EFFECT OF THE PROTEINASE INHIBITOR GORDOX ON THE PANCREAS IN EXPERIMENTAL PANCREONECROSIS

A. S. Kanayan and T. A. Markaryan

UDC 616.37-002.4-085.355:577.152.34.042.2]-036.8

KEY WORDS: experimental pancreonecrosis; pancrease; gordox.

In the last decades much experimental and clinical research has been done on the use of proteinase inhibitors in the treatment of pancreatitis. These preparations have been shown to reduce mortality [6, 9-11, 14], to shorten the period of hospital treatment of edematous pancreatitis, but to have no significant effect on the outcome of destructive pancreatitis [3, 4, 7]. With time doubts have been expressed on the desirability of the use of proteinase inhibitors in pancreatitis [2, 12], and in some countries these substances are no longer used [1, 13].

The object of this investigation was to study the effect of the new antienzyme preparation gordox (from Gedeon Richter, Hungary) on the course of the pathological process in the pancreas in experimental pancreonecrosis.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats (71 animals) weighing 180-200 g. Pancreonecrosis was induced by cooling the splenic portion of the pancreas (2/3 of the weight of the organ) with ethyl chloride [5]. In group 1 (31 rats) gordox was injected intraperitoneally in a dose of 750 units/100 g body weight twice during the first day — immediately after injury to the pancreas and 2 h later; subsequently a single dose of the drug was given daily. The remaining 40 rats served as the control (group 2). Animals of both groups were decapitated 1, 3, and 6 h after the beginning of the experiment and on the 1st and 3rd days, after preliminary starvation for 18-24 h. Amylase activity in the blood was determined by Carraway's method and the pancreas was fixed in 10% Lillie's buffered formalin and embedded in paraffin wax. Paraffin sections 4 μ thick were stained with hematoxylin and eosin, by the Jenner—Giemsa method, and for fibrin and zymogen granules by the Gram—Weigert method.

TABLE 1. Blood Amylase Level and Quantity of Peritoneal Exudate in Rats with Pancreonecrosis Treated and Not Treated with Gordox (M ± m)

Time of investigation	Group of animals	Amylase, units/ml	P	P_1	Volume of exudate, ml	P_1
1 h	Intact	1636,3±506,07	—	—	—	—
	Control	8194,7±1446,45	<0,01	—	1,67±0,16	
	Experiment	6461,5±497,86	<0,001	>0,2	3,86±0,48	<0,01
3 h	Control	14545,0±912,1	<0,001	—	4,3±0,24	—
	Experiment	5825,6±551,22	<0,001	<0,001	5,3±0,86	>0,2
6 h	Control	13910,0±497,86	<0,001	—	5,6±0,16	—
	Experiment	3651,22±275,6	<0,02	<0,001	2,4±0,96	<0,02
1 day	Control	7759,5±1003,99	<0,001	—	2,4±0,77	—
	Experiment	2175,96±245,69	>0,2	<0,001	—	—
3 days	Control	3429,3±393,5	<0,05	—	1,83±0,81	—
	Experiment	1962,66±368,5	>0,5	<0,05	—	—

Legend. P) Relative to intact animals; P_1) relative to control.

Central Research Laboratory, Erevan Postgraduate Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkistov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol 95, No. 4, pp. 60-63, April, 1983. Original article submitted October 26, 1982.

 0007-4888/83/9504-0478$07.50

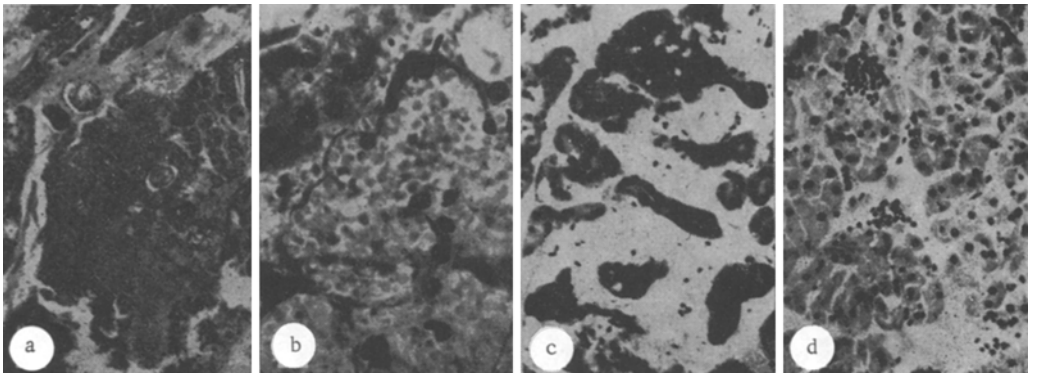

Fig. 1. Pancreas of rats of control and experimental group 3 h after cooling. a) Massive hemorrhage into gland (control). 32 ×; b) erythrocytes fill lumen of acinus (control). 320 ×; c) imbibition of splenic segment with blood, extravascular erythrocytes undergoing hemolysis (control rat). 128 ×; d) microfocal hemorrhages into gland in rat of group 1. 128 ×; hematoxylin and eosin.

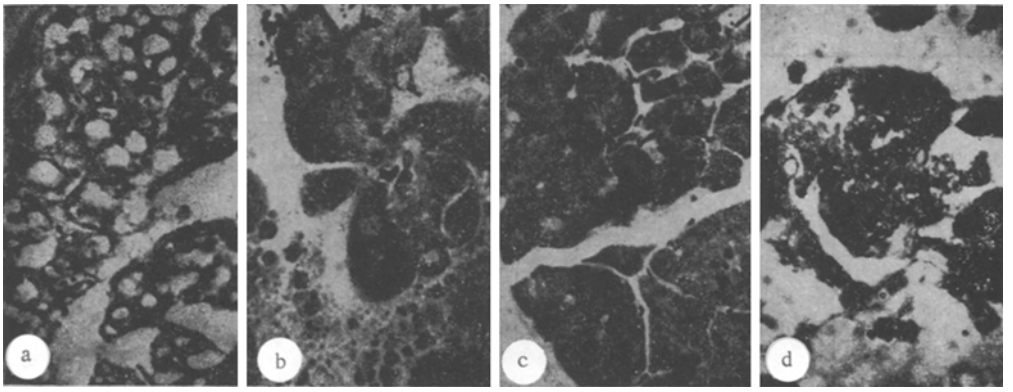

Fig. 2. Mosaic pattern of pathological changes in pancreatic acinar cells in rats of control group. a) Lysis of apical parts of acinar cells with dilatation of lumen of acini. 128 ×; b) focal necroses in basal cytoplasm of acinar cells, with cell debris in interacinar space. 320 ×; c) lobules formed by diffusely oxyphilic acinar cells deprived of zymogen granules. 128 ×; d) Diffuse zymogen granules throughout territory of acinus. 320 ×. Staining: a-c) with hematoxylin and eosin, d) by Gram—Weigert method.

EXPERIMENTAL RESULTS

The quantity of peritoneal exudate in the control rats reached a maximum 6 h after the experiment began (Table 1), but in animals receiving gordox it was much less than in the control, and by the end of the 1st day no exudate could be discovered. Replacement of hemorrhagic by serohemorrhagic exudate in the control occurred after 24 h, whereas in rats of the experimental group the exudate was serohemorrhagic in character after the first hour of the experiment.

The effect of gordox on the dynamics of the blood amylase in the experimental rats was highly positive (Table 1). As early as after 3 h the enzyme level was significantly below the control value ($P < 0.001$) and after 24 h it returned to normal.

Foci of steatonecrosis appeared in the control animals after 3 h in the pancreas and adjacent parts of the omentum, and after 6 h they became disseminated in character, with the widest spread and greatest intensity toward the end of the 1st day of the experiment. Treatment with gordox limited the spread of steatonecrosis to the pancreas from the omentum. Macroscopically the splenic part of the pancreas in the control rats 3-6 h after the beginning of the experiment was in a state of hemorrhagic necrosis, whereas the duodenal part was in a

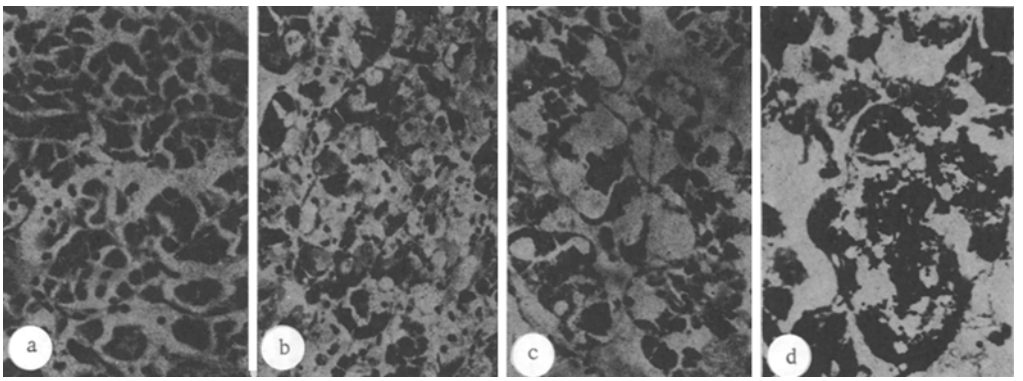

Fig. 3. Pancreas of group 1 rat 3 h after cooling. a) Loss of structural pattern of acini (hematoxylin and eosin); b) pronounced vacuolation of cytoplasm and necrosis of individual acinar cells (Jenner–Giemsa); c) periacinar edema (hematoxylin and eosin); d) "amputation" of apical portion containing zymogen granules into lumen of acinus (Gram–Weigert). Magnification: a-c) 128 ×, d) 320 ×.

state of vitreous edema. After 1 h the hemorrhagic necrosis was replaced by parenchymatous, and the hemorrhages had become focal in character. Under the influence of gordox, infiltration of the splenic segment with blood was less marked and focal hemorrhages could be detected only until 24 h after the beginning of the experiment, and later none were found. Vitreous edema was the predominant feature in the animals of this group.

Histological examination of the zone of injury in the control rats 1 h after the beginning of the experiment revealed pronounced interlobular and interacinar edema. Considerable microcirculatory disturbances were observed: congestion and stasis in the capillaries and multiple massive hemorrhages into the stroma and parenchyma of the gland (Fig. 1a, b). After 3 h imbibition of blood by the splenic segment was observed (Fig. 1c) and multiple microthrombi were present in the interacinar capillaries, accompanied by thrombosis of the veins and saturation of the arterial walls with plasma. After 6 h no microthrombi could be detected, but as before a large part of the organ was occupied by hemorrhages. Later (24 h) hemolysis of the erythrocytes occurred and the recent hemorrhages were focal in character. In the animals of group 1 edema of the stroma also was pronounced, but the edema fluid stained poorly. Hemorrhages into the stroma and parenchyma were tiny (Fig. 1d) and infrequent. After 6 h most of the extravascular erythrocytes were in a state of hemolysis. Congestion and stasis were slight, and solitary microthrombi only could be found after 6 h. After 24 h edema of the pancreas was slight in degree and foci of extravasation of erythrocytes were solitary and small. The histological structure of the pancreas in the control group of rats 1 h after cooling was disturbed on account of loss of the normal structural pattern of the acini. The lumen of some acini was dilated as a result of lysis of the apical part of the acinar cells (Fig. 2a). Acini formed by oxyphilic cells deprived of zymogen were numerous (Fig. 2b). In the basal portion of some acinar cells round structures bounded by a membrane and containing oxyphilic or basophilic granular contents (evidently autophagosomes) were found. Some acini had diffusely distributed zymogen granules (Fig. 2d), released into the stroma. The nuclei of the acinar cells were pycnomorphic and hyperchromic. Later (after 3 and 6 h) vacuolation and lysis of the acinar cells increased and foci of coagulation necrosis were found. Necrobiotic changes extended to the boundary zones of the pancreas. After 24 h the whole of the splenic segment was in a state of coagulation necrosis, with a sluggish perifocal inflammatory reaction. By this time activation of fibroblasts was noted, and was particularly marked on the 3rd day of the experiment.

Under the influence of gordox disturbance of the structural pattern of the acini was very pronounced (Fig. 3a). Often "amputation" of the apical portions of the acinar cells was observed: Their basal portions were flattened and strongly basophilic (Fig. 3d). Lysis of the acinar cells was observed, with preservation of the outlines of the acina, and the formation of large vacuoles filling most of the cytoplasm of the acinar cell was more in evidence (Fig. 3c). Autophagosomes were found much more often than in the control. Periacinar edema (Fig. 3c) — "detachment" of acinar cells from the basement membrane with the formation of a pale, finely granular zone [8] — was a frequent discovery, whereas this phenomenon was not found in the control. Nuclei of the acinar cells were much better preserved. The changes

in the acinar cells described above were observed 1 h after the beginning of the experiment and gradually increased, in the course of which the vacuolar transformations of the cytoplasm of the acinar cells gave way to coagulation necrosis.

Hence, on the 1st and 3rd days of the experiment the zone of damage to the pancreas in the animals of both groups was in a state of coagulation necrosis. However, treatment with gordox was accompanied by a more marked perifocal inflammatory reaction, with infiltration of zones of necrosis in the parenchyma and adipose tissue with polymorphs. In the zone of necrosis the stroma and efferent ducts were well preserved and interacinar capillaries, forming vascular buds, could often be seen.

Early administration of gordox in experimental pancreonecrosis thus prevents release of pancreatic enzymes into the blood stream, prevents dissemination of foci of steatonecrosis, and considerably improves the microcirculation in the pancreas. Under the influence of gordox coagulation necrosis of the gland develops against a background of edematous pancreatitis, bypassing the stage of hemorrhagic pancreonecrosis. Gordox stimulates the perifocal inflammatory reaction.

LITERATURE CITED

1. P. Banks, Pancreatitis [Russian translation], Moscow (1982).
2. V. M. Lashchevker, Acute Pancreatitis [in Russian], Kiev (1978).
3. V. M. Mavrodi, "Diagnosis and treatment of acute pancreatitis," Author's Abstract of Candidate's Dissertation, Krasnodar (1970).
4. V. A. Nikolaev, "Combined treatment of different forms of acute pancreatitis," Author's Abstract of Candidate's Dissertation, Moscow (1972).
5. P. S. Simavoryan, "Some aspects of the pathophysiology of pancreatitis," Candidate's Dissertation, Erevan (1973).
6. V. I. Filin, Acute Diseases and Injuries of the Pancreas [In Russian], Leningrad (1982).
7. V. V. Chaplinskii and A. I. Gnatyshak, Acute Pancreatitis [in Russian], Moscow (1972).
8. M. Anderson and W. Schiller, Am. J. Surg., 115, 118 (1968).
9. H. Howat, Prescribers' J., 9, 82 (1969).
10. C. Imrie and M. Mackenzie, Digestion, 22, 32 (1981).
11. A. McCutcheon and D. Race, Ann. Surg., 158, 233 (1963).
12. J. Trapnell, Clin. Gastroenterol., 1, 147 (1972).
13. J. Trapnell, C. Rigby, C. Talbot, et al., Br. J. Surg., 61, 177 (1974).
14. T. White, Pancreatitis, London (1966), pp. 140-186.