Morphofunctional Basis for the Use of Hyaluronic Acid in the Treatment of Acute Experimental Pancreatitis

G. A. Gavrilenko, A. A. Stadnikov, and B. A. Stadnikov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 4, pp. 464-467, April, 1997
Original article submitted December 22, 1995

Hyaluronic acid exerts beneficial effects on the time-course of morphological and functional changes in the pancreas caused by acute pancreatitis. It reduces damage to the microvasculature, activates formation of connective tissue in necrotic areas, and provides separation of these areas, thus creating favorable conditions for reparative histogenesis of the glandular epithelium.

Key Words: *hyaluronic acid; experimental acute pancreatitis; light microscopy; electron microscopy*

Unfortunately, there are no effective methods for the control of acute pancreatitis determining the course and outcome of the disease [6,7,9]. The relationships between local pathological process and changes in the microvasculature, glandular epithelium, and interstitium of the pancreas are poorly understood [1,5]. Substances of the tissue origin producing pronounced beneficial effects at the level of interstitial connective tissue and substantially improving the regenerative capacity of pancreatic epithelial structures have not been used in the treatment of acute pancreatitis. Meanwhile, there is evidence that naturally occurring carbohydrate biopolymers of connective tissue effectively stimulate reparative histogenesis. For example, hyaluronic acid (HA) [2-4] produces a "cementing" effect on histohematic barriers and reduces vascular permeability and edema. However, the potential utility of HA in the treatment of acute pancreatitis has not been evaluated.

In this study we analyzed cellular and tissue rearrangements occurring in the exocrine pancreas after administration of HA to rats with acute experimental pancreatitis.

Department of Surgery No. 1 and Department of Histology, Cytology, and Embryology, State Medical Academy, Orenburg

MATERIALS AND METHODS

Random-bred albino rats weighing 280-300 g were used. Acute pancreatitis was induced by injecting autologous bile into excretory ducts under pressure and producing microtraumas in selected areas of the pancreas. Laparotomy was performed under Hexenal anesthesia (0.05-0.1 g/kg body weight, intraperitoneally). Operated rats were divided into three groups: untreated controls (group 1, n=13), rats given a subserous injection of HA (4 ml) in the region of the pancreas 24 h after surgery (group 2, n=40), and rats subjected to a second laparotomy 24 h after the first one to inject 4 ml of HA in 0.25% Novocain (1:5, v/v) into the pancreas and after 48 h injected intraperitoneally with 7 ml of this mixture into the pancreatic region (group 3, n=41). Hyaluronic acid was prepared from umbilical cords of human fetuses [8].

Pancreatic tissue specimens were examined by light and electron microscopy 24, 48, and 72 h after surgery or treatment. For light microscopy, paraffin sections were stained with Mayer's hematoxylin and eosin, van Gieson stain, methylene green and pyronin by the method of Brachet, potassium periodate and Schiff's reagent, or with toluidine blue at pH 2.7, 4.0, and 7.3. For electron microscopy, tissue

0007-4888/97/0004-0403$18.00

specimens were fixed in 2.5% glutaraldehyde and postfixed with 1% osmium tetroxide, dehydrated, and embedded in Epon 812—Araldite. Ultrathin sections were cut in an LKB-5 ultratome, contrasted with uranyl acetate and lead citrate, and examined in an EMB-100AK electron microscope.

RESULTS

In group 1 (untreated controls), light and electron microscopy revealed changes characteristic of acute pancreatitis [1,7]. There were signs of destructive processes, which affected parenchymal elements at the periphery and then spreaded along interlobular and intercellular spaces toward the center of the pancreas. Small foci of necrosis were the initial and most pronounced pathological changes. The connective tissue was strongly edematous, and the fibrous structures and the basal matrix showed signs of disintegration. Edema and destruction of capillary endothelial cells led to loosening and partial destruction of the basement membranes with formation of numerous fenestrae and pores (Fig. 1). Capillaries were filled with formed elements and often contained microthrombi. Paranecrotic and destructive changes in pancreatic cells progressively increased under the actions of lysosomal enzymes, hypoxia, and secretory products from disintegrated terminal portions of the gland.

Along with necrotic areas, the pancreas contained large numbers of what appeared to be functionally active acinar cells with large amounts of RNA and secretory peptide granules. Individual pancreatic cells and acini with mucous secretion were seen near the areas of destruction. Seventy-two hours after surgery, small focal changes in the gland showed a tendency toward generalization

After 24 h, in rats treated with HA or HA—Novocain mixture pancreatic morphology was similar to that in control rats. After 48 h, necrotic and edematous areas in treated rats (groups 2 and 3) were 3 to 5 times less extensive than in group 1. Histiocytic macrophages and plasma cells were activated without any sign of erythrocytic diapedesis. Cells of the fibroblast lineage were also activated (as indicated by increased size of the nuclei and nucleoli and by the presence of mitotic figures); the activation started at the periphery of the pancreas and then extended to the interlobular layers of connective tissue. Mucopolysaccharide biopolymers within fibrous structures were also formed from the periphery toward the center, which was confirmed by tests for glycos-

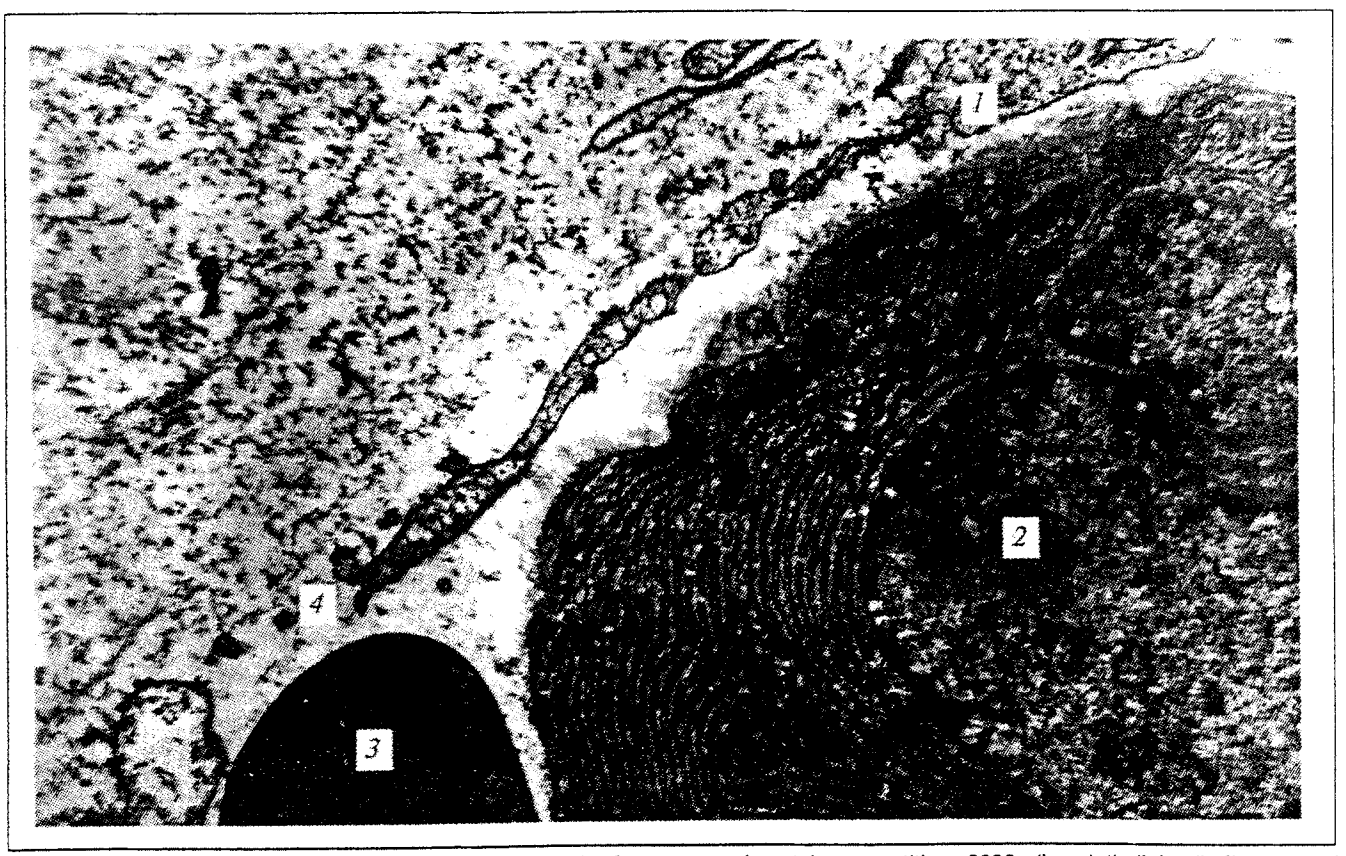

Fig. 1. Ultrastructure of pancreatic blood capillary after 48 h of acute experimental pancreatitis. ×2200. 1) endothelial cell; 2) pancreatic cell; 3) erythrocyte in the pericapillary space; 4) endothelial cell pores.

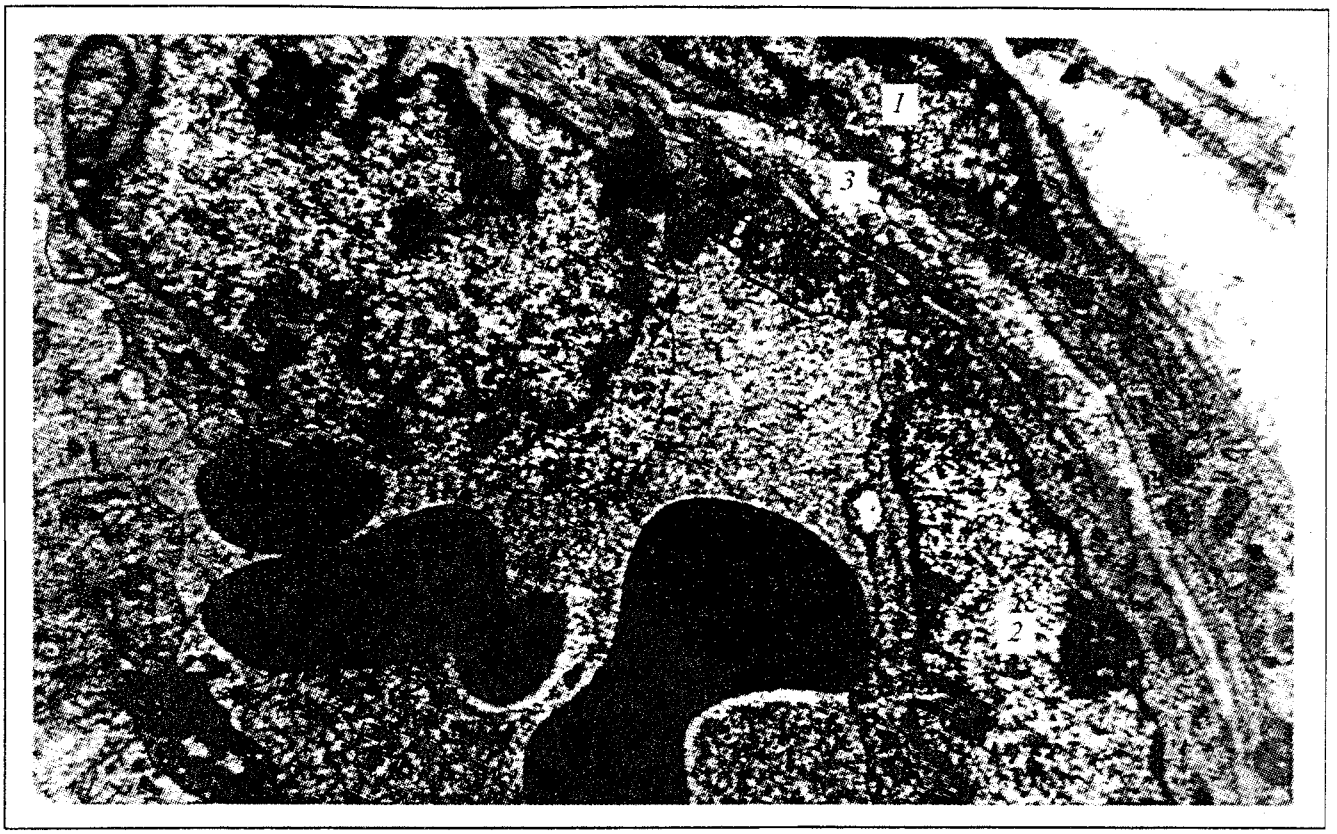

Fig. 2. Ultrastructure of blood capillary wall in juxtanecrotic area after 72 h of acute experimental pancreatitis in a rat treated with hyaluronic acid+Novocain. ×16,000. *1*) poorly differentiated fibroblast; *2*) endothelial cell nucleus; *3*) basal membrane.

aminoglycans and neutral glycoproteins. In group 3, compared with group 2, cambial cells of the pancreatic parenchyma and stroma as well as the vasculature near necrotic areas were better preserved.

After 72 h, the connective tissue elements showed signs of augmented proliferation, which provided favorable conditions for generation of new epithelial strands, tubules, and cysts. Morphofunctional differentiation of connective tissue was accompanied by enhanced synthesis of nonsulfated glycosaminoglycans and neutral glycoproteins. This led to delimitation of necrotic areas and reduction in pancreatic edema. It should be emphasized that cells of the pancreatic excretory ducts became more resistant to necrobiotic processes after local administration of HA.

After 24 and 48 h, ultrastructural studies revealed less intense destruction of collagen fibrils and much less pancreatic cells with signs of autolysis. Capillary endothelial cells were less edematous and had fewer fenestrae and pores. Morphological integrity of capillary endothelium was preserved even in areas adjacent to necrotic foci (Fig. 2). The basal membranes were clearly defined, and their amorphous and fibrillary components were adequately organized. Perivascular cells were activated, undergoing transformation into functionally active fibroblasts.

After 48 and 72 h, oval, elongated, and round fibroblasts were seen. They had a well-developed rough endoplasmic reticulum. Round and oval fibroblasts were filled with numerous ribosomes and polysomes and were mitotically active. Proliferating epithelial cells formed contacts with each other, sometimes forming structures resembling excretory ducts.

From these observations we have concluded that HA has beneficial effects on stromal and parenchymal elements of the pancreas. Hyaluronic acid promoted preservation of the pancreatic "capillaries—acinar cell—interstitial connective tissue" functional microregion, stabilized basal membranes of endothelial cells, activated formation of connective tissue in damaged areas, and caused separation of these areas from the rest of the pancreas, thus creating favorable conditions for the repair of the glandular epithelium.

REFERENCES

1. G. N. Akzhigitov, *Acute Pancreatitis* [in Russian], Moscow (1974).
2. G. A. Beburishvili, *Eksper. Khir.*, No. 4, 91-93 (1968).
3. V. V. Vinogradov, *Byull. Eksp. Biol. Med.*, **45**, No. 5, 111-115 (1958).

4. V. S. Kasavina, V. M. Lirtsman, and L. I. Muzykant, *Eksper. Khir.*, No. 4, 12-15 (1959).
5. V. M. Lashchevker, *Vestn. Khir.*, No. 1, 93-94 (1976).
6. V. S. Mayat, Yu. P. Antonov, and G. A. Buromskaya, *Khirurgiya*, No. 10, 5-10 (1983).
7. V. S. Savel'ev, Yu. V. Ognev, and V. M. Buyanov, *Acute Pancreatitis* [in Russian], Moscow (1983).
8. L. G. Smirnova, *Zh. Mikrobiol.*, No. 10, 52-56 (1951).
9. A. A. Shalimov, S. A. Shalimov, V. S. Zemskov, and S. E. Podiryatov, *Khirurgiya*, No. 4, 3-6 (1979).

Interaction of Microorganisms with Enterocytes After Oral Administration of Pesticides

I. M. Baibekov, B. Z. Kasymov, and T. A. Sagatov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 4, pp. 467-470, April, 1997
Original article submitted November 22, 1996

Intragastral administration of the pesticides Sumi-alpha and Omait to rats significantly increases the number of parietal microorganisms in the jejunum, ileum, and particularly in the cecum. Electron microscopy shows that parietal microorganisms invade goblet cells during secretion and then enter prismatic cells via the lateral plasma membrane. The number of parietal microorganisms entering enterocytes after Sumi-alpha is higher than after less toxic Omait, reaching the maximum 5 h after administration.

Key Words: *parietal microorganisms; enterocytes; pesticides*

Pesticides produce various adverse effects on animals and humans. Worldwide, about 4 mln tons of pesticides are used in each year, but only about 1% of them reach the target. Annual record of pesticide poisoning is almost 500,000 cases [5,7,8]. However, pesticides are indispensable for modern agriculture.

A vast majority of pesticides have adverse effects after ingestion with water or food. Ingested pesticides directly affect the epithelial lining of mucous membranes and so-called parietal microorganisms (PM) of the digestive tract [1-3]. Interactions of PM with mucosal structures of the small intestine, from which most nutrients [8] and pesticides are absorbed, are analyzed in the present study.

MATERIALS AND METHODS

Using optical and transmission electron microscopy, interactions of PM with enterocytes of the jejunum, ileum, and cecum in Wistar rats (>100 g) were studied after oral administration of the moderately toxic pesticide Omait (Juniroll Chemicals; LD_{50}=815 mg/kg for rats) or the highly toxic pesticide Sumi-alpha (Sumimoto Chemicals; LD_{50}=75 mg/kg for rats) in a dose of 1.0 ml/100 g body weight [4]. Both pesticides are supplied as a concentrated emulsion.

The pesticides were administered intragastrally via a gastric tube. Intact rats served as controls. Five hours, and 1, 3, 7, 15, and 30 days after administration the rats were decapitated, and intestinal specimens were collected. They were fixed in 2.5% glutaraldehyde, postfixed 1% osmium tetroxide, double stained, embedded in Epon—Araldite, and processed for electron microscopy (Hitachi H-600). Semithin sections stained with methylene blue—fuchsin were examined by light microscopy, with measurement of the relative volumes of PM (expressed in %) in areas located 40-45 μ from the plasma membranes of enterocytes [1,2].

RESULTS

In the control rats, the occurrence of PM was the highest in iliac crypts (2.5±0.1%) and cecum (5.2±0.1%). In pesticide-treated rats, PM were present in

Laboratory of Pathological Anatomy, V. Vakhidov Research Center of Surgery, Ministry of Health of Republic of Uzbekistan, Tashkent

0007-4888/97/0004-0406$18.00