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The morphology of acinar cells during acute pancreatitis in rats induced by intraductal infusion of peracetate

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Many experimental models have been created to explain the pathophysiology of acute pancreatitis (AP). Investigations have been undertaken in this laboratory into the influence of strong oxidants introduced into the pancreas retrogradely through the bile-pancreatic duct. In these experiments a potentially toxic metabolite of ethanol-peracetic acid was used to induce AP. Wistar rats were treated with 1 mM and 40 mM peracetate and with a solvent as a control for 1 and 3 hours respectively. After a period of observation the samples of pancreata were examined in a light and electron microscope together with the content of sulphydryl groups as a marker of intracellular oxidative stress. The morphological examination showed profound changes in the histology of the pancreas and also in its subcellular structures, especially in groups 3 and 4 (with a higher concentration of peracetate). The changes included parenchymal haemorrhage and widespread acinar cell necrosis. The level of the sulphydryl groups decreased in the rats treated with peracetate. This suggests that the severity of the disease strongly depends on the intensity of the oxidative stress. The results confirmed the axial role of oxygen-derived free radicals in the pathogenesis of AP.

Key words: experimental acute pancreatitis, free radicals, peracetate

INTRODUCTION

Acute pancreatitis (AP) remains one of the conditions in which the causes cannot be tackled but only the symptoms relieved and the complications treated [2].

Free radical involvement in the pathogenesis of AP is well documented [12, 14, 18]. One of the conclusions we have reached after many years of laboratory work on this subject is that antioxidant administration inhibits the development of cerulein-induced acute pancreatitis [15]. In this study we created a new model of experimental pancreatitis, based on the pro-oxidant properties of peracetic acid (PAA), a possible metabolite of ethanol in the human body, retrogradely injected into the rat bile-pancreatic duct.

MATERIAL AND METHODS

All the procedures concerning animals were approved by Local Ethical Committee (LEC) and performed according to the instructions authorised by the LEC.

48 male Wistar rats (weight 270–300 g) were randomly divided into 6 groups with 8 animals in each group. The rats from Groups 1 and 2 received

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0.5 ml of 1 mM peracetate dissolved in physiological saline. In Groups 3 and 4 the peracetate concentration was 40 mM. In Groups 5 and 6 only the solvent was administered. Groups 1, 3 and 5 were observed for 1 hour, whereas Groups 2, 4 and 6 were observed for 3 hours.

All the animals were kept at room temperature, in standard humidity and with a 12 hour dark-light cycle. They were fed with the standard diet for laboratory animals and water was given *ad libitum*. The animals were anaesthetised with vaporised diethyl ether and Ketamine (12 mg/kg) and Xylasine (10 mg/kg) were given intraperitoneally. A modification of the method proposed by Puig-Divi et al. [10] was used as a surgical procedure. The abdominal wall was opened with a small midline incision. The gut was pulled outside, the duodenal end of the bile pancreatic duct (BPD) was identified and a thin cannule was inserted by piercing the duodenal wall. The cannule was then fixed with a temporary tie. Another tie was placed on the proximal pancreatic end of the BPD near the liver. Afterwards the solutions were injected into BPD lumen at 0.5 ml over a period of 15 min by means of a syringe pump. After infusion the cannule was removed and the hole in the duodenum was closed with a circle suture. The gut was washed with 0.9% NaCl before being repositioned into peritoneal cavity. The abdomen was closed with a continuous suture. After the survival period the animals were sacrificed by exsanguination under ether anaesthesia and macroscopic changes in the pancreas were evaluated *in situ*. Samples of the pancreatic tissue were taken and divided into two parts. Those in the first, assigned for light microscopic examination, were fixed in 10% formalin solution and embedded in paraffin. Histological sections were stained with haematoxylin and eosin. Those in the second, to be examined by electron microscope, were cut into specimens measuring about 1 mm^3, pooled and incubated with 3% glutaraldehyde in 0.1 M sodium cocadylate buffer, pH 7.3. After 10 hours the specimens were rinsed in the same buffer and postfixed with 1% osmium tetroxide. After postfixation the samples were washed in veronal acetate buffer and dehydrated in ascending concentrations of alcohol. Thereafter they were embedded in Epon 812. Ultrathin sections were cut with a glass knife on Reichert Om U3 ultramicrotome, stained with uranyl acetate and lead citrate and examined with a JEM 1200 EX II electron microscope. From the rest of the gland a 20% homogenate was made for biochemical measurements. Sulphydryl group contents were evaluated using a modification of the method described by Jocelyn [6].

The unpaired t-student test was used for statistical analysis.

RESULTS

Morphological examinations

Oedema and focal necrosis of the pancreas were visible macroscopically. These changes were more pronounced in the groups which received 40 mM peracetate solution.

Light microscopic examination showed evident changes in pancreatic tissue in groups treated with peracetate. Acinar cell vacuolisation, confluent necrosis, leukocyte and erythrocyte penetration were determined. The changes increased with the time of observation.

For the purpose of comparison, a scale proposed by Kusske et al. [7] was used. From each light microscopic probe 10 fields were viewed and analysed, taking into account 4 parameters (graded from 1 to 4 patients):

— oedema (O — from focal oedema to complete separation of the lobules);
— inflammation (I — infiltration of leukocytes, % of field area);
— necrosis (N — percentage of damaged lobules);
— haemorrhage (H — erythrocyte penetration into the parenchyma, % of field area).

All the morphological parameters mentioned above are summarised in Table 1. The electron microscopic observations revealed profound changes in the acinar cells. In rats which received peracetate oedema of the rough endoplasmatic reticulum (Fig. 1A) and disruption of the cristae of some mitochondria were visible. Dilatation of the intracellular space was also observed, with the presence of blood elements (erythrocytes and platelets) in this space (Fig. 1B). In Groups 3 and 4, treated with 40 mM peracetate solution, the changes were considerably greater and complete destruction of subcellular organelles was even observed (Fig. 1C, D). These changes increased with the time of observation. Pancreata from the control groups did not reveal any pathomorphological changes (Fig. 1E).

Biochemical measurements

Sulphydryl group content was significantly diminished in pancreata taken from rats infused with peracetate. The differences between these groups and the control were statistically significant only after 1 hour of observation. The results are shown in Table 2.

Table 1. Summary of symptoms of pancreatitis: Light microscopic image scale — see text

	Group	Oedema	Inflammation	Light microscope image Hemorrhage	Necrosis
1 h	Peracetic acid 1 mM	1	1	0	1
	Peracetic acid 40 mM	1	2	3	1
	Control	0	0	0	0
3 h	Peracetic acid 1 mM	2	2	2	2
	Peracetic acid 40 mM	2	3	4	3
	Control	1	0	0	0

Table 2. Sulphydryl group content in pancreatic tissue (mean ± SEM)

Group	1 h	3 h
Control	58.08 ± 3.26	55.6 ± 7.68
Peracetate — 1 mM	49.85 ± 3.85*	46.84 ± 6.14
Peracetate — 40 mM	47.8 ± 1.082*	48.35 ± 5.768

*$p < 0.05$ — in comparison with the control group

DISCUSSION

The crucial point of severe acute pancreatitis is an inappropriate activation of hydrolytic enzymes [8, 11]. However, we are not sure where this detrimental process takes place. Some authors indicate the cytoplasm of acinar cells [4] but others suggest that it could be the extracellular space [5]. Free radical involvement in intracellular events leading to leakage of enzymes and activation has been documented in many studies [12, 13, 18]. In our laboratory we created a new model of experimental AP using a well known pro-oxidant agent, tert-butyl hydroperoxide [16], retrogradely injected into the bile-pancreatic duct. This method is commonly used to administer pancreatitis-inducing agents, such as bile acids [17]. Bergenfeldt et al. [3] announced in a clinical case report, that the digestion of a large amount of acetate could induce acute pancreatitis, resulting in severe complications.

Peracetate is a metabolite of ethanol, which could occur in the pancreas when a high concentration of acetic acid is present. It can be expected as an oxidative stress-generating agent. Allen et al. [1] confirmed its activity in the erythrocytes of alcoholic men. In this study we investigated whether acute pancreatitis in rats could be induced by retrograde peracetate administration into BPD. We found that even a low concentration of this compound, as little as 1 mM, caused profound changes in the pancreatic tissue. When these phenomena were compared with previous experiments with cerulein and tert-butyl hydroperoxide, we found that peracetate infusion generated more profound damage of the pancreatic tissue, with widespread necrosis and haemorrhage. The measurement of protein and lipid peroxidation markers confirmed that peracetate-induced oxidative stress occurred inside acinar cells. We therefore concluded that an oxidative-derived metabolite of ethanol — peracetate induced severe necrotising pancreatitis in the rats. The most interesting aspect of the experiments is the concentration of peracetate used. This was approximately one thousand-fold lower than the concentration of bile acids broadly used to induce acute necrotising pancreatitis [17]. Such a concentration approached values which could possibly arise in the human body [14]. Thus, this compound could be regarded as a postulated "toxic metabolite" in the pathogenesis of AP in humans [9]. However, peracetate activity mechanisms in the pancreas are still unclear. The protein oxidation marker evaluation suggested that it most probably "attacked" important protein structures of the membranes. Initial studies have indicated that it could provoke severe acute pancreatitis, lethal for the majority of animals during the first 48 hours of the developed disease (unpublished data).

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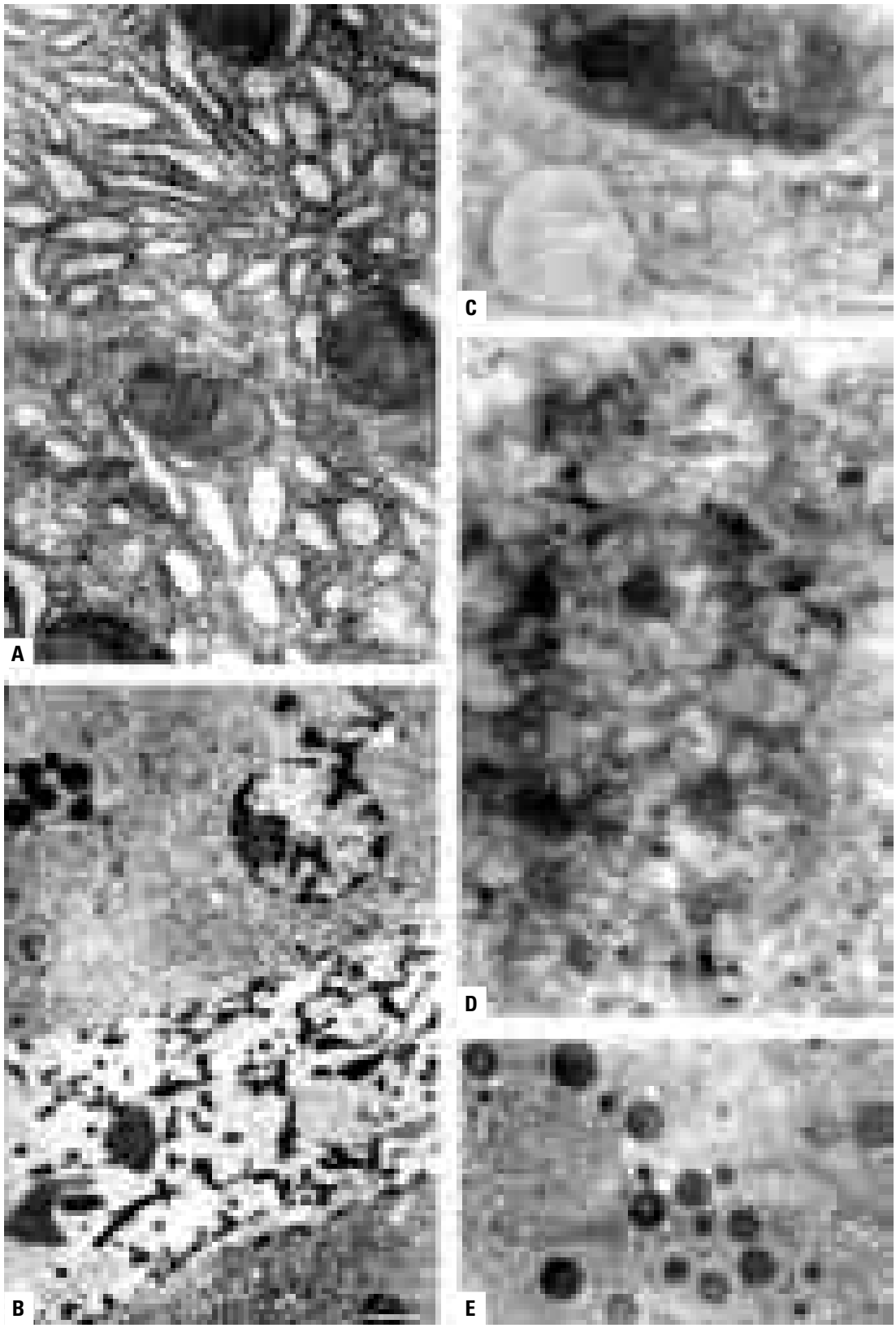

Figure 1. Electron micrograph of acinar cells from the pancreas: **A.** Group 1 — oedema of the rough endoplasmatic reticulum (1 mM peracetate, 1 hour of observation); **B.** Group 2 (1 mM peracetate, 3 hours of observation) — endoplasmatic reticulum dilatation and zymogen granule depletion. Blood elements (erythrocytes and platelets) are present in the intracellular space; **C.** Group 3 (40 mM peracetate, 1 hour of observation) — profound damage of subcellular organelle: vacuolisation of mitochondriae, destruction of the endoplasmatic reticulum; **D.** Group 4 (40 mM peracetate, 3 hours of observation) — "debris" of mitochondrial cristae in an acinar cell; **E.** Control group (0.9% NaCl, 3 hours of observation) — no structural changes are visible. Scale bars A, C, D — 500 nm; B, E — 2 μm.

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