GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Antiradical Effect of Allopurinol at Early Stages of Experimental Acute Pancreatitis

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Pretreatment with allopurinol partly prevented generation of free oxygen radicals in the pancreas of dogs with experimental acute pancreatitis. Allopurinol holds promise for the prevention of acute postoperative pancreatitis.

Key Words: *allopurinol; acute postoperative pancreatitis; free oxygen radicals*

Free radicals are involved in all pathogenetic mechanisms of acute pancreatitis (AP) [4] from the early stage of the disease [2,5,9] to its systemic complications.

There are several sources of free oxygen radicals (FOR) in the acinar cell and microvessels of the pancreas. Superoxide in the microcirculatory bed is produced by xanthine oxidoreductase, vascular NADPH oxidase, cyclooxygenase, nitric oxide (NO) synthase, and mitochondrial electron transport chains [11]. The interaction of superoxide with NO results in generation of highly toxic peroxynitrite [10]. The peroxidase reaction catalyzed by superoxide dismutase (SOD, extracellular isoform in the vascular bed) leads to generation of hydroxyl radicals [7]. Mitochondrial electron transport chains, NO synthase, xanthine oxidoreductase, and cytosolic isoform of SOD are the sources of FOR in the acinar cell. It remains unclear which enzyme sources of FOR initiate the development of AP. Several authors reported that xanthine reductase plays the leading role in this process [4]. However, contradictory results were obtained in using a xanthine oxidoreductase inhibitor allopurinol (ALP) for the prevention of experimental AP [3,8]. It should be emphasized that ALP inhibits superoxide generation only at the molybdenum site. Superoxide can be produced at the flavin site due to enzyme activity of NADH oxidase [6]. ALP at concentrations >500 μM acts as a hydroxyl radical scavenger. This specific feature is important at the early stages of AP. Hydroxyl not only causes direct damage, but also acts as a messenger in the synthesis of various signal molecules mediating the systemic inflammatory response. Therefore, scavenging of this radical can be used for the prevention of AP.

Here we studied antiradical activity of ALP in the early stage of experimental AP.

MATERIALS AND METHODS

The liver of intact albino rats and pancreas of dogs served as the tissue source of SOD. AP was modeled in 16 dogs weighing 6-30 kg [1]. The animals received intravenous bolus infusion of ALP in a dose of 10 mg/kg. Infusion started 15 min before the experiment and continued for 15 min after the initiation of AP.

Control pancreas samples were taken before AP. Specific activity of tissue SOD was measured [1]. The extracellular and cytosolic isoforms of this enzyme were identified by separation on Sephadex 32. Electrophoresis of purified SOD was performed

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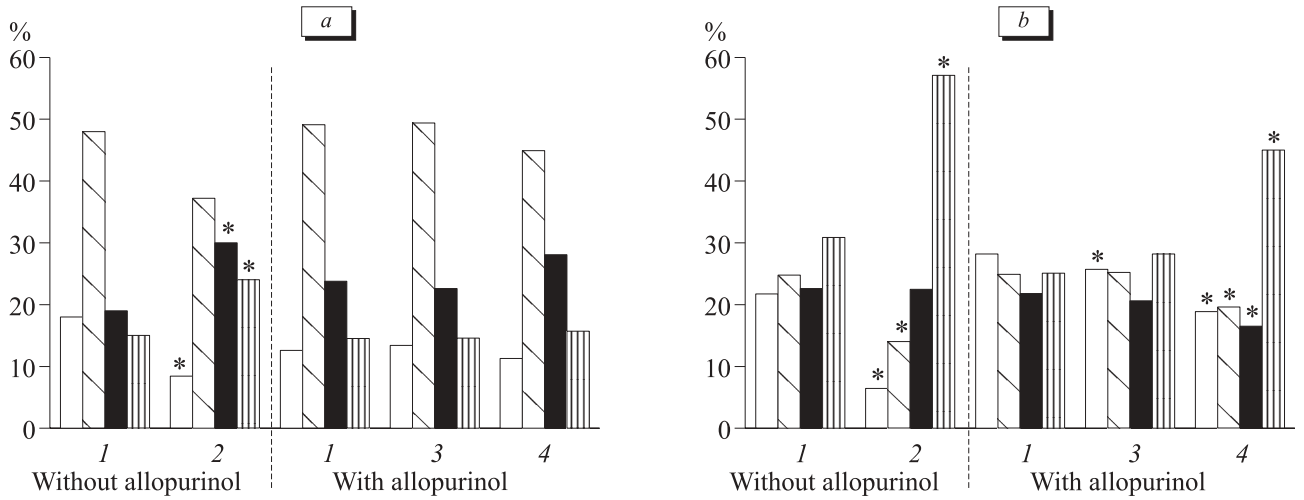

Fig. 1. Fractional composition of extracellular (*a*) and cytosolic SOD (*b*) in the pancreas of dogs before (*1*) and 15 min after modeling experimental acute pancreatitis (*2-4*). Light bars, fraction 1; shaded bars, fraction 2; dark bars, fraction 3; vertical shading, fraction 4. Here and in Fig. 2: **p*<0.05 compared to the control.

by the method of Ornstein and Davis. The gels were stained with Coomassie brilliant blue G-250 and densitometried. The amount of individual fractions was estimated by the method of peak weighing.

Experiments with *in vitro* activation of SOD by FOR were performed using 3 samples of purified SOD from liver tissue of intact rats, xanthine oxidase (ICN), and NADH. Preparations of SOD containing 3.0 mg SOD were incubated with 10 mU xanthine oxidase and 0.4 mM NADH in Tris-HCl buffer (0.1 M, pH 7.4). The final volume of this mixture was 2.5 ml. ALP in a final concentration of 130 μM was added to the incubation medium before xanthine oxidase administration. The enzyme was repeatedly isolated from the incubation medium immediately after addition of xanthine oxidase and by the 30th minute of incubation.

The results were analyzed by nonparametric tests (paired Wilcoxon test, sign test, and *U* test) and Student's *t* test.

RESULTS

Our previous studies showed that specific SOD activity in the pancreatic tissue rapidly increases in dogs with AP (over 15 min). These changes were related to SOD activation by free radicals [1]. The degree of enzyme activation decreased and was statistically insignificant after pretreatment with AP. However, differential study of specific SOD activity revealed statistically significant differences between the macroscopically intact and abnormal area of the pancreas (severe edema) 15 min after AP modeling and intravenous infusion of ALP. Specific SOD activity in visually intact areas of the gland decreased compared to the control (307±66 and 398±62, $p<0.01$). These data show that ALP has a positive effect on the development of oxidative stress. At the same time, specific SOD activity increased in edematous tissues (460±38, statistically insignificant compared to the control). Significant differences were found between specific SOD activity in the macroscopically abnormal and intact area ($p<0.01$). SOD from various tissue areas was electrophoretically separated before and after AP modeling. Experimental AP without ALP pretreatment was accompanied by a shift to dissociation of both extracellular (Fig. 1, *a*) and cytosolic SOD (Fig. 1, *b*). Pretreatment with ALP prevented dissociation of high-molecular-weight extracellular SOD. However, ALP only partly prevented dissociation of cytosolic SOD (especially in the macroscopically abnormal area).

It can be hypothesized that xanthine reductase is not the major source of FOR in the acinar cell at the early stages of AP. The positive effect of ALP can be related to trapping of hydroxyl radicals. Incubation of preparatively isolated SOD in the superoxide-generating system containing xanthine oxidase and NADH in the absence of ALP was

TABLE 1. Specific SOD Activity during Incubation in the Superoxide-Generating System (xanthine oxidase and NADH, $M\pm m$, $n=10$)

Experimental conditions	Before incubation	After incubation
Without ALP	429±16	489±22*
500 μM ALP	438±17	423±23

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

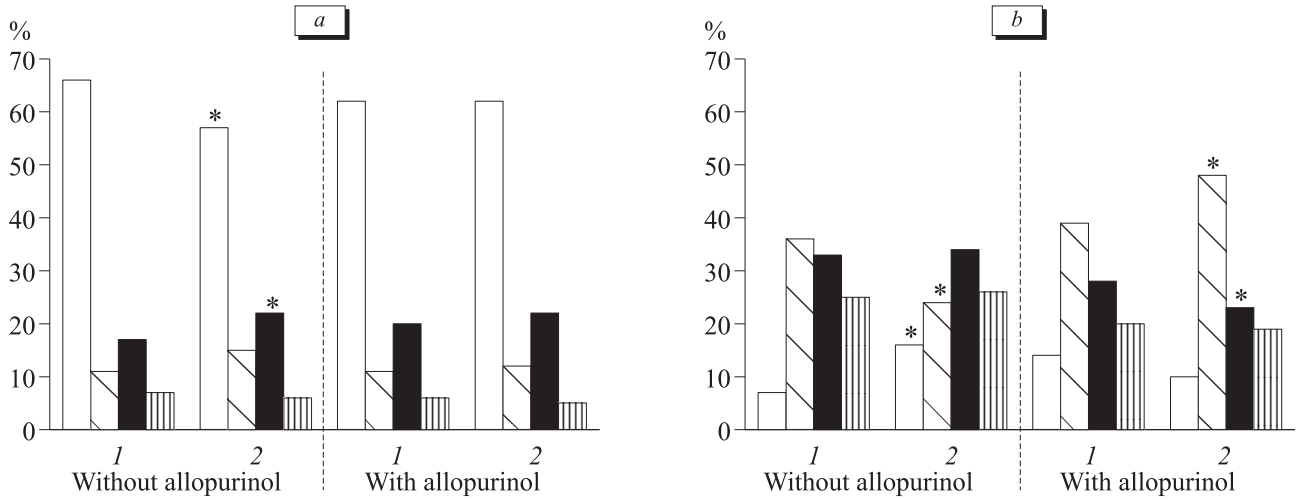

Fig. 2. Fractional composition of cytosolic (*a*) and extracellular SOD (*b*) during incubation in the superoxide-generating system (xanthine oxidase and NADH) in the presence and absence of allopurinol: control (*1*) and experiment (*2*).

followed by an increase in specific SOD activity and transformation of the cytosolic and extracellular fraction to a lower-molecular-weight fraction. Addition of ALP to the incubation medium prevented the increase in specific enzyme activity (Table 1) and dissociation of high-molecular-weight fractions of SOD (Fig. 2). Superoxide generation in this system was primarily related to NADH oxidase activity at the flavin site not inhibited by ALP. Hence, prevention of SOD activation was associated with scavenging activity of ALP relative to the hydroxyl radical generated in the peroxidase reaction.

Our results suggest that ALP is highly potent in preventing activation of extracellular and cytosolic isoforms of SOD in the model superoxide-generating system. ALP only partly prevented generation of FOR in the pancreatic tissue during AP. Probably, cytosolic SOD is *in vivo* activated by other radicals, *e.g.* peroxynitrite formed in the reaction of NO with the hydroxyl radical. Our assumption is supported by data that NO synthases are activated at the early stages of AP [10]. Mitochondrial electron transport chains in acinar cell can serve as a source of superoxide radicals.

Antiradical agent ALP has a limited positive effect on the pancreas. Therefore, monotherapy with ALP can be prescribed only when the risk of postoperative AP is low. Otherwise, ALP should be used in combination with other drugs. Antiradical agents with a wide range of effects hold much promise in this respect.

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