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Effects of glutamine alone on the acute necrotizing pancreatitis in rats

Etem Alhan, MD,[a,*] *Arif Usta, MD,*[b] *Serdar Türkyılmaz, MD,*[a] *Birgül Vanizor Kural, PhD,*[c] *and Cengiz Erçin, MD*[d]

[a] *Department of Surgery, Karadeniz Technical University, Trabzon, Turkey*
[b] *Department of Surgery, State Hospital, Karabük, Turkey*
[c] *Department of Biochemistry, Karadeniz Technical University, Trabzon, Turkey*
[d] *Department of Pathology, Kocaeli University, Kocaeli, Turkey*

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ABSTRACT

Background: The effects of the glutamine on the acute pancreatitis are controversial in the clinical and experimental studies. The aim of this study was to investigate the influence of glutamine alone on acute necrotizing pancreatitis (ANP) induced by glycodeoxycholic acid in rats.

Material and methods: Fifty-two male Sprague–Dawley rats weighing 300-350 g were used. Rats were divided into four groups as sham + saline, sham + glutamine, ANP + saline and ANP + glutamine. ANP in rats was induced by glycodeoxycholic acid. The extent of acinar cell injury, mortality, systemic cardiorespiratory variables, functional capillary density, renal/hepatic functions, and changes in some enzyme markers for pancreatic and lung tissue were investigated during ANP in rats.

Results: The induction of ANP resulted in a significant increase in the mortality rate, pancreatic necrosis, and serum activity of amylase, alanine aminotransferase, interleukin-6, lactate dehydrogenase in bronchoalveolar lavage fluid, serum concentration of urea, and tissue activity of myeloperoxidase and malondialdehyde in the pancreas and lung, and a significant decrease in concentrations of calcium, blood pressure, urine output, pO2, and functional capillary density. The use of glutamine alone improved these changes.

Conclusions: Glutamine demonstrated beneficial effect on the course of ANP in rats. Therefore, it may be used by itself in the treatment of acute pancreatitis.

1. Introduction

In most cases, acute pancreatitis is a mild and self-limiting disease, but severe necrotizing forms associated with a significant mortality rate are infrequent. However, in recent years, the mortality of acute necrotizing pancreatitis (ANP) has been reported to vary from 6.2%–20.8% inspite of the improved fluid management, respiratory care, and nutritional support [1,2].

Auto digestion of the pancreas and impairment in pancreatic microcirculation are two important parts in the pathophysiology of acute pancreatitis [2–4]. Excessive upregulation of the cytokines and secondary mediators such as histamines prostaglandins, thromboxanes, leukotrienes, nitric oxide, and platelet-activating factors play an important role during the course of acute pancreatitis [1,2,5,6]. In the early stage of the disease, hypovolemia, resulting from fluid sequestration into the abdominal cavity, and in the late stage, a sepsis caused by

* *Corresponding author*. Department of Surgery, Farabi Hospital, Karadeniz Technical University, 61080 Trabzon, Turkey. Tel.: +90 462 377 5442; fax: +90 462 325 2270.
E-mail address: alhanea@gmail.com (E. Alhan).
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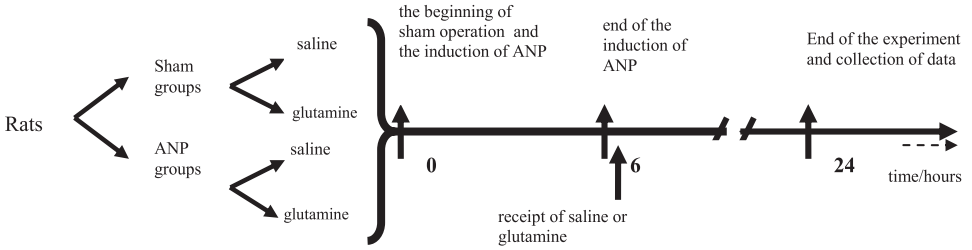

Fig. 1 – Experimental design and time schedule of the study.

bacteria translocated from the gut resulting in systemic inflammation may cause to clinical multiorgan failures [1,2,7].

Glutamine has long been known as a nonessential amino acid, which serves an immunonutrient during catabolic stress such as trauma, sepsis, and burn. The requirements of glutamine increases, therefore, glutamine may be accepted essential under these conditions [8,9]. Glutamine plays multiple functions in the human body. It is a source of fuel for lymphocytes and enterocytes [8]. Glutamine has an antioxidant effect as a precursor for glutathione and a cytoprotective effect with induced expression of heat shock proteins [10,11]. Glutamine supplementation during severe illness improves gut barrier function, lymphocyte function, and decreases some of the proinflammatory cytokines with inhibition of nuclear factor-κB and p38 mitogen-activated protein kinase [12,13].

The cytoprotective, antioxidant effects of the glutamine, the protective effect on the integrity of the intestinal mucosa, the improving effects on the immune system, and the inhibition of proinflammatory cytokines result in the use of the treatment of experimental and clinic acute pancreatitis. Although the experimental studies reported positive results on the course of acute pancreatitis, the results with clinical studies are controversial [14–25]. Glutamine in experimental and clinical studies was used as a supplement to enteral or parenteral nutrition. There is no study that administers the glutamine alone on the pancreatitis in the literature.

Therefore, in this study we examined the effect of glutamine alone on the extent of acinar cell injury, mortality, systemic cardiorespiratory variables, functional capillary density (FCD), renal and/or hepatic functions, and changes in some enzyme markers for pancreatic and lung tissue during ANP in rats.

2. Materials and methods

2.1. *Animals*

Fifty-two male Sprague–Dawley rats weighing 300–350 g were used. They were housed in rooms maintained at 21 ± 1°C and a 12 light–dark cycle. Animals were fasted overnight before the experiment, but had free access to water. The care was provided in accordance with the Ethics Committee of Karadeniz Technical University, Trabzon, Turkey (Number 607, date June 22, 2012).

2.2. *Experimental procedures*

Anesthesia was induced with vaporized ether and maintained by an intraperitoneal injection of ketamine 50 mg/kg (Ketalar; Eczacıbası, Istanbul, Turkey). The right internal jugular vein and carotid artery were cannulated (Luer Lock, ID 0.5 mm; Braun AG, Melsungen, Germany). The catheters were tunneled subcutaneously to the suprascapular area. During the experiment, the animals were housed in metabolic cages, which enabled the quantitative assessment of urine production.

Acute pancreatitis was induced by an intravenous infusion of cerulein (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) at a dose of 5 μg/kg/h over 6 h superimposed on a standard infusion of 1.2 mL/kg glycodeoxycholic acid (10 mmol/L, Sigma, St. Louis, MO) into the biliary–pancreatic duct for 10 min at 30 mm Hg as described by Schmidt *et al.* [26]. A special infusion pump for pressure and volume control (IVAC P 7000; Alaris Medical Systems, Hampshire, United Kingdom) was used. Cerulein was reconstituted in physiological saline and infused at 8 mL/kg/h as the baseline hydration. The animals of the sham group were given intraductal saline followed by a 6-h intravenous infusion of saline.

The rats were randomized into four experimental groups (Fig. 1). Those in the first group (sham + saline, $n = 10$) had arterial and venous lines placed and were given intraductal saline followed by a 6-h intravenous infusion of saline. After the 6 h period, saline was infused intravenously at 6 mL/kg/h for the last 18 h. At the 24 h from the beginning of the experiment, the cardiorespiratory function was assessed by monitoring the arterial blood gases, mean arterial pressure (MAP), renal function by the collection of urine using metabolic cages, and survival. The rats with MAP <80 mm Hg, pO_2 <80 mm Hg, pCO_2 >50 mm Hg, and pH <7.3, were excluded from the study. At the end of the 24 h, the rats were again anesthetized by ketamine and laparotomy was performed. The pancreas and spleen were exposed on an adjustable stage. The orthogonal polarization imaging video microscope (Cytoscan A/R; Cytometrics, Philadelphia, PA) was attached to the moveable shaft and the microcirculation was recorded in six different capillary regions of the exocrine pancreas at least 20 s [27]. The images were stored in audio video interleave format on the computer (Sony VGN-FW 230 J/H, Japan). Thereafter, the blood samples were taken from the carotid artery for the measurements of serum concentrations of electrolytes, calcium, urea, creatinine, glucose and activities of amylase, alanine aminotransferase (ALT), and interleukin (IL)-6. At the end of the 24 h after the blood withdrawn, a midline sternotomy was performed and the left main bronchus was clamped. Bronchoalveolar lavage (BAL) of the right lung was performed with 2 mL phosphate-buffered saline containing 0.07 M ethylenediamine tetra acetic acid and this procedure was repeated twice. The combined lavage of approximately 6 mL was centrifuged at