Free Radical Oxidation and Activity of Enzymes Catalyzing Biotransformation of Xenobiotics in the Liver of Rats with Experimental Acute Pancreatitis

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The development of experimental acute pancreatitis in rats was accompanied by intensification of lipid peroxidation, activation of superoxide dismutase and glutathione reductase, inhibition of glutathione transferase, and deceleration of aniline N-hydroxylation by cytochrome P-450 in the liver. Antioxidants inhibited lipid peroxidation and antioxidant enzymes, but did not prevent inhibition of detoxifying enzymes.

Key Words: *pancreatitis; liver; lipid peroxidation; antioxidant protection; detoxifying enzymes*

Clinical observations indicate that severe acute pancreatitis (AP) of various etiologies leads to liver damages. On the other hand, hepatic dysfunction promotes progression of AP [1,3,4]. The pathogenetic mechanisms of liver insufficiency during AP are poorly understood. Previous studies suggest that activation of free radical oxidation and damage to hepatocyte membranes are involved in the pathogenesis of liver insufficiency [9]. Therefore, hepatoprotectors possessing antioxidant properties should be added to standard therapy of AP [4].

For evaluation of the role of free radical processes in the pathogenesis of liver diseases, we studied lipid peroxidation (LPO) and activities of antioxidant and detoxifying enzymes in hepatocytes of animals with experimental AP. Commercial preparations Ubiquinone Compositum and Thioctacid 600T containing radical scavengers ubiquinone and thioctic acid trometamol salt, respectively, were used as antioxidants.

MATERIALS AND METHODS

Experiments were performed on 57 outbred albino rats. The animals were anesthetized with 12.5 mg calipsol, and the bile duct was ligated on both sides from the pancreas. Secretion was stimulated with 0.005 mg 0.05% neostigmine methylsulfate (subcutaneously), 0.2 ml 96% ethyl alcohol and 2 ml meat broth (though a gastric tube). The duct was drained with a PVC tube to preserve normal passage of the bile. Sham-operated rats served as the control.

The dynamics of pancreatitis was studied in preliminary experiments. Plasma amylase (Lachema kits) and lipase activities were measured on days 2 and 5 after AP modeling [5]. Histological examination of the pancreas was performed on days 2, 5, and 10. The tissue was fixed in 12% formalin and embedded into paraffin and Epon. The slices were stained with hematoxylin and eosin, methylene blue, and acid fuchsin.

Some animals with AP received antioxidants, and others were untreated. Thioctacid 600T and Ubiquinone Compositum were injected intraperitoneally in daily doses of 0.25 and 0.01 ml, respectively, for 5 days. Autopsy was performed on day 5 under ether anesthesia. The liver was perfused with 1.15% KCl and immediately frozen. The content of conjugated dienes (CD) [7] and specific activity of superoxide dismutase (SOD) [11] were measured in liver tissue homogenates. The rate of NADPH-dependent LPO was determined in mitochondrial and lysosomal fractions after centrifugation at 12,000*g* for 20 min [2]. Cytochrome P-450 activity was estimated in the mi-

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TABLE 1. Metabolic Parameters of Rat Liver under Normal Conditions and 5 Days after AP Modeling (per mg protein, *M*±*m*)

Parameter	Control	AP	
		no treatment	treatment with antioxidants
CD content, ΔE_{233}	0.0116±0.0007	0.0188±0.0017*	0.0147±0.0018
SOD specific activity, arb. U	134.47±5.52	210.69±13.73*	158.07±12.93
Glutathione reductase activity, μmol/min	0.952±0.077	1.297±0.091**	1.104±0.117
NADPH-dependent LPO rate, nmol malonic dialdehyde/min	2.555±0.187	3.511±0.398**	2.286±0.288
Glutathione transferase activity, μmol/min	1.002±0.069	0.783±0.078**	0.6780±0.0527*
Cytochrome P-450 N-hydroxylase activity, arb. U	0.074±0.005	0.043±0.004*	0.047±0.005*

Note. *p<0.001 and **p<0.05 compared to the control.

crosome-enriched fraction (centrifugation at 30,000*g* for 60 min) by the rate of aniline N-hydroxylation [10]. Glutathione reductase and glutathione transferase activities were measured in the supernatant [5]. The results were analyzed by Student's *t* test.

RESULTS

Plasma amylase (84.4±14.2 *vs*. 12.9±0.7 cat in the control, p<0.001) and lipase activities (30.7±8.6 *vs*. 73.0±1.7 μmol/min/liter, p<0.05) increased 2 days after modeling of AP. Histological examination revealed interstitial edema of the pancreas, reduced number of zymogenic granules in pancreatic cells, and focal steatonecrosis of the mesentery. On day 5 lipase activity returned to normal, while amylase activity remained high (27.1±13.3 cat, p<0.01). The pancreas was characterized by interstitial edema. Small pancreatic cells containing few zymogenic granules were characterized by pronounced atrophy (vacuolization of the cytoplasm, irregular distribution of the nuclear chromatin, and karyorrhexis); focal necroses and hemorrhages were seen. The interstitial layer was diffusely infiltrated with intensively phagocytizing mononuclear leukocytes. Acinar atrophy and hypertrophy of the connective tissue were revealed on day 10. Thus, this experimental design provided the development of AP in rats. Destructive processes in the pancreas were most pronounced on day 5 after surgery.

At this term the content of CD, rate of NADPH-dependent LPO, and activities of SOD and glutathione reductase in the liver of untreated rats with AP were higher than in controls. By contrast, cytochrome P-450 and glutathione transferase activities decreased in these animals. In rats with AP receiving antioxidant preparations the intensity of LPO and activities of antioxidant enzymes did not differ from the control, while the rate of aniline N-hydroxylation and glutathione transferase activity decreased (Table 1).

These data attested to intensification of free radical oxidation in the liver of untreated rats with AP. These processes manifested in acceleration of NADPH-dependent LPO in mitochondrial membranes and accumulation of CD. Activity of antioxidant enzymes SOD and glutathione reductase increased, while the rate of aniline N-hydroxylation by cytochrome P-450 and glutathione transferase activity decreased. Previous studies showed that cytochrome P-450 activity in the liver decreases during experimental AP [6]. Deceleration of substrate utilization by enzymes of phase I and II xenobiotic biotransformation [8] attested to severe impairment of hepatic detoxifying functions and, probably, contributes to the development of liver insufficiency.

The question arises as to whether the changes in detoxifying enzyme activity are associated with damages caused by free radicals, *e.g.*, LPO metabolites. Our findings indicate that antioxidants inhibit lipid peroxidation and antioxidant enzymes in the liver, but do not prevent deceleration of aniline N-hydroxylation by cytochrome P-450 and decrease in detoxifying enzyme activity. Thus, dysfunction of these enzymes is not related to the direct effect of free radicals produced in the liver. Our findings suggest that oxidative stress is not the major pathogenetic mechanism underlying the development of liver insufficiency during AP.

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