# Effects of endothelin-1 or of its receptor A a selective antagonist, on histological and ultrastructural patterns in experimental acute pancreatitis in rats

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## Abstract

The role of endothelin-1 (ET-1) and of its receptor A ($ET_A$) blockade in oedematous acute pancreatitis (AP) remains unclear. In 40 male Wistar rats with i.p. cerulein-induced AP, lasting 4 hours, ET-1 2x0.5 nmol/kg and 2x1.0 nmol/kg or selective $ET_A$ antagonist LU 302146, 10 mg/kg and 20 mg/kg was given i.p. simultaneously with cerulein. Histological and ultrastructural studies of pancreatic specimens were done. ET-1 decreased the inflammatory infiltration, but not the mean scores of necrosis and vacuolization in AP. The ultrastructural damage of acinar cells was less evident after ET-1 than in untreated AP. Selective $ET_A$ antagonist slightly aggravated the vacuolization and necrosis of acinar cells and some ultrastructural alterations in AP. In conclusion, ET-1, in contrast to selective $ET_A$ antagonist, exerts some protective effect in the early course of oedematous cerulein-induced acute pancreatitis in rats.

**Key words:** acute pancreatitis, cerulein, endothelin-1, ETA antagonist, ultrastructure.

## Introduction

The role of ET-1 and of its $ET_A$ antagonists in acute pancreatitis remains controversial and not fully elucidated [1, 2]. In some experiments, a selective $ET_A$ antagonist attenuated inflammatory changes in the pancreas with cerulein-induced AP, thus improving the course of the disease [3, 4, 5]. On the contrary, Kogire et al. [6] found that selective $ET_A$ blockade worsened, whereas ET-1 improved histological changes in that model of AP. This problem has important clinical implications for either a possible treatment of AP or prevention of the disease progression from the oedematous stage to more severe, necrotic forms with poor prognosis, by early modification of ET-1 action.

Ultrastructural examination could be a reliable method for elucidation of this problem, however, such studies have not yet been conducted. Therefore, the aim of the present study was to assess and compare the effects ET-1 and of selective $ET_A$ antagonist on ultrastructural and histological alterations in the pancreas in early cerulein-induced AP in rats.

## Material and methods

The experiments, approved by the institutional Bioethical Commission, were carried out on 40 male, Wistar rats, 240 - 280 g of body weight (b.w.). AP was induced by two i.p. injections of cerulein (Sigma) at a dose of 40 µg/kg b.w. in 1 hour interval [7]. Group I. Control group (C) without AP (n=6). Group II. Rats with AP untreated (n=10). Group III and IV. Rats with AP treated with ET-1 (Sigma) i.p. 2 x 0.5 or 2 x 1.0 nmol/kg b.w., simultaneously with cerulein (n=6 in each group). Group V and VI. Rats with AP treated with LU 302146 (generously donated by Knoll AG) i.p. 10 or 20 mg/kg b.w. once, with the first i.p. cerulein injection (n=6 in each group). The rats were sacrificed in general anaesthesia by quick decapitation 4 hours after the first injection of cerulein. Pancreas specimens were taken for histological and ultrastructural examinations, performed as in our previous work, including a statistical analysis [8].

## Results and discussion

The results of histological scoring of pancreatic changes are presented in Table 1. The ultrastructural patterns of acinar cells in the control group did not show any signs of damage. In

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*Table 1*. Histological changes of the pancreas in cerulein acute pancreatitis (AP) in rats*.

| No | Group | Oedema | PMN infiltration | Necrosis | Vacuolization |
|---|---|---|---|---|---|
| I | Control (n=6) | 0-1 (0.12Ä0.03) | 0-1 (0.06Ä0.02) | 0-0 (0.00Ä0.00) | 0-1 0.08Ä0.03 |
| II | AP untreated (n=10) | 1-3 (2.04Ä0.07) | 0-3 (1.61Ä0.08) | 0-2 (0.58Ä0.06) | 1-3 (1.88Ä0.08) |
| III | AP + ET-1 2x0.5 nmol/kg (n=6) | 2-3 (2.62Ä0.05) | 0-3 (1.47Ä0.09) | 0-2 (0.57Ä0.06) | 1-3 (1.96Ä0.07) |
| IV | AP + ET-1 2x1.0 nmol/kg (n=6) | 1-3 (2.12Ä0.08) | 0-2 (1.04Ä0.05) | 0-2 (0.54Ä0.06) | 1-3 (1.92Ä0.08) |
| V | AP + LU 302146 10 mg/kg (n=6) | 0-3 (1.96Ä0.09) | 0-3 (1.43Ä0.09) | 0-2 (0.86Ä0.07) | 1-3 (1.91Ä0.09) |
| VI | AP + LU-302146 20 mg/kg (n=6) | 0-3 (2.14Ä0.07) | 0-3 (1.41Ä0.07) | 0-2 (0.82Ä0.06) | 1-3 (2.19Ä0.07) |

*Values are expressed as the ranges of scores and means ± SEM.
Statistical significance of important differences:
*Oedema: I / II, III, IV, V, VI $p < 0.001$; II / III $p < 0.001$; III / IV $p < 0.001$.*
*PMN infiltration: I / II, III, IV, V, VI $p < 0.001$; II / IV $p < 0.001$; II/VI $p < 0.05$.*
*Necrosis: I / II, III, IV, V, VI $p < 0.001$, II / V, VI $p < 0.01$.*
*Vacuolization: I / II, III, IV, V, VI $p < 0.001$, II / VI $p < 0.01$.*

*Figure 1*. Part of acinar cell with dilated channels of RER and mitochondria with destruction of their cristae (arrow). Group II - AP untreated group. Original magnification x 3000.

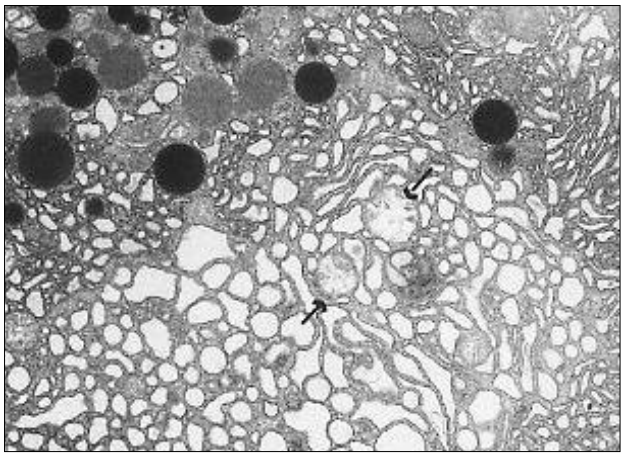


*Figure 2*. Vesicular transformation and circular arrangement of endoplasmic reticulum. Group III - AP, treated with the lower dose of ET-1. Original magnification x 7000.

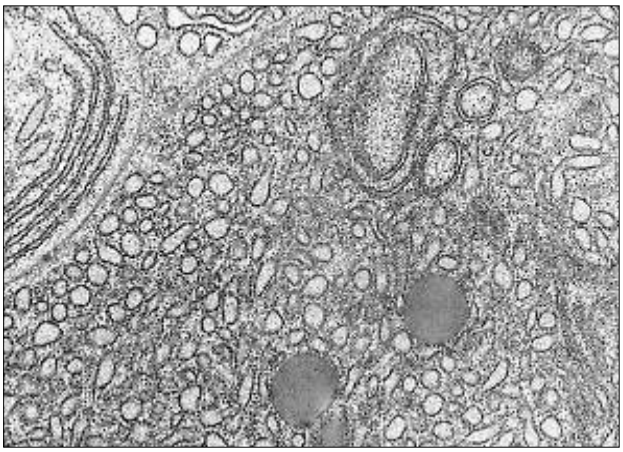


*Figure 3*. Nearly normal appearance of pancreatic acinar cells. Group. IV - AP, treated with the higher dose of ET-1. Original magnification x 3000.

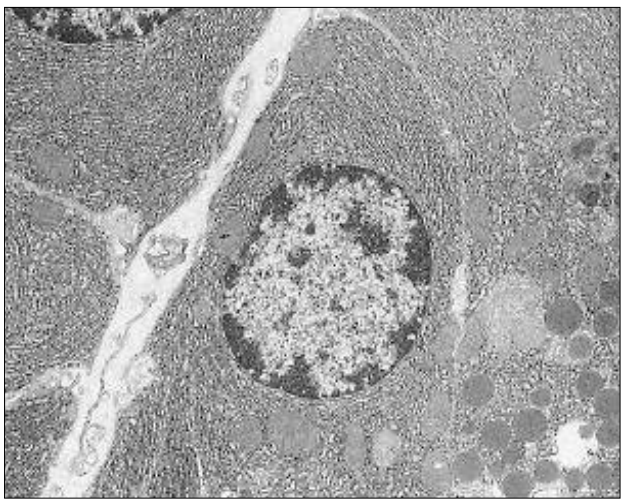
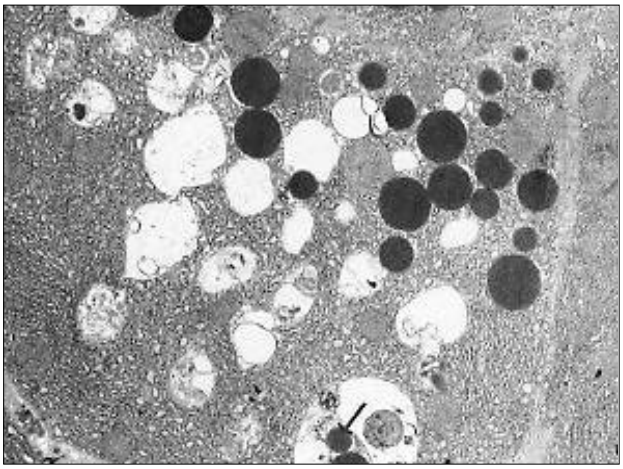


*Figure 4*. Numerous vacuoles in the cytoplasm of pancreatic acinar cell. Zymogen granules in one of them are seen (›). Group VI - AP, treated with the higher dose of $ET_A$ antagonist. Original magnification x 3000.



untreated AP, the majority of acinar cells contained quite numerous vacuoles. Zymogen granules were less numerous than those in the control group. The cisternae of Golgi apparatus were sometimes dilated. Channels of rough endoplasmic reticulum (RER) were usually dilated (Fig. 1) and disorganized. A necrosis of acinar cells was only sporadically observed. In the group, treated with a lower dose of ET-1, zymogen granules were scarce. The cytoplasm contained numerous phagosomes, autophagous vacuoles and vacuoles with amorphic content. Channels of RER were usually dilated and showed vesicular transformation or concentric arrangement (Fig. 2). Some of the mitochondria showed features of swelling. Necrosis of acinar cells was sporadic. In the group, treated with a higher dose of ET-1, those lesions were slightly less pronounced: phagosomes and vacuoles were less numerous, RER channels were dense and regularly arranged and mitochondria were normal (Fig. 3). The ultrastructural changes after the treatment with lower dose of selective $ET_A$ antagonist resembled those, observed in the animals with untreated AP. However, after the treatment with higher dose, those lesions were more expressed. Many acinar cells showed features of total disintegration. The cytoplasm contained numerous autophagous vacuoles (Fig. 4). Zymogen granules had varied electron density; sometimes, they were found in autophagous vacuoles or were loosely spread in the interstitial space. The changes in RER, Golgi apparatus and mitochondria were similar to those, noted in the untreated group.

Summarizing our study, ET-1 exerted a distinct protective effect on histological changes in the pancreas, as evidenced by the decrease of inflammatory cells infiltration, more evident after the higher dose of this agent, despite of the slight increase of edema score after the lower dose of ET-1. On the contrary, a slight aggravating effect on the scores of necrosis and vacuolization could be observed after both doses of selective ETA antagonist. The ultrastructural examination supported less advanced alterations of pancreatic acinar cells in AP treated with ET-1, especially after the higher dose of this agent. It was manifested by a decreased number of phagosomes and autophagous vacuoles, as signs of focal degradation of the cytoplasm. The channels of RER were more regular and rarely dilated and the mitochondria were better preserved. Those findings indicate that ET-1 administration can partially ameliorate the course of early acute oedematous pancreatitis. The treatment with $ET_A$ antagonist increased slightly the vacuolization of acinar cells but did not significantly affect the oedema. The score of necrosis was slightly increased after both ETA antagonist doses. The ultrastructural changes, such as: a decreased number of zymogen granules, disorganization of RER, autophagosomes and cytoplasmic vacuoles were also more prominent than those in the rats with untreated AP. Those observations did not support the assumption on the beneficial effect of $ET_A$ blockade in cerulein-induced AP, while even suggesting some undesirable effects in the early course of oedematous AP, taking into account the investigated doses and the time of application. Generally, our results support and extent the observations of Kogire et al. [6] and Martignoni et al. [1].

In conclusion, our results indicate evident beneficial effects of ET-1 on histological and ultrastructural changes of the pancreas in the early course of cerulein-induced AP. On the other hand, treatment with selective $ET_A$ antagonist in the investigated dosage exerted no alleviating influence on those changes, while the ultrastructural examination was even suggestive of its undesirable effects in early oedematous AP. Therefore, either in the prevention of oedematous pancreatitis (i.e., post-ERCP acute pancreatitis) or in the treatment of the early course of AP, ET-1, rather than its $ET_A$ antagonists, is to be taken into consideration.

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