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Original article

Dissecting the effect of moxifloxacin in mice with infected necrosis in taurocholate induced necrotizing pancreatitis

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ABSTRACT

Objectives: To investigate the limited benefit of antibiotics in ameliorating the outcome of acute necrotizing pancreatitis, we analyzed antibiotic therapy in primarily infected necrotizing pancreatitis in mice with respect to the local pancreatic pathology as well as systemic, pancreatitis induced adverse events.

Methods: Sterile pancreatic necrosis (SN) was induced by retrograde injection of 4% taurocholate in the common bile duct of Balb/c mice. Primarily infected pancreatic necrosis (IN) was induced by co-injecting 10^8 CFU/ml *Escherichia coli*. 10 mg/kg of moxifloxacin was administered prior to pancreatitis induction (AN). After 24 h, animals were sacrificed to examine serum as well as organs for signs of SIRS.

Results: Moxifloxacin significantly reduced bacterial count in pancreatic lysates of animals with infected pancreatic necrosis (IN $4.1 \cdot 10^7 \pm 2.4 \cdot 10^7$ vs. AN $4.9 \cdot 10^4 \pm 2.6 \cdot 10^4$ CFU/g; $p < 0.001$). However, it did not alter pancreatic histology or pulmonary damage (Histology score: IN 23.8 ± 2.7 vs. AN 22.6 ± 1.7). Moxifloxacin reduced systemic immunoactivation (Serum IL-6: IN 330.5 ± 336.6 vs. 38.7 ± 25.5 pg/ml; $p < 0.001$), hypoglycemia (serum glucose: IN 105.8 ± 12.7 vs. AN 155.7 ± 39.5 mg/dl; $p < 0.001$), and serum aspartate aminotransferase (IN 606 ± 89.7 vs. AN 255 ± 52.1; $p < 0.05$). These parameters were significantly increased in animals with necrotizing pancreatitis.

Conclusion: In the experimental setting, initial antibiotic therapy with moxifloxacin in acute infected necrotizing pancreatitis in mice does not have a beneficial impact on pancreatic pathology or pulmonary damage. However, other systemic complications induced by infected necrosis in acute pancreatitis are reduced by the administration of moxifloxacin.

1. Introduction

The natural course of acute necrotizing pancreatitis is characterized by two phases. The first 14 days after onset of disease are characterized by a systemic inflammatory response syndrome (SIRS) which is caused by the massive systemic release of inflammatory mediators [1,2]. This results in pulmonary, cardiovascular and renal insufficiency. Due to the steady progress in intensive care medicine, the outcome of patients suffering from SIRS early in the course of pancreatitis has improved [3], but a subset of patients continues to develop an infection of the pancreatic necrosis. This occurs around the second and third week after onset of pancreatitis symptoms and in 40–70% of patients with necrotizing pancreatitis [4–7]. The risk of infection is related to the extent of intra- and extra-pancreatic necrosis [1,8]. Bacteria are believed to translocate from the intestine into the pancreatic necrosis [9]. Thus, bacterial contamination is typically mediated by enteric microorganisms such as *Escherichia coli*, *Pseudomonas* spp. *Klebsiella* spp., *Proteus* and *Enterobacter*, but gram positive and fungal infections are observed as well [10]. Infected pancreatic necrosis and septic conditions deriving from pancreatic infection are indications for surgical necrosectomy [3,11–14].

Infection of pancreatic necrosis increases the mortality by more than 30%. Hence, there is a special interest in preventing and effectively treating bacterial infections of pancreatic necrosis. However, use of prophylactic antibiotic treatment has failed to show a significant reduction of infection rates and subsequently prophylactic antibiotic therapy was not able to reduce overall mortality [15,16]. This may be caused by the reduced diffusion of antibiotics into necrotic pancreatic tissue. Therefore,

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antibiotics with good tissue penetration are required [17]. The quinolone moxifloxacin combines good diffusion and penetration into the pancreas in humans as well as mice with a broad antibiotic spectrum and interferes with the inflammatory response due to interactions with macrophages [18–20]. Therefore, we investigated the effect of initial treatment with moxifloxacin on local pancreatic damage as well as on systemic complications of acute pancreatitis with infected pancreatic necrosis in mice.

2. Material and methods

2.1. Animals

All animal procedures were conducted according to the Federation of European Laboratory Animals Science Associations guidelines and approved by the local animal welfare committee. 30 Male Balb/c mice (Charles River, Sulzfeld, Germany) with an average weight of 23.91 ± 1.98 g and an average age of 12–16 weeks were housed under standard conditions with a 12 h dark/light circle and standard pellet diet and water ad libitum and were randomized into groups of 5 animals for each treatment arm as described below. Anesthesia was administered using Forene (Abbot GmbH & Co KG, Wiesbaden, Germany) and buprenorophin (Temgesic–Essex Pharma GmbH, München, Germany) 0.15 mg/kg. During the experiment 0.15 mg/kg buprenorphin was administered every 8 h as 0.5 ml injections.

2.2. Treatment procedures

After shaving the abdomen and disinfecting the skin, a midline laparotomy was performed and the proximal common bile duct was temporarily clamped by using a microvessel clip as previously described [21]. Sham treated animals in group 1 received 2 injections of 2 mL/kg 0.9% sodium chloride (Braun AG, Melsungen, Germany) into the common bile duct. In group 2, animals received 2 mL/kg saline injection as well as 2 mL/kg 1.0×10^8 CFU/ml *E. coli* solution (XL blue, Stratagene). Administering 2 mL/kg 4% taurocholate followed by 2 mL/kg saline induced sterile necrosis in group 3. Animals in group 4 were injected with 2 mL/kg taurocholate (Sigma ultra, Sigma Aldrich Chemie GmbH, Steinheim, Germany) and 2 mL/kg saline. Additionally, animals in group 4 received prior intravenous injections of 10 mg/kg moxifloxacin into the lateral tail vein (Bayer AG, Leverkusen, Germany). In group 5, infected necrosis was induced by injection of 2 mL/kg taurocholate followed by injection of 2 mL/kg 1.0×10^8 CFU/ml *E. coli* solution (XL blue, Stratagene). Finally, animals in group 6 received prior intravenous injection of 10 mg/kg moxifloxacin followed by induction of infected necrosis by injection of 2 mL/kg taurocholate and 2 mL/kg *E. coli* solution. Thus, all animals received a total of 4 mL/kg injection volume. Furthermore, injection pressure was controlled and monitored and did not exceed 20 mmHg. After the infusion into the common bile duct, the needle was withdrawn, and the puncture site was closed using 8/0 Prolene (Johnson & Johnson Medical GmbH, Norderstet, Germany). The microvessel clip was removed and physiological bile flow was restored. Finally, the abdomen was closed.

For 24 h, animals were housed alone and given free access to water. To prevent aspiration, animals were not allowed to eat during this time. 24 h after induction of pancreatitis, animals were sacrificed by cardiac puncture under general anesthesia using Forene. After re-laparotomy, organs were harvested and a bronchoalveolary lavage was performed.

2.3. Macroscopic findings

At re-laparatomy, macroscopic signs of pancreatitis were scored. These included edema of the pancreas, areas of necrosis, hemorrhages, mesenterial necrosis. All parameters were scored from 0 to 3 modified according to Wacke et al. [18].

2.4. Histological findings

For histomorphological analysis, tissue samples of the pancreas, liver, spleen, kidney and lung were formalin fixed and embedded in paraffin. 4 μm sections were stained with hematoxylin and eosin (H&E, Merck, Darmstadt, Germany). *N*-acetyl-chloracetate esterase (NACE) staining was performed on formalin-fixed tissue sections using a naphthol ammonia scrubber distillate chloroacetate staining kit according to the supplier's instructions (Sigma Chemicals Ltd., Dorset, Great Britain). Histological examination of 3 randomly numbered and blinded sections of each animal were conducted by 2 independent observers. Severity of pancreatitis was diagnosed by scoring edema, leukocyte infiltration, parenchymal necrosis, fatty tissue necrosis and hemorrhages following the scoring system of Spormann et al. [22,23]. With this scoring system, edema and leukocyte infiltrate are graded on a scale from 0 to 3, while acinar cell necrosis, fatty tissue necrosis and hemorrhage are graded from 0 to 7.

2.5. Bacterial findings

For bacterial examination, samples of the pancreas were immediately cooled to 4 °C. Tissue samples were weighed, homogenized and plated in various dilutions on LB-Tetracyclin-Agar plates. Colony forming unites (CFUs) were identified and counted after 24 h of incubation.

2.6. Serum lipase and amylase, serum aspartate aminotransferase, IL-6, BAL

Blood gas analysis was performed with 0.1 ml blood immediately after the cardiac puncture using an ABL 725 Radiometer (Wittich, Germany). Serum lipase and amylase were determined by routine clinical chemistry methods, as was serum aspartate aminotransferase (AST).

2.6.1. Enzyme-linked immunosorbent assays

Serum-interleukin-6 concentration was determined using an ELISA in duplicate form (IL-6 – BioLegend, San Diego, USA). Albumin concentration in BAL fluid was determined in duplicate by enzyme-linked immunosorbent assay (ELISA) (Albumin–Bethyl Laboratories, Montomery, USA). Myeloperoxidase (MPO) concentration in BAL fluid was determined by MPO ELISA (Hycult Biotechnology, Uden, Netherlands) with undiluted samples. The assays were performed in duplicate according to the supplier's instructions.

2.7. Statistics

The data were analyzed using IBM® SPSS® Statistics (Version 16 for Mac OS, LEAD Technologies, Chicago, USA) and are displayed as mean ± SEM. Statistical significance was analyzed using Student-*t* test, the Fisher exact test, or Mann–Whitney *U* test. Significance is assumed for $p < 0.05$.

3. Results

The operative procedure was performed successfully in all 30 animals. To examine postoperative drinking behavior and