright green fluorescence attests to the presence of cells containing histamine, yellow-green and yellow fluorescence indicates dopamine- and serotonin-containing cells, respectively.

The level of NADPH diaphorase$^+$ MC also increased significantly during this period (13%); there were intact cells and MC at one of degranulation stages (Fig. 1, *e*). In addition to cNOS$^+$ cells, MC with iNOS activity were detected (Fig. 1, *c*). No degranulating cells were detected in this population at this term.

Macrophages, histiocytes, and lymphocytes appeared among polynuclear leukocytes and lymphocytes on day 5 after implantation, which indicated the development of productive inflammation. Young granulation tissue with numerous fine-walled vessels was forming. Macrophages containing fragments of phagocytosed cell nuclei and hemosiderin predominated at the periphery of the inflammation focus (Fig. 1, *g*). The count of MC saturated with heparin decreased during this period (20±5), degranulation coefficient in labrocyte population (detected by methylene blue staining) was 0.7% ($p<0.05$). The level of fluorescent MC decreased significantly (Fig. 2, *a*), though cells with bright green fluorescence still predominated over cells with yellow-green and yellow fluorescence. The level of NADPH diaphorase$^+$ MC slightly decreased, while the level of iNOS$^+$ MC increased significantly (Fig. 2, *a*), with moderately degranulating forms scattered among them.

By day 10, the count of polymorphonuclear leukocytes in the connective tissue adjacent to the implant dropped, while the counts of lymphocytes, macrophages, and fibroblasts increased. A fine network of collagen fibrils appeared in the focus of inflammation, collagenization of intercellular matrix was in progress, and organization of a fibrous capsule enveloping the endoprosthesis started. The counts of cells in all MC populations with iNOS activity decreased significantly.

On day 30, the signs of interstitial inflammation (lymphocytes, macrophages, histiocytes) were less pronounced, cell counts decreased significantly; devastation of vessels was observed, the process of collagen fibril compactization and formation of a capsule from loose connective tissue enveloping the propylene threads was in progress (Fig. 1, *h*). Eosinophils appeared in cell infiltrate, presumably, this was a variant of the transplantation immunity reaction. The count of MC decreased during this period to the minimum level (2-3 per field of view), their profile field constituting 200-250 μ^2, no degranulation thereof detected. Other methods detected just solitary MC.

Changes in MC population were also observed on the contralateral side (Fig. 2, *b*). By the end of day 1 after endoprosthesis implantation, the count of these cells increased in comparison with the control, though not so obviously as in the implantation zone. The count of MC in this region surpassed the normal throughout the observation period, intact cells

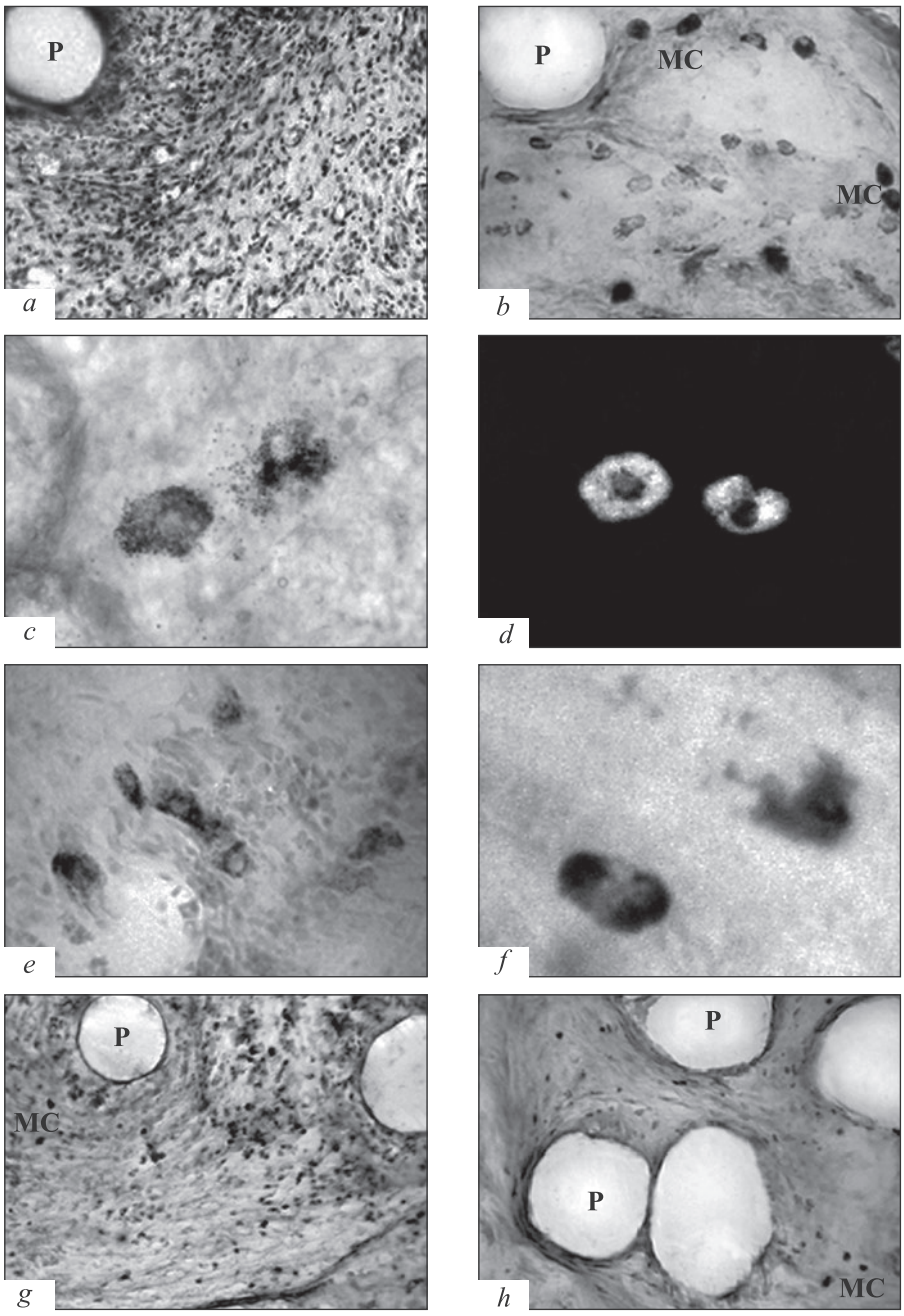

Fig. 1. Morphological picture at the polypropylene mesh fixation boundary. Staining with hematoxylin and eosin, ×32 (*a*, *g*, *h*); methylene blue, ×100 (*b*), ×400 (*c*); by Furness and Costa's method, ×400 (*d*); for NADPH diaphorase detection, ×200 (*e*), for iNOS detection, ×400 (*f*). *a*) Exudative reaction in the form of aseptic serous inflammation by the end of day 1; *b-f*) MC in the implant zone by the end of day 1; *g*) day 5 of experiment; *h*) day 30. P: polypropylene.

or cells with slight and moderate functional activities predominating. Mast cells were most numerous on day 5 of the experiment. Later, their count decreased and approached the control level on day 30. The level of catecholaminergic MC was just a little higher than in the control. The relative number of diaphorase-positive MC varied from 4 to 7% ($p<0.05$) cells during various periods of observation, and these were cells mainly in one of the functional activity stages. Mast cells expressing iNOS were solitary and found not in all animals during all periods of observation.

Our data suggest that immediately after the intervention MC stimulated the processes aimed at wound healing. Secretion of MC was definitely regulatory. These cells migrated to the zone of injury, after which their functional activity was stimulated. The MC granules contained inflammation mediators: biogenic amines (histamine, serotonin), arachidonic acid cleavage products, cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-5, and TNFα), growth factors (fibroblast growth factor, transforming growth factor, and vascular endothelial growth factor), neuropeptides, phospholipids, proteoglycans, and other bioactive substances [11] characterized by a variety of effects on the adjacent tissues, primarily on the regeneration processes. Production of cytokines and growth factors by

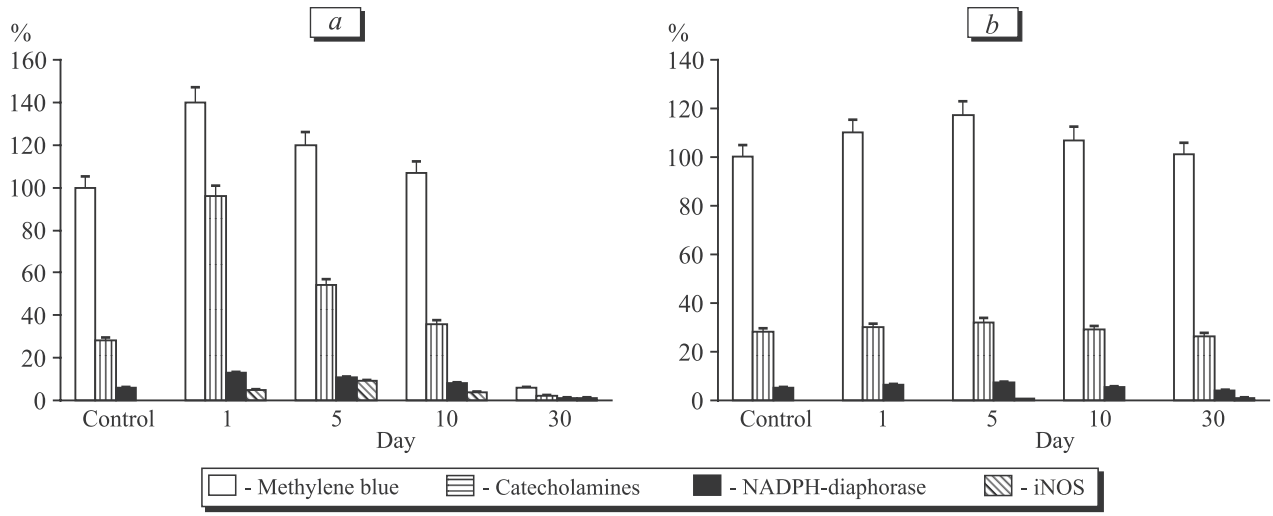

Fig. 2. MC (%) detected by various methods during different periods after polypropylene mesh implantation in the zone of implantation (*a*) and on the contralateral side (*b*). The level of MC detected by methylene blue staining in the target region in the control, is taken for 100%.

MC seemed to determine the angiogenesis processes in the zone of injury and mobilization of fibroblasts to the zone of young cicatrix formation; fibroblasts started intense production of collagen, this leading to the formation of a connective tissue capsule enveloping the implant [4,6]. Though MC are rare in the cicatricial tissue [1], it seemed that their stimulation of fibrillogenesis was due to their mainly activatory effect on the fibroblast functions rather than to their collagen production.

It seems that the regulatory effect of MC on the nearest microenvironment was mediated by NO [12], capable of regulating the activities of pro- and anti-inflammatory cytokines through activation of their receptors [10]. Our data indicated that the maximum expression of $cNOS^+$ cells was observed in the polypropylene mesh zone by the end of day 1. The content of $iNOS^+$ MC peaked on day 5 of the experiment, which seemed to be compensatory. No MC with iNOS activity were detected on the contralateral side throughout the entire period of the implant take.

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