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Original article

Association between probiotics and enteral nutrition in an experimental acute pancreatitis model in rats

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ABSTRACT

Background/objectives: Recently, a randomized controlled trial showed that probiotic prophylaxis was associated with an increased mortality in enterally fed patients with predicted severe pancreatitis. In a rat model for acute pancreatitis, we investigated whether an association between probiotic prophylaxis and enteral nutrition contributed to the higher mortality rate.
Methods: Male Sprague–Dawley rats were allocated to four groups: 1) acute pancreatitis ($n = 9$), 2) acute pancreatitis and probiotic prophylaxis ($n = 10$), 3) acute pancreatitis and enteral nutrition ($n = 10$), and 4) acute pancreatitis, probiotic prophylaxis and enteral nutrition ($n = 11$). Acute pancreatitis was induced by intraductal glycodeoxycholate and intravenous cerulein infusion. Enteral nutrition, saline, probiotics and placebo were administered through a permanent jejunal feeding. Probiotics or placebo were administered starting 4 days before induction of pancreatitis and enteral nutrition 1 day before start until the end of the experiment, 6 days after induction of pancreatitis. Tissue samples and body fluids were collected for microbiological and histological examination.
Results: In all animals, serum amylase was increased six hours after induction of pancreatitis. After fulfilling the experiment, no differences between groups were found in histological severity of pancreatitis, degree of discomfort, weight loss, histological examination of small bowel and bacterial translocation (all $p > 0.05$). Overall mortality was 10% without differences between groups ($p = 0.54$).
Conclusion: No negative association was found between prophylactic probiotics and enteral nutrition in acute pancreatitis. No new clues for a potential mechanism responsible for the higher mortality and bowel ischaemia in the PROPATRIA study were found.

Introduction

Acute pancreatitis runs a mild course in the majority of patients. However, 20% of patients develop a severe pancreatitis with the presence of peripancreatic or pancreatic necrosis and (multiple) organ failure [1]. If the necrosis becomes infected, this is associated with a mortality of 15–25% and a morbidity rate of 50–100% [2–5]. Infection of necrotic pancreatic tissue is caused by bacterial translocation from the intestines and is thought to be preceded by three pathophysiological processes: 1) bacterial overgrowth of the small bowel due to decreased bowel motility, 2) dysfunction of the local mucosal and systemic immune system, and 3) increased intestinal permeability, resulting in bacterial translocation to other sites, such as the pancreas [6–8]. Reduction of bacterial translocation may reduce the rate of secondary infection of the pancreatic necrosis and decrease mortality and morbidity.

In 2006, our study group started a multicenter placebo-controlled randomized trial (PROPATRIA) on probiotic prophylaxis in patients with predicted severe pancreatitis [9]. Based on the results of a smaller trial, the aim of the study was to reduce the

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number of infectious complications. However, no difference in infection rate between the two groups was observed [10]. Strikingly, probiotic prophylaxis turned out to be associated with an unexpected high mortality rate, possibly related to the presence of bowel ischaemia (4% vs. 0% in the placebo arm, $p = 0.004$) [10]. These unexpected and unexplained findings prompted others to stop planned and ongoing trials on probiotic prophylaxis in severely ill patients [11,12].

In previous experiments we observed that prophylactic use of probiotics improved survival in rats [13,14]. The underlying mechanism for the negative effect of the combination of enteral probiotics with enteral nutrition in patients with predicted severe pancreatitis is unknown and stands in strong contrast to the previous findings of the protective effect of prophylactic probiotics in rats with acute pancreatitis. In order to address this mechanism, we investigated the relation between probiotic administration and enteral nutrition in a rat model of acute pancreatitis.

Materials and methods

Animals

Male specific pathogen-free Sprague–Dawley rats (Harlan, Horst, the Netherlands) with a mean bodyweight of 328 g (range 91 g) and age between 10 and 12 weeks were kept under constant housing conditions (temperature 22 °C), relative humidity (60%) and a 12-h light–dark cycle). Prior to the first surgical procedure, rats were allowed to adjust to these conditions for at least one week. During this week, all animals had unlimited access to water and food. Rats were randomly divided into four groups: 1) acute pancreatitis (jejunal cannula, 0.9% sodium chloride and placebo, $n = 9$), 2) acute pancreatitis and probiotics (jejunal cannula and 0.9% sodium chloride, $n = 10$), 3) acute pancreatitis and enteral nutrition (jejunal cannula and placebo, $n = 11$) and 4) acute pancreatitis, probiotics and enteral nutrition (jejunal cannula, $n = 11$). Animals were terminated at the end of the experiment, 6 days after induction of acute pancreatitis. The experimental design, as shown in Fig. 1, was approved by the Regional Animal Ethics Committee of the Radboud UMC and was conducted under the guidelines of the Dutch Council for Animal Care and the National Institutes of Health.

Enteral nutrition

The animals allocated to group 3 and 4 received sterile enteral nutrition (Nutrison Multi Fibre, Nutricia, Zoetermeer, the Netherlands). Animals in groups one and two received (sterile) saline as substitution to the enteral nutrition. The saline and enteral nutrition were infused through the permanent jejunal cannula from day -1 to the end of the experiment on day 6 (Fig. 1). On day -12, a swivel jacket was fitted and the rat was able to adjust to the jacket for one week. The swivel jackets were checked daily and, if necessary, adapted to the body size of the rat. From day -1 to the end of the experiment, each animal was connected to a swivel system for nine hours per day (usually from 9.00 a.m. to 6.00 p.m.). As shown in Fig. 2, the complete swivel system consisted of a syringe pump, a swivel device and a swivel mount, all interconnected by tubing. The system was used to allow free movement of the animal through the cage during connection (all parts of the swivel system and swivel jacket: Instech Laboratories Inc, Plymouth, PA, USA). When connected, the syringe pump continuously administered the enteral nutrition or the saline with an infusion rate of 1.5 ml/h. During the 9 h of connection to the swivel system rats were withheld from other food. However, when disconnected, animals had unlimited access to food (RMH 11110, Hope Farms, Woerden, The Netherlands). Throughout the whole experiment, whether connected or disconnected to the swivel system, all animals had free access to water.

Probiotics and placebo

The probiotics (*Ecologic® 641*, Winclove Probiotics, Amsterdam, the Netherlands) consisted of six viable and freeze-dried probiotic strains; four lactobacilli (*Lactobacillus acidophilus* (W70), *Lactobacillus casei* (W56), *Lactobacillus salivarus* (W24), *Lactobacillus lactis (W58)*), and two bifidobacteria strains (*Bifidobacterium bifidum* (W23) and *Bifidobacterium infantis* (W52)). The placebo consisted of carrier substance only (corn-starch). Directly before administration of the probiotics and the placebo, both products were reconstituted in sterile water for 15 min at 37 °C. A single probiotics dose in a volume of 1.0 ml contained a total of 5×10^9 Colonic Forming Unit (CFU) bacteria. According to van Minnen et al., both probiotics and

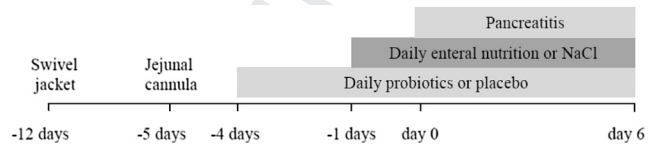

Fig. 1. Experimental design. At the start of the experiment, 12 days prior to induction of acute pancreatitis, a swivel jacket was fitted to all animals, which were subsequently allowed to adjust to this jacket for seven days. On day -5, a jejunal cannula was fitted. From day -4 onwards, daily probiotics or placebo were administered through the permanent cannula. One day before induction of the pancreatitis, animals received daily enteral nutrition or saline which continued until the end of the experiment. On day 0, acute pancreatitis was induced and seven days later, on day 6, all surviving animals were anesthetized to allow sterile removal of organs and blood samples, followed by termination.

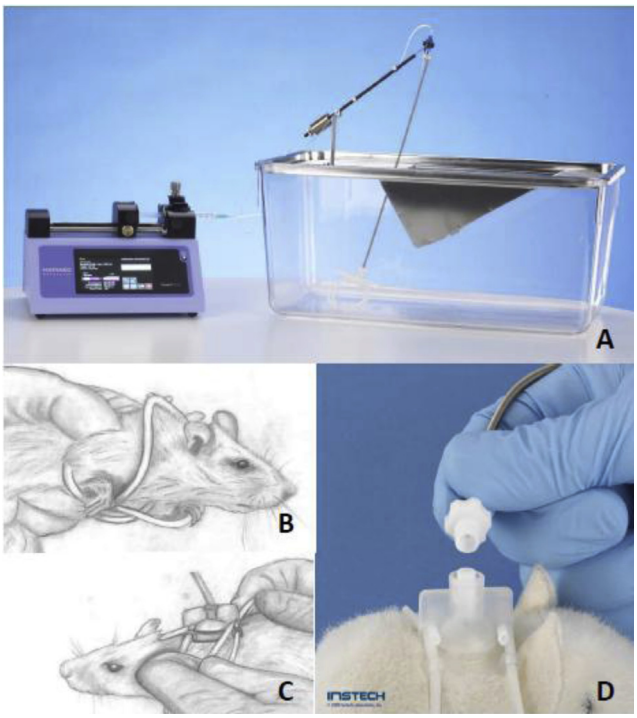

Fig. 2. Swivel system. A: The complete swivel system consisted of a syringe pump, a swivel mount with a swivel device, all interconnected with tubing. B and C: fitting the swivel jacket to the proportion of the rat. D: Connection of the swivel arm to the swivel jacket. Illustrations provided by Instech Laboratories Inc, Plymouth, PA, USA.

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