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Original article

Effect of percutaneous catheter drainage on pancreatic injury in rats with severe acute pancreatitis induced by sodium taurocholate

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ABSTRACT

Objective: This study investigated the effect of percutaneous catheter drainage (PCD) on pancreatic injury in severe acute pancreatitis (SAP) rats.
Methods: Sixty Wistar rats were equally randomized into three groups: a sham operated control group, an SAP control group, and a PCD group. The levels of inflammatory cytokines, the activity of group II phospholipase A2 (PLA2) in blood and ascitic fluid, and the pancreas level of group II PLA2 and trypsin activity were measured 24 h after the operation. The apoptosis of the pancreatic cells, the expression of cycloxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), active caspase-3, Bcl-2 and Bax in the pancreas was detected. Pancreatic pathological changes were observed.
Results: The levels of proinflammatory cytokines, the activity of group II PLA2 and trypsin activity in pancreas in the SAP group were higher than those in the PCD group. The histopathological results revealed that the pancreatic injury was alleviated in the PCD group. The expression of COX-2 and iNOS in the pancreatic tissue in the SAP control rats was higher than that in the PCD rats. The expression of Bcl-2 was decreased and the expression of active caspase-3 and Bax was increased in the pancreas of PCD rats. The apoptosis index of the pancreatic cells in the PCD rats was higher than that in the SAP control rats.
Conclusion: PCD can relieve SAP-induced pancreatic injury by inhibiting inflammatory reactions, and promoting apoptosis of pancreatic cells.

Introduction

Acute pancreatitis (AP) can be induced by the activation of trypsogen for various reasons [1]. Clinical data have shown that most attacks of AP are mild and self-limiting [2]. However, in about 15–20% of cases, severe acute pancreatitis (SAP) occurs, and this often results in multiple systemic complications [3]. In the course of SAP, patients can experience hypovolemia or hypovolemic shock and septic shock [4], in addition to intraperitoneal or extraperitoneal fluid collection [5]. Formerly, the preferred medical treatment for patients with SAP was laparotomy debridement surgery. However, this increased the probability of anesthetic accident and the chance of infections, resulting in elevated mortality rates [6]. A minimally invasive method, percutaneous catheter drainage (PCD), is now promoted in the clinic. Compared with open surgery, PCD is associated with a lower probability of secondary infection and anesthetic accidents [7].

The intraperitoneal and extraperitoneal fluid that collects in AP contains trypsin, proinflammatory factors, and other harmful components, which have an adverse effect on intra-abdominal organs [8]. Apoptosis is one kind of protective phenomenon in pancreatic diseases [9]. Caspase-3 is a key mediator of apoptosis. Proapoptotic Bax and antiapoptotic Bcl-2 are known critical death regulators. They are able to undergo homodimerization and heterodimerization. The ratio of pro- to antiapoptotic proteins determines the occurrence of apoptosis [10]. Inducible nitric oxide synthase (iNOS) and cycloxygenase-2 (COX-2) play an important role in the development of inflammation. The pancreas is the initial site affected in SAP. To shed light on the effect of PCD on pancreatic injury in SAP, we established a rat PCD model of SAP and observed the alteration of pancreatic cell apoptosis. We also identified the expression of active caspase-3, Bax and Bcl-2 in the pancreas and determined the expression of iNOS and COX-2 in the pancreatic tissue and other inflammation-related indicators after PCD treatment.

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Materials and methods

Ethics statement

All procedures involving the rats were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Chengdu Military General Hospital (Permit Number: 2013–19), which specifically approved this study. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Establishment of animal model

Sixty male Wistar rats (210 ± 10 g) were kept in a temperature-controlled environment with a 12 h light–dark cycle. They were allowed free access to food and water 12 h before the operation. The rats were randomly divided into three groups: a control group ($n = 20$), an SAP group ($n = 20$), and a PCD group ($n = 20$). All the rats were anesthetized with an intraperitoneal injection of 2.5% sodium pentobarbital (Sigma Corp., Ronkonkoma, NY, USA) before the operation. The improved Aho method was used to induce SAP in 40 rats. The method of animal model preparation was as follows: In the SAP group, we identified the duodenal papilla inside the duodenum duct wall. A No. 5 needle was used to drill a hole in the avascular area of the mesentery. A segmental epidural catheter was inserted into the duodenum cavity through the hole and inserted retrogradely into the biliary-pancreatic duct through the papilla. This was followed by a retrograde transfusion of 5% sodium taurocholate (0.1 ml/100 g) with a microinjection pump at 0.2 ml/min. The hole in the lateral duodenal wall was then sutured. In the PCD group, we established an SAP rat model first and then placed an infusion tube (diameter of 0.3 cm) with side holes inferior to the pancreas. A plastic catheter (internal diameter of approx. 0.3 cm) with side holes and an outer cannula were placed below the pancreas through a puncture in the skin on the right side of a median abdominal incision. The catheter hole was sutured, and the catheter was subcutaneously fixed and covered with a spring (inner diameter of approx. 0.4 cm) to avoid being bitten by the rat. The spring was fixed on a plastic sheet of 2 cm × 2 cmat the end of the tube near the rat's skin. Finally, the four corners of the plastic sheet were sutured to the rat's skin. The catheter was connected to a 50 ml blood transfusion bag to drain the ascitic fluid (Fig. 1). In the control group, each rat's pancreas was exposed for the same amount of time as the other groups. After closing the abdominal incision, the rats in each group were subcutaneously injected with 37 °C saline (4 ml/100 g) to replace fluids. All rats were consecutively monitored during a 24-h long observational period.

Histological examination

In all the three groups, part of the tissue of the pancreas near the pancreatic duct was excised at 24 h after operation and fixed in 4% buffered paraform, embedded in paraffin, and sectioned for 4 μm thick slices. Histological examination was performed by hematoxylin and eosin (HE) staining and was analyzed using an optical microscope (CH20 model, the Olympus Corporation, Japan). Two pathologists performed the histologic examinations in a double-blinded manner. The pancreatic injury was scored as follows [11]: Inflammation: 0 = absent, 1 = aggregation of eukocytes in the

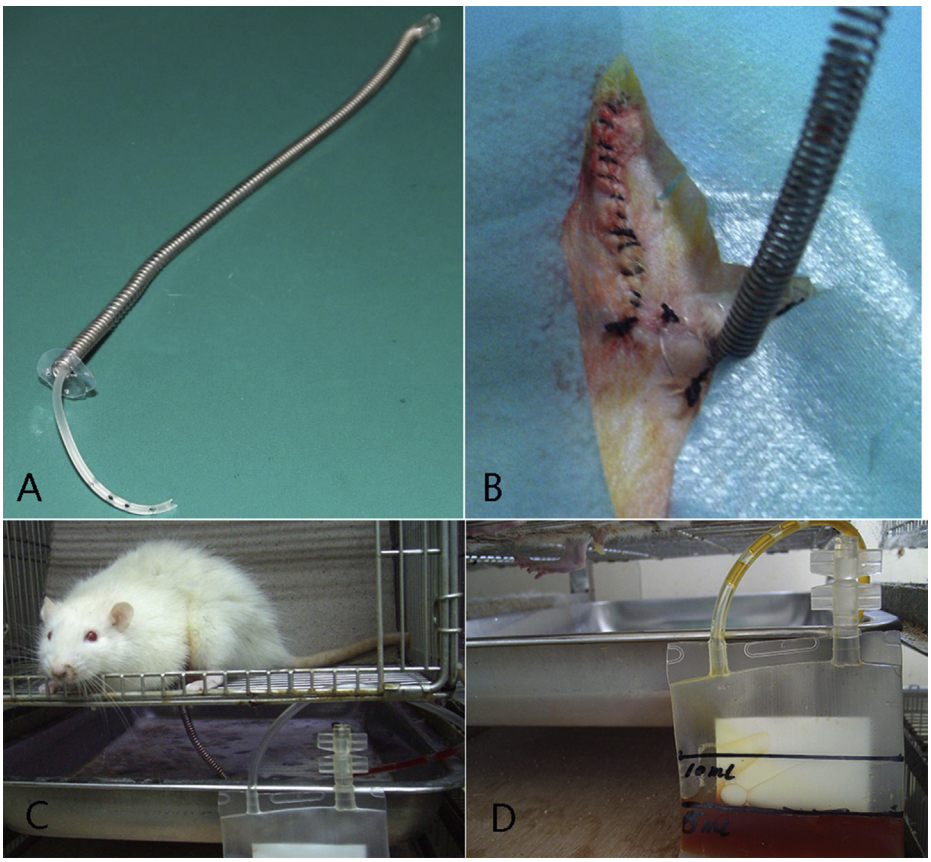

Fig. 1. A: PCD device for rats; B: Place the PCD device into the peritoneal cavity of rats and fix it to the abdominal wall; C: Rats treated with PCD; D: Ascites drained by PCD.

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