

Poly(ADP-ribose) polymerase inhibition modulates experimental acute necrotizing pancreatitis-induced oxidative stress, bacterial translocation and neopterin concentrations in rats

Mehmet Yasar¹, Bulent Uysal², Umit Kaldirim³, Yesim Oztas⁴, Serdar Sadir², Mehmet Ozler², Turgut Topal², Omer Coskun⁵, Abdullah Kilic⁶, Tuncer Cayci⁷, Yavuz Poyrazoglu⁸, Sukru Oter², Ahmet Korkmaz² and Ahmet Guven⁹

¹Noncommissioned Officer Health College; ²Department of Physiology; ³Department of Emergency Medicine, Gulhane Military Medical Academy, Etlik, Ankara 06018; ⁴Department of Clinical Biochemistry, Hacettepe University School of Medicine, Sıhhiye, Ankara 06100; ⁵Department of Infectious Diseases; ⁶Department of Clinical Microbiology; ⁷Department of Clinical Biochemistry, Gulhane Military Medical Academy, Etlik, Ankara 06018; ⁸Department of General Surgery, Elazig Military Hospital, Elazig 23300; ⁹Department of Pediatric Surgery, Gulhane Military Medical Academy, Etlik, Ankara 06018, Turkey
Corresponding author: Bulent Uysal. Email: mbuysalmd@gmail.com

Abstract

Various studies have been performed to find out novel treatment strategies for acute necrotizing pancreatitis (ANP). Inhibition of poly(ADP-ribose) polymerase (PARP) is shown to reduce inflammation in several pathological conditions. We aimed to evaluate the efficacy of benzamide, a PARP inhibitor, in an experimental model of ANP. Thirty Sprague–Dawley rats were divided into three groups: sham-operated, ANP and ANP + benzamide groups. All groups except the sham-operated group were subjected to the ANP procedure, induced by infusing of 1 mL/kg of 3% sodium taurocholate into the common biliopancreatic duct. The ANP + benzamide group received 100 mg/kg/day benzamide intraperitoneally for a total of three days after induction of pancreatitis. The surviving animals were killed at the fourth day and the pancreas was harvested for biochemical, microbiological and histological analysis. Blood samples were also obtained from the animals. In the ANP group, a significant increase was observed in concentrations of serum amylase and neopterin and tissue oxidative stress indices (malondialdehyde, superoxide dismutase and glutathione peroxidase). Almost all of these changes were found to be reversed to near their normal values in the ANP + benzamide group. Histological injury scores were significantly higher in the ANP group than in the sham group ($P < 0.05$, ANP versus sham), and were significantly lower in the ANP + benzamide group than in the ANP group ($P < 0.05$, ANP + benzamide versus ANP). Evaluation of bacterial translocation identified significantly fewer infected sites in the ANP + benzamide group than in the ANP animals ($P < 0.01$). We observed that inhibition of PARP with benzamide reduced the severity, the mortality, the bacterial translocation rates and the neopterin concentrations in an experimental ANP model in rats. These findings suggest that it may be possible to improve the outcome of ANP by using PARP inhibitors.

Keywords: acute necrotizing pancreatitis, PARP, benzamide, bacterial translocation, neopterin, oxidative stress

Experimental Biology and Medicine 2010; **235**: 1126–1133. DOI: 10.1258/ebm.2010.010091

Introduction

Acute necrotizing pancreatitis (ANP) is a pathological condition with high morbidity and mortality. It is caused by a massive inflammatory reaction, which occurs as a result of an imbalance between the systemic release of pro-inflammatory and anti-inflammatory mediators in the ANP process, which eventually results in septic multiple organ failure.¹ In addition, reactive oxygen species (ROS), such as superoxide anions ($\cdot\text{O}_2^-$), hydroxyl radicals

($\cdot\text{OH}^-$), hydrogen peroxide (H_2O_2) and reactive nitrogen species (RNS), such as peroxynitrite (ONOO^-), have been identified as potential important factors in the pathogenesis and progress of pancreatitis.^{2,3} It is known that recruitment of activated neutrophils and monocytes into the pancreatic interstitium further exacerbates pancreatic damage via the generation of ROS/RNS.⁴

Poly(ADP-ribose) polymerase-1 (PARP-1) (also known as poly[ADP-ribose] synthetase or poly[ADP-ribose]

transferase) is a nuclear enzyme present in eukaryotic cells.⁵ PARP-1 activation is triggered by the presence of nicks and breaks in the DNA strand. This damage can be induced by reactive molecules, such as superoxide, hydroxyl radical or peroxynitrite, which trigger the PARP-1 activation.⁶ Rapid depletion of NAD^+ due to PARP activation leads to cellular ATP depletion and functional alteration of the cell, and eventually necrotic-type cell death occurs.^{7,8} To date, it has been shown that inhibition of PARP attenuates acute inflammation and tissue damage in animal models of various pathological conditions including pleuritis, lung injury, inflammatory bowel diseases and arthritis.^{9–11} In our team's previous works, beneficial effects of PARP inhibition were shown in different experimental inflammatory models.^{12,13}

This study was designed to investigate whether benzanide, a PARP inhibitor, has an ameliorative effect on ANP injury via blockage of cellular ATP depletion by using an experimental model induced by infusing sodium taurocholate into the common biliopancreatic duct.

Materials and methods

Animals

Sprague–Dawley male rats (200–250 g) were used in our study. All animal procedures were approved by the Institutional Committee on the Care and Use of Animals of Gulhane Military Medical School (Issue; 08/31). Before the experiment, animals were fed standard rat chow and water *ad libitum* and housed in cages with controlled temperature and 12-h light/dark cycle for at least one week.

Experimental groups and treatment modalities

Thirty rats were randomly assigned to three groups containing 10 rats each: sham-operated, ANP, ANP + benzanide groups. The sham-operated group served as a negative control. This group underwent laparotomy with injection of the 1 mL/kg saline into the common biliopancreatic duct, and then the animals received saline (10 mL/kg) intraperitoneally every 12 h for three consecutive days. The ANP group, as positive control, was also injected with intraperitoneal saline with the same regimen. The treatment group, namely ANP + benzanide, received benzanide instead of saline at a daily dose of 100 mg/kg intraperitoneally. Four days after the induction of pancreatitis, all surviving animals were killed.

Induction of pancreatitis

All animals were fasted overnight and anesthetized by an intramuscular injection of 50 mg/kg ketamine (Ketalar; Parke Davis, Eczacibasi, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun; Bayer AG, Leverkusen, Germany); all operations were performed at room temperature (24°C). Through a midline laparotomy, the common biliopancreatic duct was cannulated with a 28-gauge microfine catheter. An atraumatic microvascular clamp (Bulldog Artery Clamp, Harvard Apparatus, Holliston, MA, USA) was placed on

the bile duct below the liver and another around the common biliopancreatic duct at its entry into the duodenum to avoid reflux of enteric contents into the duct. Then, 1 mL/kg of 3% sodium taurocholate (Sigma, St Louis, MO, USA) was slowly infused into the common biliopancreatic duct, and the infusion pressure was kept below 30 mmHg, as measured with a mercury manometer.¹⁴ When the infusion was finished, the microvascular clamps were removed. Finally, the abdominal incision was closed, and the animals were returned to their cages to recover. Water and food were available *ad libitum*.

Sample collection

All surviving animals were killed on the fourth day of experiment via decapitation; the abdomen was opened and pancreatic tissue samples were harvested for analysis. Pancreatic tissue samples were divided into two pieces, one of them was fixed in 10% formalin solution for histological evaluation and the other was stored at -80°C deep freeze for the assay of antioxidant enzyme activity and tissue lipid peroxidation levels. In addition, samples from the pancreas, visceral peritoneum, mesenteric lymph nodes (MLN), liver and cecum were harvested to evaluate bacterial translocation. Blood samples were also obtained and some additional biochemical analyses were done on the serum samples.

Biochemical analysis

The frozen tissues were homogenized in phosphate buffer (pH 7.4) on an ice cube by using a homogenizator (Heidolph Diax 900; Heidolph Elektro GmbH, Kelheim, Germany). The supernatant was used for the assay of tissular parameters. Initially, the protein content of tissue homogenates was measured by the method of Lowry using bovine serum albumin as the standard.¹⁵

Lipid peroxidation levels were measured with the thiobarbituric acid (TBA) reaction using the method of Ohkawa *et al.*¹⁶ This method was used to obtain a spectrophotometric measurement of the color produced during the reaction of TBA with malondialdehyde (MDA) at 535 nm. The calculated MDA concentrations were expressed as nmol/mg-protein.

Superoxide dismutase (SOD) activity was assayed using a modified nitroblue tetrazolium (NBT) method as previously described by Durak *et al.*¹⁷ In brief, NBT was reduced to blue formazan by $\cdot\text{O}_2^-$, which has a strong absorbance at 560 nm. One unit (U) of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50%. The estimated SOD activity was expressed as units per gram of protein.

The glutathione peroxidase (GSH-Px) activity was measured using the method described by Paglia and Valentine¹⁸ in which GSH-Px activity was coupled with the oxidation of NADPH by glutathione reductase. The oxidation of NADPH was spectrophotometrically followed up at 340 nm at 37°C. The absorbance at 340 nm was recorded for 5 min. The activity was the slope of the lines expressed

as mmol of NADPH oxidized per minute. GSH-Px activity was expressed as U/g-protein.

Serum amylase, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein (TPRO) and albumin (ALB) concentrations were measured by an autoanalyzer using commercial kits and routine spectrophotometric techniques (Olympus AU-2700, Hamburg, Germany).

Serum neopterin (NP) concentrations were determined by a high pressure liquid chromatography (Agilent Technologies 1200 Series System, Santa Clara, CA, USA) system with a fluorescence detector as described previously.¹⁹ In brief, to 0.4 mL serum or tissue supernatant, 0.1 mL 2 mmol/L trichloroacetic acid was added and the mixture was left in an ice bath for 10 min. The precipitated protein was removed by centrifugation at 2000 *g* for 10 min. Twenty microliters of the supernatant was filtered through a 0.2 μ m filter and then injected into the chromatographic system. Separation of NP was achieved with a 250 $\times$ 4.6 mm² ID Allsphere ODS-2, C18 RP column with a particle size of 5 μ m (Alltech, Deerfield, IL, USA) fitted with a 10 $\times$ 4.6 mm² ID Allsphere ODS-2 guard column (Alltech) using 0.015 mmol/L phosphate buffer (pH: 6.4) as the mobile phase (isocratic elution) at a flow rate of 0.8 mL/min. The areas of peaks detected by fluorescent detector (Ex: 353 nm; Em: 438 nm) were used for quantification. Serum NP concentrations were expressed as nmol/L.

Histological analysis

Tissue specimens were fixed in formalin for 24 h, then embedded in paraffin and cut into 4 μ m sections. Slides were stained with hematoxylin and eosin and examined under a light microscope. Each slide was evaluated by two expert investigators blinded to the experiment groups. The treatment protocol scored the tissues for edema, inflammatory infiltration, fat necrosis, parenchymal necrosis and hemorrhage in 20 fields. The scores for each histological parameter were summed up, with a maximum score of 27, as defined by Spormann *et al.*²⁰

Bacterial quantitative cultures

Samples from the pancreas, visceral peritoneum, MLN, liver and cecum were taken from each rat under sterile conditions. All samples were weighted and homogenized in cysteine broth immediately for quantitative and qualitative culture of aerobic bacteria. Homogenized samples were diluted serially and cultured onto MacConkey (Merck, Darmstadt, Germany) and 5% sheep blood agars (Merck). All plates were incubated for up to three days at 35–37°C. After incubation, Gram-negative bacteria were identified with the API-20GN system (BioMerieux Vitek, Hazelwood, MO, USA). Gram-positive bacteria were identified to the genus level by means of standard microbiological methods. Bacterial counts were expressed in colony-forming units per gram of tissue (CFU/g). Growth of more than 10³ CFU/g of tissue was considered to indicate significant infection.

Statistical analysis

Results were expressed as median (min–max). Translocation incidence was evaluated by chi-square (χ^2) analyses and Fisher's exact test when an expected frequency was less than 5. In the other analyses, differences among the groups were analyzed by the Kruskal–Wallis test. Dual comparisons among groups with significant values were evaluated with the Mann–Whitney *U*-test; *P* < 0.05 were considered significant. All analyses were performed with the Statistical Package for the Social Sciences (SPSS) statistical program (Software version 11.0, SPSS Inc, Chicago, IL, USA).

Results

In this study, no complications occurred in the surgical technique. The courses of survival rate and weight for each group are shown in Figure 1. In the ANP group, four of 10 animals died by day 4: two of them died within the first 24 h, and the two others died by 48 and 72 h following induction of pancreatitis. In the ANP + benzamide group, two rats died in first 24 h. Additionally, there were significant differences at the fourth day in terms of the weight of rats. The mean weight of rats in the ANP group was significantly lower than those of sham-operated and ANP + benzamide groups (*P* < 0.05, ANP versus the other groups). In the ANP + benzamide group, the mean weights were significantly higher than ANP group, but those were still lower than in the sham-operated group (*P* < 0.05, ANP + benzamide versus ANP and sham-operated).

Biochemical results

Serum measurements

Serum amylase concentrations in the ANP group were significantly higher than those of the sham-operated and the ANP + benzamide groups (*P* < 0.05, ANP versus both, sham and ANP + benzamide), suggesting pancreatic tissue damage which was lessened with PARP inhibition. Nevertheless, amylase values in the ANP + benzamide group were still higher than the sham group (*P* < 0.05, ANP + benzamide versus sham). Serum AST and ALT concentrations were found to be enhanced in the ANP group (*P* < 0.05, ANP versus sham for both). Benzamide administration reversed these increases, while only ALT concentrations statistically returned to nearly sham values (*P* < 0.05, for only ALT in ANP + benzamide versus ANP group). Serum GGT concentrations also increased (*P* < 0.05), but ALP decreased (insignificantly) with pancreatitis induction; treatment with benzamide did not affect the changes of these two enzymes. The detailed results for levels of serum amylase, AST, ALT, ALP and GGT are shown in Table 1.

Table 2 summarizes the results of the analysis of serum glucose and protein levels. Upon induction of pancreatitis, levels of glucose increased and TPRO and albumin levels decreased significantly (*P* < 0.05, ANP versus sham group). In general, benzamide was able to reverse these changes, but statistical significance was only to be achieved for the albumin levels.

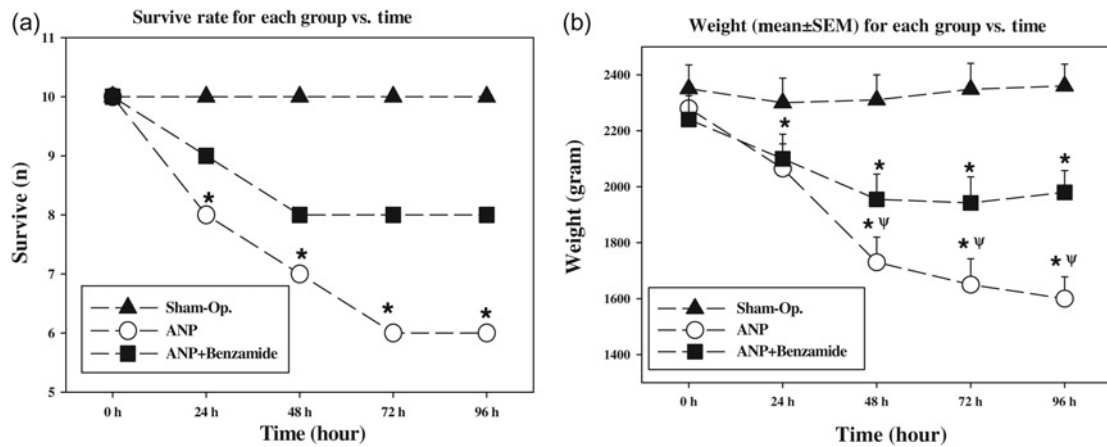


Figure 1 (a) Survival rates in all groups were shown as a Kaplan–Meier survival curves. The survival rate was significantly lower in the acute necrotizing pancreatitis (ANP) group than in the sham-operated group (log rank = 5.420, $P = 0.020$, ANP + benzamide group versus ANP group). There was no significant difference between the ANP + benzamide and the ANP group (log rank = 2.111, $P = 0.146$, ANP + benzamide group versus ANP group). (b) Mean weight of the rats in each group versus time displayed significant weight loss in the ANP group. The treatment with benzamide prevented mean weight loss in the ANP + benzamide group. * $P < 0.05$ statistically different from sham-operated group; ** $P < 0.05$ statistically different from ANP group

Serum NP concentrations were significantly increased in the ANP group ($P < 0.05$, ANP versus sham group). The increased NP concentrations significantly decreased with benzamide treatment but remained still higher than the sham values ($P < 0.05$, ANP + benzamide group versus ANP and sham groups). The serum NP concentrations are shown in Figure 2a.

Oxidative stress indices

The pancreatic tissue MDA concentrations in the ANP group were significantly higher compared with other groups ($P < 0.05$, ANP versus sham and ANP + benzamide groups) reflecting a clear oxidative stress which led to lipid peroxidation. The tissue SOD activity was found to be increased, but GSH-Px decreased significantly in ANP animals ($P < 0.05$). Although benzamide treatment reversed all changes of these oxidative stress markers significantly, all values remained significantly different from those of the sham-operated animals ($P < 0.05$, ANP + benzamide versus ANP and sham groups for MDA, SOD and GSH-Px). The outcomes of these oxidative stress parameters are displayed in Figure 2b–d.

Histological evaluation

Histological examination revealed no evidence for necrotizing pancreatitis in the sham-operated group, while all animals in the ANP group showed a severe degree of

pancreatitis with marked parenchymal necrosis, hemorrhage and leukocyte infiltration. In the benzamide-treated group, degrees of necrosis and leukocyte infiltration were lower than the ANP group ($P < 0.05$). Representative photomicrographs of the study groups are presented in Figure 3 and the detailed injury scores are shown in Table 3.

Bacterial translocation

Bacterial translocation was evaluated in the tissue samples from the pancreas, visceral peritoneum, MLN, liver and cecum. *Escherichia coli* and *Proteus mirabilis* were the most frequently isolated bacteria in tissue samples obtained from rats with pancreatitis. While infection was detected in samples of all organs from rats in the ANP group, no infection was detected in any organs of the rats in the sham-operated group ($P < 0.01$, ANP versus sham-operated). The number of infected sites was significantly lower in the ANP + benzamide group compared with the ANP group ($P < 0.01$, ANP + benzamide versus ANP). Significantly, bacterial translocation was only detected in the pancreatic site only in the ANP + benzamide group (Table 4).

Discussion

In the current study, we aimed to test effects of PARP inhibition via benzamide administration on various

Table 1 Biochemical values in serum

Groups	n	Amylase (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)
Sham-operated	10	418 (285–594)	90 (64–116)	65 (33–75)	280 (187–327)	1.3 (1.1–2.1)
ANP	6	1681 (1423–3596)*	177 (111–234)*	97 (80–100)*	178 (125–315)	3.0 (3.0–5.0)*
ANP + benzamide	8	891 (588–1499)**	122 (93–200)	63 (38–83)**	181 (147–372)	3.0 (2.0–4.0)

ANP, acute necrotizing pancreatitis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT gamma-glutamyl transferase

Data are expressed as median (min–max)

* $P < 0.05$ statistically different from sham-operated group

** $P < 0.05$ statistically different from ANP group

Table 2 Glucose and protein values in serum

Groups	N	Glucose (mg/dL)	TPRO (g/dL)	ALB (g/dL)
Sham-operated	10	102 (86–111)	8.17 (6.47–8.32)	3.7 (3.1–4.5)
ANP	6	321 (212–374)*	5.60 (3.24–6.66)*	2.1 (1.5–2.8)*
ANP + benzamide	8	212 (120–343)	6.95 (4.11–8.26)	3.0 (2.1–4.1)**

ANP, acute necrotizing pancreatitis; TPRO, total protein; ALB, albumin
Data are expressed as median (min–max)

* $P < 0.05$ statistically different from sham-operated group

** $P < 0.05$ statistically different from ANP group

pathological parameters, especially bacterial translocation rates and NP concentrations, in a rat model of ANP. The experiment showed that benzamide had a beneficial effect on pancreatic necrosis. Moreover, benzamide also had an ameliorating effect on oxidative stress indices of the pancreatic tissue in correlation with histological findings. Although oxidative stress is the initial step in the pathogenesis of pancreatic injury, overexpression of inducible nitric oxide synthase changes the nature of this stress from oxidative to nitrosative stress, because the combination of nitric oxide with superoxide results in peroxynitrite formation. Peroxynitrite then induces both apoptosis and necrosis of cells.^{21,22} In this mechanism, activation of the DNA repair enzyme PARP-1, a member of PARP enzyme family, mediates ONOO[−]-induced necrosis. PARP-1 detects and signals DNA strand breaks induced by binding to DNA of

a variety of genotoxic insults, including ionizing radiation, alkylating agents, oxidants and free radicals.^{23–26}

Upon binding to DNA, strand breaks occur, and PARP transfers ADP-ribose units from the respiratory coenzyme NAD⁺ to various nuclear proteins. From a physiological viewpoint, PARP-1 activity and poly(ADP-ribosyl)ation reactions are implicated in DNA repair processes, the maintenance of genomic stability, the regulation of gene transcription and DNA replication. An important function of PARP-1 is to allow DNA repair and cell recovery under conditions associated with a low level of DNA damage. In case of severe DNA injury, overactivation of PARP-1 depletes the cellular stores of NAD⁺, an essential co-factor in principal energy production mechanisms, including the glycolytic pathway, the tricarboxylic acid cycle and the mitochondrial electron transport chain. As a result, the loss of NAD⁺ leads to a marked reduction in the cellular pools of ATP, resulting in cellular dysfunction and death via the necrotic pathway. This is known as the ‘suicide hypothesis’ of PARP activation and seems to be a regulatory mechanism to eliminate cells after irreversible DNA injury.^{21,22} A vast amount of experimental evidence has established that the PARP-1 pathway of cell death plays a pivotal role in tissue injury and organ dysfunction in numerous disease processes.^{12,24,27,28}

NP is a marker associated with cell-mediated immunity and produced in monocytes/macrophages via the stimulation of interferon- γ . It has been shown that NP concentrations not only provide knowledge about the state of cell-mediated immunity, but also allow monitoring of

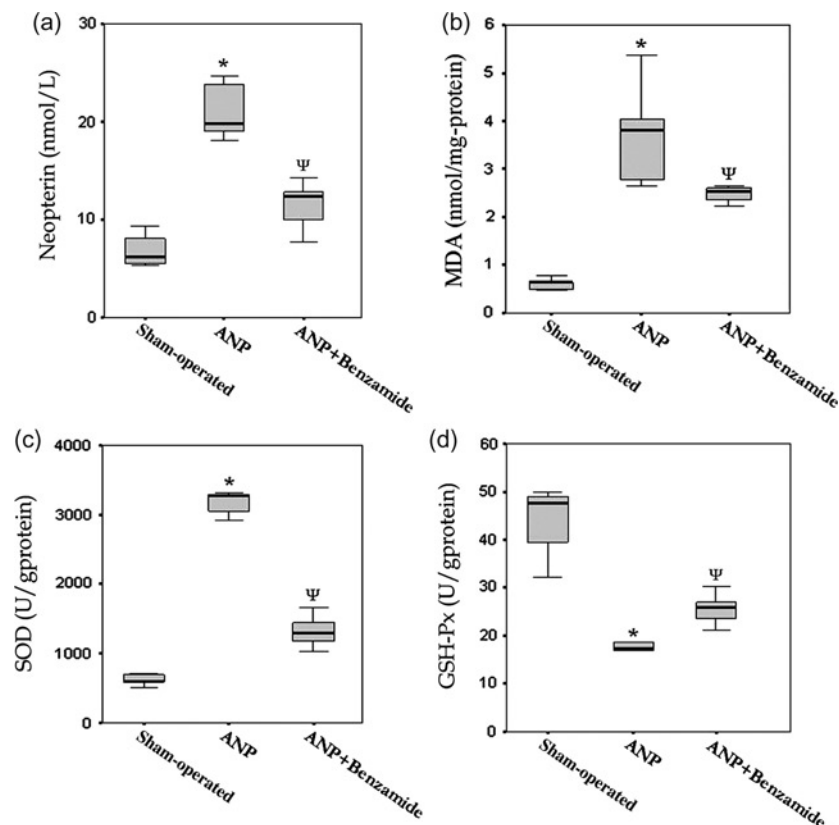


Figure 2 Serum neopterin concentrations and tissue oxidative stress indices. * $P < 0.05$ statistically different from sham-operated group; ** $P < 0.05$ statistically different from ANP group

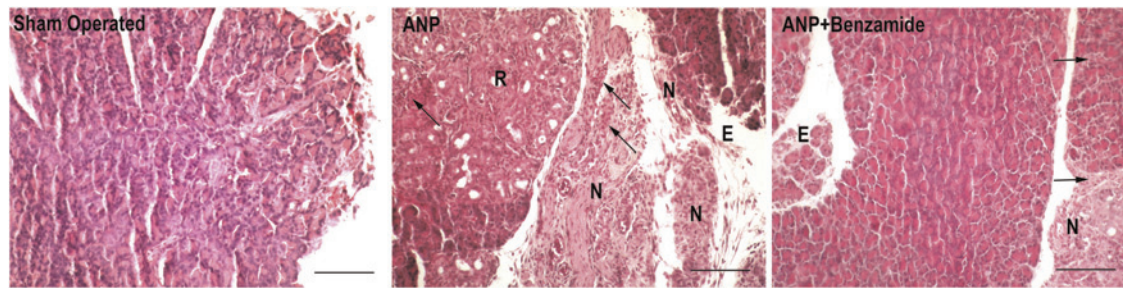


Figure 3 Representative photographs showing the histological changes in pancreatic tissues. Sham-operated group shows normal pancreatic structure. In the acute necrotizing pancreatitis (ANP) group, massive edema (E), parenchymal necrosis (N), pancreatic acinar regeneration (R) and leukocyte infiltration (black arrows) are seen. In the ANP + benzamide group, benzamide treatment normalized edema, parenchymal necrosis and leukocyte infiltration (hematoxylin and eosin, scale bars = 100 μ m) (A color version of this figure is available in the online journal)

the disease progression.²⁹ Also, it has been shown that enhanced NP concentrations reflect the severity of the acute pancreatitis.³⁰ Another study reported that NP concentrations are higher in acute pancreatitis patients with poor prognosis and are more specific for predicting disease progression than levels of C-reactive protein.³¹ A recent study by our team, testing another treatment modality in the same experimental model, also found enhanced NP concentrations in ANP-induced animals, which were in correlation with oxidative stress and histological injury.³² Thus, in the current study, due to its promising reports as a biochemical parameter to diagnose and follow-up pancreatic inflammation, serum NP concentrations were measured. NP concentrations in animals receiving benzamide came close to values in sham group. The inhibition of PARP by benzamide improved pancreatic functions, as evidenced by the reduction in amylase and NP concentrations in animals with ANP (Table 1, Figure 2). By using sources of cellular energy, namely ATP, to repair injured tissue, it seems likely that treatment with benzamide prevented pancreatic necrosis and allowed the preservation of the cellular stores of NAD. This in turn would have led to decreased activation of monocytes/macrophages, and as a consequence, reduction in levels of NP.

Necrotic pancreatic tissue derived from animals that were treated with benzamide demonstrated a decrease in the extent of necrotic sites, restoration of acinus structure and inhibition of the neutrophil recruitment. A major requirement for the initiation of the cell survival and regeneration process is the repletion of cellular ATP levels. Therefore,

the enhanced restoration of the injured pancreas in the setting of PARP inhibition may partly be due to the protection of pancreatic cells as evidence of decreased suicide hypothesis of PARP activation.

We evaluated the tissue oxidative stress marker (MDA) and the tissue antioxidant system (SOD and GSH-Px). Data revealed that the inhibition of PARP significantly ameliorated oxidative stress; in other words, PARP activation contributed to pancreatic injury. Higher SOD levels were observed in all groups except sham (Figure 2). On the other hand, GSH-Px activities in the control group were found to be reduced. These data collectively suggest that the tissue is undergoing oxidative stress. Higher SOD accompanied by lower GSH-Px can be more dangerous; SOD can reduce superoxide to hydrogen peroxide, but if hydrogen peroxide accumulates because of reduced GSH-Px levels, it can undergo a Fenton reaction to produce the hydroxyl radical. This may have occurred in the ANP group. However, in the ANP + benzamide group, both higher SOD and GSH-Px might reduce such an oxidative risk as evidenced in Figure 2.

PARP activation may contribute to pancreatic injury via several other mechanisms: PARP activation is likely to upregulate the activities of both extramitochondrial NADPH oxidase and mitochondrial NADH oxidase, which are the important superoxide-generating enzymes.³³ In addition, PARP activation alters transcriptional regulation and activates nuclear factor κ -B and activator protein-1, a transcription factor that regulates proinflammatory gene expression.³⁴ Such activation leads to increased formation

Table 3 Pathological scores

Groups	Sham-operated (n = 10)	ANP (n = 6)	ANP + benzamide (n = 8)
Edema	0 (0–1)	3 (2–3)*	2 (1–3)*
Hemorrhage	0 (0–1)	4 (3–7)*	2 (1–3)***
Leukocyte infiltration	0 (0–1)	3 (1–3)*	1 (1–2)***
Fat necrosis	0 (0–0)	3 (1–5)*	1 (1–3)***
Parenchymal necrosis	0 (0–1)	7 (5–7)*	2 (1–4)***
Total score	0 (0–4)	20 (11–25)*	8 (4–12)***

ANP, acute necrotizing pancreatitis

Data are expressed as median (min–max)

* $P < 0.05$ statistically different from sham-operated group

** $P < 0.05$ statistically different from ANP group

Table 4 Total incidence of bacterial translocation (evaluated by χ^2 analyses)

Infection sites	Groups		
	Sham-operated (n = 10)	ANP (n = 6)	ANP + benzamide (n = 8)
Peritoneum (%)	0 (0)	6 (100)*	0 (0)**
Liver (%)	0 (0)	6 (100)*	0 (0)**
MLN (%)	0 (0)	6 (100)*	0 (0)**
Pancreas (%)	0 (0)	6 (100)*	8 (100)*
Cecum (%)	0 (0)	6 (100)*	0 (0)**
Total infected site	0	30	8

ANP, acute necrotizing pancreatitis; MLN, mesenteric lymph nodes

* $P < 0.05$ statistically different from sham-operated group

** $P < 0.05$ statistically different from ANP group

of endothelin-1 and inflammatory cytokines (e.g. tumor necrosis factor- α , interleukin-6 and interleukin-1 β , all known to contribute to superoxide generation).^{34,35}

Another important outcome was the finding that bacterial translocation was significantly increased in all of the rats' tissues harvested from the pancreas, visceral peritoneum, MLN, liver and cecum after induction of ANP. Bacterial translocation is known to have critical importance for the mortality and morbidity in patients with pancreatitis.³⁶ Nevertheless, the pathogenesis of pancreatic superinfection is not completely understood.³⁷ Our findings are in agreement with the hypothesis that intra-abdominal spread by lymphatics is the pathway most likely to be involved in this process.³⁸ It has been shown that peroxynitrite is capable of mimicking many of the pathophysiological alterations associated with shock, i.e. endothelial and epithelial dysfunction, vascular hyporeactivity and cellular dysfunction; all these conditions are mediated by PARP activation.³⁹ Similarly, it has also been shown that different PARP inhibitors show a protective effect against shock-induced intestinal epithelial permeability changes.⁴⁰ Moreover, Cuzzocrea *et al.*⁴¹ have investigated the role of PARP in intestinal tight junction permeability, and this study has demonstrated that the alteration of permselectivity is most likely induced by PARP activation. Thus, it seems likely that benzamide treatment prevented bacterial translocation in the peritoneum, MLN, liver and cecum by inhibiting activation of PARP, which consequently led to an alteration in permselectivity of the intestinal mucosa. However, benzamide was not able to prevent bacterial translocation in the pancreatic tissue. The possible reason for this diversity may be that the pancreas is itself the main source of the inflammatory process.

In conclusion, inhibition of PARP with benzamide reduced severity, septic complications and mortality in the experimental ANP rat model. These findings suggest that it may be possible to improve the outcome of ANP by using novel and more effective PARP inhibitors as a promising therapeutic strategy.

Author contributions: MY was the prime mover and carried out induction of experimental models and surgical procedures. BU carried out housing, keeping and feeding of animals; injection of PARP inhibitor, sample collection; and pipetting of sample for analysis. UK carried out sample collection, preparation of figures and pipetting of samples for analysis. YO measured antioxidant indices and other biochemical parameters. SS homogenized tissue samples. MO carried out housing, keeping and feeding of animals, and sample collection. TT carried out statistical analysis and Photoshop procedures. OC and AK evaluated bacterial translocation. TC measured neopterin levels. YP carried out induction of experimental models and surgical procedures. SO and AK wrote the manuscript. AG carried out surgical procedures, preparation of tables and statistical analysis.

ACKNOWLEDGEMENTS

We thank epidemiologist Selim Kilic, MD, PhD, for statistical evaluation. This work is a part of studies supported by

the 'Gulhane Military Medical Academy Research and Progress Center' by Grant AR-2007/3.

REFERENCES

- 1 Zhu AJ, Shi JS, Sun XJ. Organ failure associated with severe acute pancreatitis. *World J Gastroenterol* 2003;**9**:2570–3
- 2 Leung PS, Chan YC. Role of oxidative stress in pancreatic inflammation. *Antioxid Redox Signal* 2009;**11**:135–65
- 3 Vaquero-Raya EC, Molero-Richard X. Reactive oxygen species in inflammatory diseases of the pancreas. A possible therapeutic target? *Gastroenterol Hepatol* 2005;**28**:473–84
- 4 Sandoval D, Gukovskaya A, Reavey P, Gukovsky S, Sisk A, Braquet P, Pandol SJ, Poucell-Hatton S. The role of neutrophils and platelet-activating factor in mediating experimental pancreatitis. *Gastroenterology* 1996;**111**:1081–91
- 5 Virag L, Szabo E, Bakondi E, Bai P, Gergely P, Hunyadi J, Szabo C. Nitric oxide-peroxynitrite-poly(ADP-ribose) polymerase pathway in the skin. *Exp Dermatol* 2002;**11**:189–202
- 6 Lindahl T, Satoh MS, Poirier GG, Klungland A. Post-translational modification of poly(ADP-ribose) polymerase induced by DNA strand breaks. *Trends Biochem Sci* 1995;**20**:405–11
- 7 Szabo C, Zingarelli B, O'Connor M, Salzman AL. DNA strand breakage, activation of poly (ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite. *Proc Natl Acad Sci USA* 1996;**93**:1753–8
- 8 Naziroglu M. New molecular mechanisms on the activation of TRPM2 channels by oxidative stress and ADP-ribose. *Neurochem Res* 2007;**32**:1990–2001
- 9 Mazzon E, Dugo L, Li JH, Di Paola R, Genovese T, Caputi AP, Zhang J, Cuzzocrea S. GPI 6150, a PARP inhibitor, reduces the colon injury caused by dinitrobenzene sulfonic acid in the rat. *Biochem Pharmacol* 2002;**64**:327–37
- 10 Cuzzocrea S, Zingarelli B, Gilad E, Hake P, Salzman AL, Szabo C. Protective effects of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthase in a carrageenan-induced model of local inflammation. *Eur J Pharmacol* 1998;**342**:67–76
- 11 Murakami K, Enkhbaatar P, Shimoda K, Cox RA, Burke AS, Hawkins HK, Traber LD, Schmalstieg FC, Salzman AL, Mabley JG, Komjati K, Pacher P, Zsengeller Z, Szabo C, Traber DL. Inhibition of poly (ADP-ribose) polymerase attenuates acute lung injury in an ovine model of sepsis. *Shock* 2004;**21**:126–33
- 12 Guven A, Demirbag S, Uysal B, Topal T, Erdogan E, Korkmaz A, Ozturk H. Effect of 3-amino benzamide, a poly(adenosine diphosphate-ribose) polymerase inhibitor, in experimental caustic esophageal burn. *J Pediatr Surg* 2008;**43**:1474–9
- 13 Oztas E, Guven A, Turk E, Uysal B, Akgul EO, Cayci T, Ersoz N, Korkmaz A. 3-Aminobenzamide, a poly ADP ribose polymerase inhibitor, attenuates renal ischemia/reperfusion injury. *Ren Fail* 2009;**31**:393–9
- 14 Chen HM, Shyr MH, Ueng SW, Chen MF. Hyperbaric oxygen therapy attenuates pancreatic microcirculatory derangement and lung edema in an acute experimental pancreatitis model in rats. *Pancreas* 1998;**17**:44–9
- 15 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;**193**:265–75
- 16 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;**95**:351–8
- 17 Durak I, Yurtarslan Z, Canbolat O, Akyol O. A methodological approach to superoxide dismutase (SOD) activity assay based on inhibition of nitroblue tetrazolium (NBT) reduction. *Clin Chim Acta* 1993;**214**:103–4
- 18 Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;**70**:158–69
- 19 Alrashed M, Abougoush M, Akgul EO, Erbil MK. Detection method of serum and urine neopterin levels by high performance liquid chromatography and clinical applications. *Gulhane MJ* 2002;**44**:273–7 (in Turkish)
- 20 Buscaglia JM, Simons BW, Prosser BJ, Ruben DS, Giday SA, Magno P, Clarke JO, Shin EJ, Kalloo AN, Kantsevov SV, Gabrielson KL, Jagannath

- SB. Etanercept, a TNF-alpha binding agent, is ineffective in the prevention of post-ERCP pancreatitis in canines. *J Pancreas* 2008;**9**:456-67
- 21 Virag L, Szabo E, Gergely P, Szabo C. Peroxynitrite-induced cytotoxicity: mechanism and opportunities for intervention. *Toxicol Lett* 2003;**140-141**:113-24
 - 22 Szabo C. Multiple pathways of peroxynitrite cytotoxicity. *Toxicol Lett* 2003;**140-141**:105-12
 - 23 Szabo C. Poly(ADP-ribose) polymerase activation by reactive nitrogen species - relevance for the pathogenesis of inflammation. *Nitric Oxide* 2006;**14**:169-79
 - 24 Korkmaz A, Yaren H, Topal T, Oter S. Molecular targets against mustard toxicity: implication of cell surface receptors, peroxynitrite production, and PARP activation. *Arch Toxicol* 2006;**80**:662-70
 - 25 Korkmaz A, Topal T, Oter S. Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of reactive oxygen and nitrogen species as well as PARP activation. *Cell Biol Toxicol* 2007;**23**:303-12
 - 26 Yaren H, Mollaoglu H, Kurt B, Korkmaz A, Oter S, Topal T, Karayilanoglu T. Lung toxicity of nitrogen mustard may be mediated by nitric oxide and peroxynitrite in rats. *Res Vet Sci* 2007;**83**:116-22
 - 27 de la Lastra CA, Villegas I, Sanchez-Fidalgo S. Poly(ADP-ribose) polymerase inhibitors: new pharmacological functions and potential clinical implications. *Curr Pharm Des* 2007;**13**:933-62
 - 28 Korkmaz A, Kurt B, Yildirim I, Basal S, Topal T, Sadir S, Oter S. Effects of poly(ADP-ribose) polymerase inhibition in bladder damage caused by cyclophosphamide in rats. *Exp Biol Med (Maywood)* 2008;**233**:338-43
 - 29 Berdowska A, Zwirska-Korczala K. Neopterin measurement in clinical diagnosis. *J Clin Pharm Ther* 2001;**26**:319-29
 - 30 Uomo G, Spada OA, Manes G, Feola B, Misso S, Cavallera A, Rabitti PG. Neopterin in acute pancreatitis. *Scand J Gastroenterol* 1996;**31**:1032-6
 - 31 Kaufmann P, Tilz GP, Demel U, Wachter H, Kreijs GJ, Fuchs D. Neopterin plasma concentrations predict the course of severe acute pancreatitis. *Clin Chem Lab Med* 1998;**36**:29-34
 - 32 Uysal B, Yasar M, Ersoz N, Coskun O, Kilic A, Cayc T, Kurt B, Oter S, Korkmaz A, Guven A. Efficacy of hyperbaric oxygen therapy and medical ozone therapy in experimental acute necrotizing pancreatitis. *Pancreas* 2010;**39**:9-15
 - 33 Obrosova IG, Drel VR, Pacher P, Ilnytska O, Wang ZQ, Stevens MJ, Yorek MA. Oxidative-nitrosative stress and poly(ADP-ribose) polymerase (PARP) activation in experimental diabetic neuropathy: the relation is revisited. *Diabetes* 2005;**54**:3435-41
 - 34 Devalaraja-Narashimha K, Singaravelu K, Padanilam BJ. Poly(ADP-ribose) polymerase-mediated cell injury in acute renal failure. *Pharmacol Res* 2005;**52**:44-59
 - 35 Chatterjee PK, Zacharowski K, Cuzzocrea S, Otto M, Thiemeermann C. Inhibitors of poly (ADP-ribose) synthetase reduce renal ischemia-reperfusion injury in the anesthetized rat *in vivo*. *FASEB J* 2000;**14**:641-51
 - 36 Beger HG, Bittner R, Block S, Buchler M. Bacterial contamination of pancreatic necrosis. A prospective clinical study. *Gastroenterology* 1986;**91**:433-8
 - 37 Medich DS, Lee TK, Melhem MF, Rowe MI, Schraut WH, Lee KK. Pathogenesis of pancreatic sepsis. *Am J Surg* 1993;**165**:46-50
 - 38 Marotta F, Geng TC, Wu CC, Barbi G. Bacterial translocation in the course of acute pancreatitis: beneficial role of nonabsorbable antibiotics and lactitol enemas. *Digestion* 1996;**57**:446-52
 - 39 Tewari M, Quan LT, O'Rourke K, Desnoyers S, Zeng Z, Beidler DR, Poirier GG, Salvesen GS, Dixit VM. Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* 1995;**81**:801-9
 - 40 Liaudet L, Soriano FG, Szabo E, Virag L, Mabley JG, Salzman AL, Szabo C. Protection against hemorrhagic shock in mice genetically deficient in poly(ADP-ribose) polymerase. *Proc Natl Acad Sci USA* 2000;**97**:10203-8
 - 41 Cuzzocrea S, Mazzon E, De Sarro A, Caputi AP. Role of free radicals and poly(ADP-ribose) synthetase in intestinal tight junction permeability. *Mol Med* 2000;**6**:766-78

(Received March 9, 2010, Accepted April 30, 2010)