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Activity of Gas Transmitters in Vessels of the Anterior Abdominal Wall after Implantation of a Polypropylene Mesh

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The distribution of NO and H_2S in the arterial vessels of the anterior abdominal wall after implantation of a polypropylene mesh was studied by immunohistochemical methods at different stages of healing of the surgical wound in mature male Wistar rats. The presence of enzymes of NO and H_2S synthesis in the wall of arterial vessels of the soft tissues of the anterior abdominal wall has been established. It has been shown that endothelial NO synthase is localized exclusively in the endothelium of both large and small vessels. Cystathionine γ lyase in small vessels is located only in the endothelial lining, whereas in large arteries and vessels of medium caliber, it is located in the endothelium and in myocytes. Inducible NO synthase appears in the artery wall only in animals with implanted polypropylene mesh by day 5 of the postoperative period, reaching the maximum by day 10. The content and localization of cystathionine γ lyase in the vascular wall of sham-operated and experimental rats did not much differ from the control values.

Key Words: *polypropylene implant; arterial vessels of the anterior abdominal wall; nitric oxide (NO); hydrogen sulfide (H_2S)*

In recent years, increasing attention of researchers is attracted to the mechanisms of interaction of implanted polypropylene mesh with soft tissues due to their active use for strengthening of muscle-aponeurotic structures in reconstructive surgery [2,3,7]. Polypropylene mesh is resistant to mechanical stress, is not destroyed by enzymes, and maintains its strength and integrity throughout patient's life. However, the incidence of wound complications after implantation of mesh endoprostheses 5% and higher even in the most experienced surgeons [12], which requires further study of the response of the surrounding tissues to the implanted material. An important role in the inflammatory reactions belongs to vascular endothelium. It is assumed that inflammation and reparative tissue regeneration in the polypropylene mesh implantation zone are associated with gas transmitters, in particular nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S). Their role in both intracellular and intracellular regulation of various physiological processes is well known [4-6].

Our aim was to study the distribution of NO and H_2S in the wall of arterial vessels of the anterior abdominal wall after implantation of a polypropylene mesh at different stages of postoperative wound healing.

MATERIALS AND METHODS

The study was performed on 27 mature Wistar male rats weighing 350±50 g in compliance with the European Convention for the Protection of Vertebrate

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Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The experiments were guided by the requirements of the World Society for the Protection of Animals (WSPA).

Polypropylene mesh Surgipro-SPMM-149 (1×1 cm) was implanted to the anterior abdominal wall of 12 rats. The tissue flap surrounding the implant in experimental rats and a similar tissue site of sham-operated animals (*n*=12) were studied in 1, 5, 10, and 30 days after surgery. The material taken from 3 rats kept under the same conditions as experimental animals served as the control. The rats were decapitated under thiopental anesthesia. The isolated specimens were fixed in 4% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.4) at 4°C for 1 h. Then, they were impregnated in cold 30% sucrose solution on 0.1 M phosphate buffer and 30-40-μ serial cryostat sections were sliced.

On consecutively prepared sections, endothelial (eNOS), inducible NO synthase (iNOS), and cystathionine γ-lyase (CSE) involved in the synthesis of endogenous H_2S were detected. For detection of eNOS and iNOS, the preparations were incubated for 15-17 h at room temperature with rabbit polyclonal antibodies against eNOS and iNOS (1:200; Cayman). For detection of CSE, the sections were successively incubated in 1% normal horse serum (Abcam) for 1 h at room temperature and mouse monoclonal antibodies (1:500; Abcam) at 4°C for 18 h. After washing, the material was incubated with biotinylated goat anti-rabbit IgG antibodies (1:200; Vectastain) for 1 h at room temperature (for detection of eNOS and iNOS) or with biotinylated equine anti-mouse IgG antibodies (1:100; VestorLabs) 2 h and with avidin—peroxidase complex (Vectastain Elite ABC Kit, VestorLabs) for 1 h at room temperature (for detection of CSE). For visualization of the eNOS and iNOS reaction products under a microscope, the sections were incubated in a blue substrate for detection of peroxidase (VIP Substrate Kit, VestorLabs); CSE immunoprecipitate was visualized using diaminobenzidine (DAB Substrate Kit for Peroxidase, VectorLabs). Then, the sections were washed, dehydrated according to standard methods, and embedded in polystyrene.

The numerical density of enzyme-positive vessels of the corresponding diameter from the total number of vessels encountered in each size group (taken as 100%) was calculated in at least 10 sections. The results were processed by methods of variation statistics using Microsoft Excel 2010, BIOSTAT (Biostatistics version 4.03). The significance of differences was evaluated by the Student's *t* test. The values were significant at $p<0.05$.

RESULTS

In control animals, a small number of arterial vessels with a diameter of 20 to 80 μ was visualized on cross-sections of the anterior abdominal wall. eNOS was present in vessels of different diameters and was localized exclusively in the endothelium. The highest intensity of the reaction was noted in large arterial branches (Fig. 1, *a*). In small arteries, the precipitate was usually seen only in a small part of the cells (Fig. 1, *b*, *c*). It should be noted that the intensity of the reaction significantly varied even in vessels of the same diameter: in some arteries, the granular precipitate, a product of the enzymatic reaction, was densely packed and clearly delineated the cells from the surrounding matrix, while in others, the precipitate weakly contours the structural elements of arterial vessels.

According to some researchers, the endothelium constantly secretes a small amount of NO necessary to maintain the basal tone of the arterial vessels. Being a lipophilic molecule, NO can freely pass through cell membranes into surrounding cells. Through myoendothelial contacts, eNOS from the endothelium penetrates into vascular myocytes, where with participation of cGMP reduces calcium levels by activating myosin light chain kinase and thereby causes local vasodilatation [8].

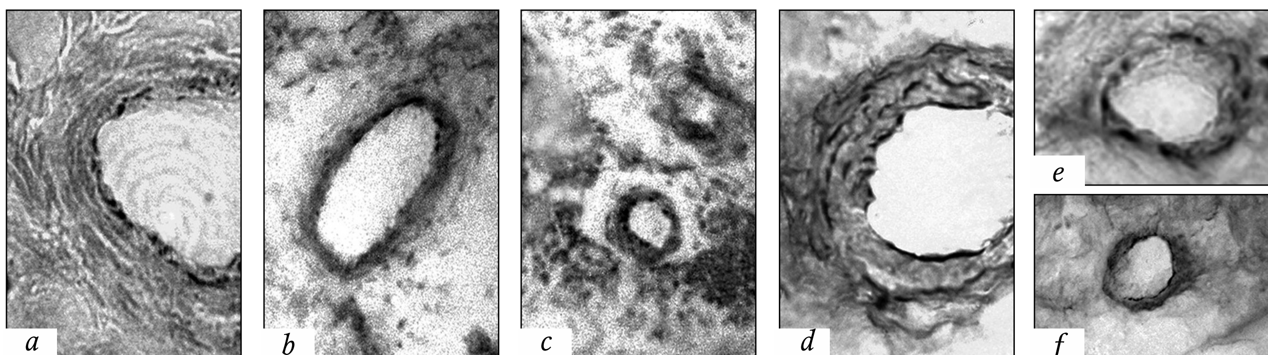

Fig. 1. Blood vessels of different diameters in the anterior abdominal wall in control (intact) animals with positive reaction to eNOS (*a-c*) and CSE (*d-f*), ×100.

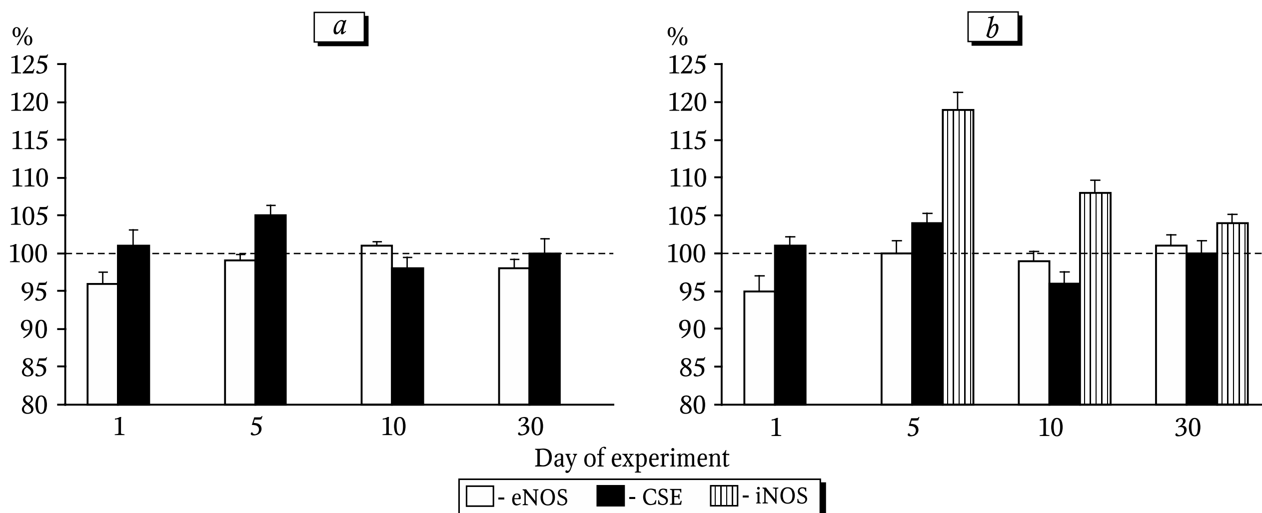

Fig. 2. Number of vessels with positive reactions for eNOS, CSE, and iNOS in sham-operated animals (*a*) and rats with implanted polypropylene mesh (*b*). Values observed in the control group are taken as 100%.

In arteries of control rats, we failed to detect iNOS by immunohistochemical methods. However, expression of CSE involved in the synthesis of endogenous H_2S from L-cysteine in the cardiovascular system was constantly observed in the wall of arterial vessels.

A fine precipitate of CSE in the vascular wall was most often found either exclusively in the endothelium, or only in smooth muscle cells. In most small vessels, CSE expression was seen only in the inner lining. As the diameter of the arterial vessels increased, the reaction product was less and less abundant in endotheliocytes, although in some cases, staining was observed in the endothelium of larger arteries. In some largest vessels, the precipitate was present in myocytes and endothelium simultaneously and staining them in various shades of brown color depending on the density of the precipitate (Fig. 1, *e-f*). The intensity of the reaction product deposition in cells lying in surface and deep layers of the muscle layer was practically the same, which, according to some researchers [11], indirectly indicated that the synthesis of CSE occurs directly in the tunica media.

It should be noted that, along with enzyme-positive arteries, when detecting both eNOS and CSE, a rather large number of vessels are observed in the wall of which the studied enzymes are not found.

In sham-operated animals, a slight decrease in the density of precipitate deposition in the endothelium of the arteries with a positive reaction to eNOS was noted by the end of day 1. It can be hypothesized that damage to the vascular endothelium triggers aggregation of blood cells, while blood coagulation causes spasm of blood vessels near the surgical wound, which contributes to a decrease in the level of NO. Starting from day 5, the endothelium of enzyme-positive arteries did not significantly differ from the control level (Fig. 2, *a*).

CSE^+ vessels did not differ from normal by the end of day 1. However, the intensity of the reaction in the muscular layer of the arteries slightly increased on day 5, and returned to the control by day 10 (Fig. 2, *a*).

Vessels with iNOS activity were not detected in sham-operated rats.

After implantation of mesh endoprostheses, eNOS in arterial vessels located near the mesh did not differ

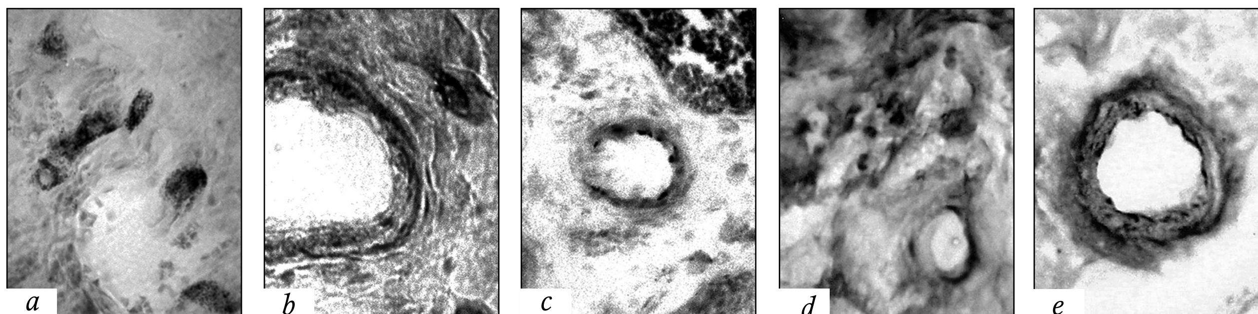

Fig. 3. Localization of iNOS (*a-c*) and CSE (*d*, *e*) in mast cells (*a*) and blood vessels (*b-e*) of rats with implanted polypropylene mesh.

much from those in sham-operated animals during the entire period of the experiment (Fig. 2, *b*). Expression of iNOS was detected only in single mast cells located near the vessels by the end of day 1. It is believed that NO that regulates activity of pro- and anti-inflammatory cytokines through the activation of their receptors, mediates the regulatory effect of mast cells on the nearest microenvironment [10]. The maximum number of $iNOS^+$ mast cells was observed on day 5 (Fig. 3, *a*), which, according to some authors [2], represents a compensatory reaction. During this period, precipitate was observed in some vessels, especially large ones, mainly in their muscle layer. There are published reports that iNOS is expression in the vascular walls during vascular pathology, especially in myocytes of large arteries with developed muscular sheath [9].

By day 10, the number of $iNOS^+$ mast cells markedly decreased (Fig. 2, *b*), while chromophilic precipitate, on the contrary, appeared not only in arterial myocytes, but also in endothelial cells. Moreover, in small arterial vessels, it was detected primarily in the endothelium, while in large vessels, it was seen in muscle cells (Fig. 3, *c*). This picture was observed up to day 30 of the experiment, although the intensity of the iNOS reaction in the vascular wall decreased.

The distribution of CSE in the vascular wall of animals with the implant, in dynamics, did not much differ from that in sham-operated rats (Fig. 2, *b*). On day 5, CSE was more intensively expressed in endothelial cells of arterial vessels ($p<0.05$) and was detected in perivascular leukocytes (Fig. 3, *d*). However, the reaction intensity ($p<0.05$) in the muscular layer of arteries decreased on day 10, and returned to the control values by day 30. It is believed that H_2S in the arterial endothelium plays the role of a primary factor initiating activity of smooth muscle cells through myo-endothelial contacts, being a mediator in the release of vasorelaxants, mainly NO [13,14], without directly affecting the tone of smooth myocytes.

Thus, the results of our study showed the presence of NO and H_2S synthesis enzymes in the wall of arterial vessels of the soft tissues of the anterior abdominal wall. Moreover, eNOS is located exclusively in the endothelium of both large and small vessels. CSE in small vessels is located only in the endothelial lining, while in large arteries and vessels of medium caliber it was found in both the endothelium and myocytes. iNOS in the arterial wall appeared only in animals with implanted polypropylene mesh and only by 5 days of the postoperative period, reaching a maximum of 10 days. Localization and content of CSE in the vascular wall of sham-operated and experimental animals did not differ from the control values.

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