Generation of Free Oxygen Radicals in the Pathogenesis of Experimental Acute Reflux Pancreatitis

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In dogs with acute reflux pancreatitis specific activity of superoxide dismutase in the pancreas rapidly increased (over 15 min). Activation of this enzyme by free oxygen radicals was demonstrated in *in vitro* experiments. Treatment with allopurinol prevented activation of superoxide dismutase. Our results indicate that superoxide ions are generated in the pancreas at the early stage of acute reflux pancreatitis due to activation of xanthine oxidase.

Key Words: *acute pancreatitis; superoxide dismutase; allopurinol; free oxygen radicals*

The development of acute pancreatitis (AP) is associated with the release of free oxygen radicals (FOR) from damaged pancreatic acinar cells. Technical difficulties in detecting FOR hamper detailed investigations of the dynamics and role of this process in the pathogenesis of AP [3,4,6].

Here we evaluated the intensity of FOR generation at the early stage of AP by activation of superoxide dismutase (SOD), the enzyme scavenging superoxide ions $O_2^{\bullet -}$.

MATERIALS AND METHODS

Experiments were performed on 10 dogs weighing 6-20 kg. The animals were anesthetized with 5 mg/kg calipsol and 10 mg/kg sodium thiopental. AP was modeled by injecting bile with 15% ethyl alcohol into the pancreatic duct. The pancreas was perfused through the inferior pancreaticoduodenal artery with 0.9% NaCl. Blood-free tissue specimens were taken for biochemical assay. The control group included animals without AP narcotized with the same drugs. Intravenous infusion of xanthine oxidase inhibitor allopurinol (10 mg/kg) was started 15 min before AP modeling and terminated 15 min after injection of bile-alcohol mixture into the pancreatic duct [4].

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For measuring specific SOD activity, the tissue was homogenized in 0.1 M Na-phosphate buffer and the enzyme was preparatively isolated [7]. SOD activity was evaluated by inhibition of epinephrine autoxidation [7]. The optimal concentration of SOD was selected so that 2-fold dilution of the solution 2-fold decreased enzyme activity. The results of 2 parallel measurements were averaged. Protein concentration was measured by the biuret method. SOD activity was expressed in relative units (rel. units) and calculated per 1 mg protein. Electrophoresis of purified SOD was performed by the method of Ornstein and Davis [9]. The gels were stained with Coomassie brilliant blue G 250 and densitometried on a UT 7608 device. Unstained gels were cut into fragments (width 1.5 mm), proteins were extracted with distilled water, and specific SOD activity in the extract was measured. Solutions enriched with $O_2^{\bullet -}$ radicals were obtained by UV irradiation of distilled water for 10 min (DDS-30 lamp).

The results were analyzed by Mann—Whitney test.

RESULTS

Electrophoresis of the enzyme preparatively isolated from the pancreas of control animals showed 8 bands (Fig. 1). SOD activity was detected in fractions 1-8 (except for fraction 2, 11% total protein). Therefore, about 90% of preparatively isolated protein was pre-

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sented by SOD. This procedure allowed us to estimate specific SOD activity with high accuracy.

SOD activity in control dogs varied from 62 to 1069 rel. units (490±153 rel. units). In animals with AP specific SOD activity in the pancreas increased by several times (1082-7652 rel. units, mean 4522±2331 rel. units, $p<0.05$). This attests to rapid activation of the enzyme scavenging superoxide ions at the early stage of AP, which is probably a response to FOR release.

To verify this assumption we compared SOD activities in distilled water and in water enriched with $O_2^{\bullet}$ radicals. In UV-irradiated water specific activity of the enzyme increased 2-fold for 20 min (from 615 to 1200 rel. units). Therefore, sharp activation of SOD over the first 15 min after AP modeling can be (at least partially) explained by generation of FOR in the damaged pancreatic tissue and can serve as a marker of this process.

To identify the source of superoxide ions in the pancreatic tissue, we assayed the dynamics of SOD activity in 3 animals intravenously injected with allopurinol. Activation of SOD was absent or insignificant 15 min after AP modeling (Table 1), which attested to FOR generation due to activation of xanthine oxidase that catalyzes oxidation of hypoxanthine into xanthine with the formation of $O_2^{\bullet}$. Our results are consistent with published data (E. Folch *et al*. [4]) on irreversible conversion of xanthine dehydrogenase into xanthine oxidase in experimental pancreatitis.

Radicals can be generated by xanthine oxidase localized on the luminal surface of endothelial cells of microvessels and by acinar cells [1,6]. G. Telek *et al.* [11] used cerium chloride to visualize cells generating H_2O_2 and $O_2^{\bullet}$. It was shown that acinar cells act as the primary source of FOR in the pancreatic tissue 1 h after AP modeling. Then, neutrophilic leukocytes are activated. Unfortunately shorter time intervals were not analyzed. Hence, FOR release occurring within the first few minutes after induction of AP is the very early event in the pathogenesis of this disease. FOR trigger various processes: they induce spasm of arterioles [2], activate nuclear factor kappa B, the key regulator of cytokine induction [5,10], and produce direct damaging effects on intracellular structures [12]. Some authors reported that FOR play the major role in activation of zymogens for proteolytic enzymes [8].

TABLE 1. SOD Activity in Animals with AP Receiving Allopurinol (rel. units)

Animal	Before AP	Fifteen minutes after the incidence of AP
1	319	309
2	699	624
3	1069	1121

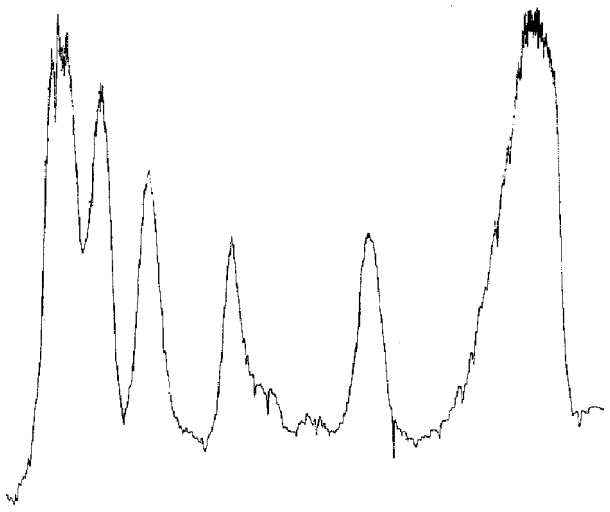

Fig. 1. Densitogram of SOD fractions from canine pancreas.

The early stage of AP proceeds very rapidly and at later stages of the disease antioxidants are little effective [6]. The search for new medicinal preparations that block the pathways activated by FOR rather than trap and scavenge these particles is of considerable importance.

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