Acute Pancreatitis, Bacterial Translocation, and Different Octreotide Regimens: An Experimental Study

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Abstract

Purpose. To determine the effect of octreotide, octreotide with zinc, levamisole, and misoprostol on the bacterial translocation that develops in rats with acute pancreatitis (AP).

Methods. A total of 36 rats were divided into six groups, each consisting of six rats. Only laparotomy was performed on the first group. Acute pancreatitis was performed on the second group. Octreotide was given to the third, fourth, fifth, and sixth groups. Octreotide, octreotide with zinc, levamisole, and misoprostol were given to groups III, IV, V, VI, respectively. Rats were euthanized 48 h after the occurrence of AP. Blood and mesenteric lymph node samples were collected for polymerase chain reaction (PCR). Pancreatic tissue and terminal ileum were obtained for histopathological examinations.

Results. The severity of pancreatitis and mucosal damage of the terminal ileum was higher in group II than groups I, III, IV, V, and VI, histopathologically \( P < 0.05 \). There wasn’t a significant difference with respect to OA with Zn or L or M and OA group \( P > 0.05 \). A significant difference was found in PCR positivity in blood and mesenteric lymph node between groups I and II \( P < 0.05 \).

Conclusions. In AP, administering octreotide alone significantly prevented the bacterial translocation by preventing mucosal damage. The zinc, levamisole, or misoprostol with octreotide did not influence the results.

Key words Bacterial translocation · Octreotide · Zinc · Levamisole · Misoprostol

Introduction

Bacterial translocation (BT) can be defined as transmission of the intestinally located living or nonliving microorganisms or their by products (such as endotoxins) from the epithelial mucosa to the lamina propria, and then from there to the mesenteric lymph nodes, and finally to the distant organs.1,2 Serious acute pancreatitis (AP) is known cause of bacterial translocation.3

It is thought that the reason for an infectious mortal complication is bacterial translocation in serious acute pancreatitis.4 Somatostatins (S) have been reported to be able to stimulate the reticuloendothelial system and they have cyto- and organoprotective effects.5 However, there is no consensus in the literature about their usefulness because the results are varied. Zinc is another drug that may have a protective effect for the BT.6 There are numerous publications on the important role that zinc plays in the immunological response.7,8 Levamisole (L) is an antihelmintic and an immunostimulatory drug. Clinically, it is used by immune insufficient patients and for cancer chemotherapy. Misoprostol (M), which is a PGE1 analog, is known to have protective effects on gastrointestinal mucosa. The cytoprotective effects of prostaglandins are attributed to an increase of the gastrointestinal blood stream, the mucus secretion, the bicarbonate secretion, and the cyclic adenosine monophosphate (cAMP) production.9,11

The purpose of this experimental study was to investigate the use of octreotide, octreotide with zinc, octreotide with levamisole, and octreotide with misoprostol, whose effects are known to be cytoprotective and immunostimulatory on the bacterial translocation in acute pancreatitis.
Materials and Methods

Animals

All the experimental procedures were performed in accordance with the laboratory animal use and care principles (National Institutes of Health Publication no. 86–23, revised 1985) and were approved by the Ankara Education and Research Hospital ethics council. A total of 36 male Wistar Albino rats with a weight of 200–250 g were used in this study. The rats were fed with standard laboratory fodder during the preoperative period. The laboratory environment’s heat was kept at 22–24°C for the duration of the study. All the rats were euthanized after 48 h of acute pancreatitis. Exitus was not observed within 48 h in the control and treatment groups. Two new rats were added and acute pancreatitis occurred because two rats died in the 15th and 20th hour in the acute pancreatitis group. The rats were divided into six groups and each group had six rats:

Group I: Control group (C)
Group II: Acute pancreatitis group (AP)
Group III: Acute pancreatitis group in which octreotide was used (AP+O)
Group IV: Acute pancreatitis group in which zinc was used with octreotide (AP+O+Zn)
Group V: Acute pancreatitis group in which levamisole was used with octreotide (AP+O+L)
Group VI: Acute pancreatitis group in which misoprostol was used with octreotide (AP+O+M)

Operation

None of the rats were restricted with respect food and water from 7 days before the operation to the second postoperative day, which was the day they were euthanized. A laparotomy was performed for all the groups after 50 mg/kg ketamine (intramuscular) (Ketalar, Eczacıbaşı, İstanbul, Turkey) anesthesia, following 12 h of starvation.

Induction of Acute Pancreatitis

The pancreas and the pancreaticobiliary duct were visualized after the laparotomy and then acute pancreatitis was induced (Groups II, II, IV, V, VI). The main biliary duct was suspended with Prolene by 6–0 spherical needle. A 26-gauge catheter was entered from the pancreaticobiliary duct by the transduodenal route. When the catheter was seen in the pancreatic duct, 4.5% Na-taurocholate (0.1 ml/100 g) (Sigma–Aldrich, Steinheim, Germany) was manually given to the pancreatic duct with very slow speed and minimal pressure. After the perfusion, the catheter was taken out and the transduodenal entrance hole was sutured with Prolene by 6–0 spherical needle. The abdominal wall of the rat was sutured with 3–0 silk by a sharp needle. Only a laparotomy was performed for group I (Control group). Acute pancreatitis was induced in group II, but no drug was given. Before 7 days and after 2 days of the acute pancreatitis induction, octreotide (1.5 μg/kg/day) (0.05 cc) (Sandostatin; Novartis, İstanbul, Turkey) was given to groups III, IV, V, and VI at 8-h intervals subcutaneously. At the same time, zinc (5 mg/day) (Zinc Nutrimed, Nutrifarma, İstanbul, Turkey), in addition to octreotide via orogastric intubation, was given to group IV, levamisole (25 mg/kg/day) (Ketrax, Abdi İbrahim, İstanbul, Turkey), in addition to octreotide via orogastric intubation, was given to group V, and misoprostol (200 μg/kg/day) (9-oxo-11α, 16-dihidroxy-16-methyl-prot-13E-en-1-oic acid-methyl ester) (Cytotec, Ali Raif, İstanbul, Turkey), in addition to octreotide via orogastric intubation with a 12-h interval, was given to group VI. A relaparotomy was performed for groups III, IV, V, and VI 48 h after the induction of acute pancreatitis. One-milliliter blood samples from the right ventricle and from the mesenteric lymph node (MLN) samples, which were located nearest to the terminal ileum, were collected for the polymerase chain reaction (PCR) analysis. The serum amylase, urea, creatinine, alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and interleukin (IL)-6 levels are shown in Table 1. For the histopathological analysis, a 2-cm distal segment of the terminal ileum and all of pancreatic tissue was removed and established in 10% formalin fluid. To get DNA from the blood samples, the samples were placed in tubes containing ethylenediamine tetraacetic acid. Immediately after that step, they were spun with a NucleoSpin Blood kit (Macherey Nagel, Düren, Germany). To do this, the manufacturer’s suggestions were applied after 25 μl (20 mg/ml) of protease K was added to the 200 μl of blood. To get DNA from the tissue samples, approximately 25 mg of the MLN was taken and divided into small particles by a lancet. It was then incubated with the protease K (AppliChem, Darmstadt, Germany) for one night. The next day, it was treated with the Nucleospin Tissue kit (Macherey Nagel), according to the manufacturer’s protocol which is explained below.

Polymerase Chain Reaction (PCR)

The presence of Escherichia coli in the samples was examined by the amplification of the beta-galactosidase gene region using the PCR technique. Briefly, the bacterial DNA was extracted from all the samples by using a Nucleospin DNA extraction kit (Macherey Nagel) according to the manufacturer’s instructions.