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The effects of p53 inhibition using pifithrin-α on acute necrotizing pancreatitis in rats*

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Aim: To investigate the effects of p53 inhibition using pifithrin-α (PFA) on acute necrotizing pancreatitis (ANP) induced by glycodeoxycholic acid in rats. The role of p53 during acute pancreatitis is unclear.

Materials and methods: Rats were divided into 4 groups, 2 of which were initially given only saline (the sham group) and 2 of which were given glycodeoxycholic acid in order to induce ANP. Each group was then identified according to treatment plan: sham+saline, sham+PFA, ANP+saline, or ANP+PFA. The study then investigated the extent of acinar cell injury, apoptosis, mortality, systemic cardiorespiratory variables, functional capillary density (FCD), renal/hepatic functions, and changes in some enzyme markers for pancreatic and lung tissue during ANP in rats.

Results: The induction of ANP resulted in a significant increase in mortality rate, pancreatic necrosis, apoptosis, serum activity of amylase, alanine aminotransferase (ALT), interleukin (IL)-6, lactate dehydrogenase (LDH) in bronchoalveolar lavage (BAL) fluid, serum concentration of urea, and tissue activity of myeloperoxidase (MPO) and malondialdehyde (MDA) in the pancreas and lung. In addition, ANP induction caused a significant decrease in concentrations of calcium, blood pressure, urine output, pO2, and functional capillary density (FCD). The use of PFA did not alter these changes.

Conclusion: PFA demonstrated no effect on the course of ANP in rats. Therefore, it has no value in the treatment of acute pancreatitis.

Key words: Acute pancreatitis, p53, pifithrin-α

Pifithrin-α ile oluşturulan p53 inhibisyonunun sıçanlarda akut nekrotizan pankreatite etkileri

Amaç: p53 geninin akut pankreatit seyri esnasında rolü bilinmemektedir. Çalışmanın amacı pifithrin-α (PFA) kullanarak sağlanan p53 inbisyonunun glikodeoksikolik asitle oluşturulan akut nekrotizan pankreatite(ANP) etkisi araştırmaktır.

Yöntem ve gereç: Sıçanlar sham+salin, sham+PFA, ANP+salin ve ANP+PFA olmak üzere 4 gruba ayrıldı. ANP glikodeoksikolik asitle oluşturuldu. Pankreatik injuri, apoptozis, mortalite, sistemik kardiovasküler parametreler, fonksiyonel kapiller dansite (FCD), böbrek, karaciğer fonksiyonları ve pankreas ve akciğer dokusunda bazı enzim belirteçleri incelendi.

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Bulgular: Pankreatit indüksiyonu mortaliteyi, pankreatik nekrozu, apopitozisi, serum amilaz, üre, alanin aminotransferazı (ALT), interlökin(IL)-6, bronkoalveolar lavajda laktik dehidrogenazı (LDH), pankreas ve akciğer dokularında myeloperoksidaz (MPO), malondialdehit (MDA) önemli olarak artırdı. Serum kalsiyum, kan basıcı, idrar debisi, pO2, FCD azalttı. PFA kullanılması bu değişikleri değiştirmedi.

Sonuç: PFA kullanılması pankreatit seyrini etkilemedi. Dolayısıyla PFA nın akut pankreatit tedavisinde yeri olmadığını düşündürmektedir.

Anahtar sözcükler: Akut pankreatit, p53, pifithrin-α

Introduction

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

Autodigestion of the pancreas and the impairment of pancreatic microcirculation are 2 important parts in the pathophysiology of acute pancreatitis (2-4). The severity of the local inflammation and the systemic complications are related to a number of factors: the excessive up-regulation of cytokines; secondary mediators including histamines, prostaglandins, thromboxanes, leukotrienes, nitric oxide, platelet-activating factor, and polymorphonuclear leukocyte functions; the amount of pancreatic acinar necrosis; and the development of bacterial contamination (1,2,5,6). In the early stage of the disease, hypovolemia, resulting from fluid sequestration in the peripancreatic areas and abdominal cavity, may cause increased metabolic demand; later, a septic complication caused by bacteria translocated from the gut may prompt systemic inflammation, potentially progressing to clinical multiorgan failure (1,2,7).

Acinar cell death is a hallmark of both human and experimental acute pancreatitis (8,9). The pathogenesis of acinar cell death in pancreatitis is not clearly understood. Acinar cell death in acute pancreatitis occurs by both necrosis and apoptosis. Apoptosis is essentially cell suicide, the result of a built-in self-destruct mechanism consisting of a genetically programmed sequence of biochemical events. As the cell dies, it curls up, the chromatin in the nucleus condenses into a dense mass, cytoplasm shrinks, blebbing of the plasma membrane occurs and, finally, phagocytosis by macrophages takes place (9). There are 2 main routes to cell death: the first involves the stimulation of death receptors by external glands such as CD95 (Fas), tumor necrosis factor (TNF) itself, or TRAIL (TNF-α-related apoptosis-inducing ligand), while the other arises within the cell and involves the mitochondria (10-13). Both of these routes activate initiator caspases and both converge on a final common effector caspase pathway, and there is crosstalk between the death receptor pathway and the mitochondrial pathway (14). The mitochondrial pathway is activated by internal factors such as DNA damage, which results in the transcription of gene p53 (15). The p53 protein activates a subpathway that causes cytochrome c to be released from the mitochondrion (12). This, in turn, combines with protein apoptotic protease-activating factor-1 and together they activate initiator caspase 9 (11-13,16). The effector caspases (caspase 3) start a pathway that results in the cleavage of cell constituents, DNA, cytoskeletal components, and enzymes. This reduces the cell to a cluster of membrane-bound entities that are eventually phagocytosed by macrophages (16). In undamaged cells, survival factors such as cytokines, hormones, and cell-to-cell contact factors continuously activate the antiapoptotic mechanism. Withdrawal of survival factors' stimulation causes cell death through the mitochondrial pathway (17,18).

Apoptotic cell death during acute pancreatitis has been reported in a number of pancreatitis-relevant models (9). The importance of apoptosis during acute pancreatitis and its relationship with multiorgan failure have not been conclusively defined. Some

experimental studies have indicated that caspase 8 activation, oxidative stress, the Bcl-2 family, stress-induced protein genes, nuclear factor KB, neutrophil activation, and TNF-α may be involved, leading to apoptosis during acute pancreatitis (9,19-24).

Transcription factor p53 induces the expression of multiple genes whose products are implicated in cell cycle arrest or apoptosis (25). Most of the encoding proteins of the p53-induced apoptosis-related genes affect the mitochondrial pathway and, in addition, produce membrane death and decoy receptors of the TNF receptor family (25). The role of p53 during acute pancreatitis is unclear. Pifithrin-α (PFA) selectively inhibits p53 transcriptional activity in various mouse cell lines, prevents DNA damage-induced apoptosis in those cells, and improves hepatic microcirculation after lipopolysaccharide exposure (26,27).

Therefore, in this study, we examined the effect of PFA as a p53 gene inhibitor on the extent of acinar cell injury, apoptosis, mortality, systemic cardiorespiratory variables, functional capillary density (FCD), renal/hepatic functions, and changes in enzyme markers for pancreatic and lung tissue during ANP in rats.

Materials and methods

This study used 52 male Sprague-Dawley rats, each weighing 300-350 g. They were housed in rooms maintained at 21 ± 1 °C with a light:dark cycle of 12:12 h. Animals fasted overnight before the experiment but had free access to water. All care was provided in accordance with the Ethics Committee of Karadeniz Technical University, Trabzon, Turkey.

Anesthesia was induced with vaporized ether and maintained by an intraperitoneal injection of ketamine at a dosage of 50 mg/kg (Ketalar, Eczacıbası, İstanbul, 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 a quantitative assessment of urine production.

Acute pancreatitis was induced by an intravenous infusion of cerulein (Sigma & Aldrich Chemie GmbH, Steinheim, Germany) at an hourly dose of 5 µg/kg over a period of 6 h, superimposed on a standard infusion of 1.2 mL/kg glycodeoxycholic acid (10 mmol/L, Sigma, St. Louis, MO, USA) into the biliary-pancreatic duct for 10 min at 30 mmHg as described by Schmidt et al. (28). 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 hourly at 8 mL/kg 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 4 experimental groups (Figure 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. Following the 6-h period, 1 mL of saline was given intraperitoneally and saline was infused intravenously at 6 mL/kg for the last 18 h. Cardiorespiratory function was assessed 18 h later by monitoring the arterial blood gases, mean arterial pressure (MAP), heart rate

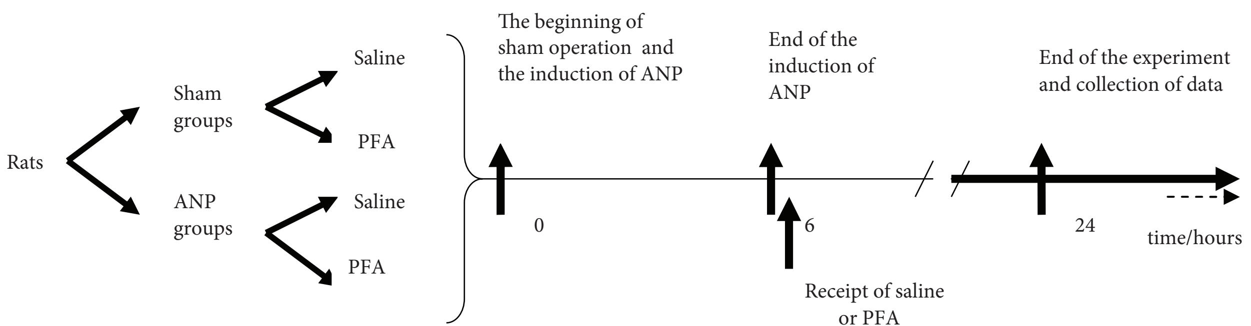

Figure 1. Experimental desing and time schedule of the study. PFA: pifithrin-α; ANP: acute necrotizing pancreatitis.

(HR), renal function by the collection of urine using metabolic cages, and survival. Rats with MAP < 80 mmHg, pO_2 < 80 mmHg, pCO_2 > 50 mmHg, and pH < 7.3 were excluded from the study. The rats were again anesthetized by ketamine and laparotomy was performed. The pancreas and spleen were exposed on an adjustable stage. An orthogonal polarization imaging video microscope (Cytoscan A/R, Cytometrics, Philadelphia, PA, USA) was attached to the moveable shaft and the microcirculation was recorded in 6 different capillary regions of the exocrine pancreas for at least 20 s (29). The images were stored in AVI format on the computer (Sony VGN-FW230J/H). Thereafter, blood samples were taken from the carotid artery in order to measure the serum concentrations of electrolytes, calcium, urea, creatinine, and glucose as well as the activities of amylase, alanine aminotransferase (ALT), and interleukin (IL)-6. A midline sternotomy was performed and the left main bronchus was clamped. Bronchoalveolar lavage (BAL) of the right lung was performed with 2 mL of phosphate-buffered saline containing 0.07 M ethylene diamine tetraacetic acid (EDTA), and this procedure was repeated twice. The combined lavage of approximately 6 mL was centrifuged at 1500 rpm for 20 min at 4 °C, frozen at −20 °C, and assessed subsequently for lactate dehydrogenase (LDH) measurement (30). The left lung was harvested for the measurements of the activity of the myeloperoxidase (MPO) and malondialdehyde (MDA) levels. The excised lung tissues were rinsed in saline, blotted dry, frozen in liquid nitrogen, and stored frozen at −80 °C until thawed in order to measure MPO and MDA activity. At the end of the experiment, the entire pancreas was removed. The pancreas was divided into 2 parts, the first for the histological examination and the other for the measurements of MPO and MDA activity.

The second group (sham+PFA, n = 10) was treated according to the protocol of Group 1, but, instead of saline, 2.2 mg/kg PFA (Sigma, Germany, code number P4539) was given intraperitoneally after 6 h (26,27). The third group (ANP+saline, n = 16) was treated according to the protocol of Group 1 after the induction of ANP. In the fourth group (ANP+PFA, n = 16 group), ANP was induced and PFA was given, as in Group 2. Saline or PFA was given to the pancreatitis groups 6 h after the induction of pancreatitis. Since ANP in small animals occurs 4-6 times faster than in humans and most patients with acute pancreatitis are admitted 24-36 h after the onset of pancreatitis, this period closely mirrors the clinical situation (31).

Blood pressure and HR were measured with a pressure monitor (Petas KLM 200, İstanbul, Turkey) by connecting the arterial line to a pressure transducer. The blood gases were analyzed using a Ciba Corning 865 analyzer (Chiron Diagnostics Co., East Walpole, MA, USA). The serum activities of amylase and ALT, and the concentrations of glucose, creatinine, urea, calcium, LDH in BAL, and electrolytes were measured by an autoanalyzer (Vitros 750 autoanalyzer, Johnson & Johnson, Rochester, NY, USA).

Orthogonal polarization imaging has been suggested for recording and quantifying changes in microcirculation (29). The technique uses the optical filtration of polarized light absorbed by hemoglobin so that red blood cells appear dark. The recorded images were analyzed using the MAS image analysis software system, developed by Dr. Iwan Dobbe, Professor Can Ince, and Dr. K.R. Mathura at the Academic Medical Center, University of Amsterdam, Netherlands. FCD, identified as the best parameter for the measurement of microcirculation, was defined by Messmer (29) as the length of red blood cell-perfused capillaries (cm) per observation area (cm^2). We thus selected FCD as the parameter for measurement of the microcirculation.

Serum IL-6 concentrations were measured with a commercial ELISA kit (IL-6, BioSource Cat. No. BMS 625) and an ELISA reader (Sanofi Diagnostic Pasteur LP 35, Marnes-la-Coquette, France).

The tissue-associated MPO activity was assessed by a modification of the method described by Schierwagen et al. (6). Protein concentrations of supernatants were measured by Lowry's method (6). MPO activity was expressed as U/mg protein.

The lipid peroxidation in tissues was assessed by measuring the concentration of MDA using a colorimetric reaction with thiobarbituric acid in a modification of the method described by Buege and Aust (6). The protein concentrations of supernatants were measured by Lowry's method (6). MDA concentrations were expressed as nmol/mg protein.

Finally, half of the pancreas was fixed in 10% buffered formalin and, after sectioning, it was stained with hematoxylin and eosin. The histopathological evaluation was conducted by 2 experts in pancreatic pathology (C.E., D.F.). They were blind to both the induction technique and the additional drugs given. Edema, acinar necrosis, and inflammation were assessed using a scoring system from 0 (no pathologic changes) to 3 (maximum inflammatory infiltration, total necrosis of the pancreas), as previously described (28). Apoptosis was observed with the immunohistochemical staining method using the Fas CD95 mouse monoclonal antibody (NCL-Fas-310, Sitogen, İstanbul, Turkey) (32). Membranous staining in small bowel cells was used as the positive control. For the evaluation of Fas CD95, comprehensive scoring made use of the method of Shibakita et al. (33). All histological slides were examined by 2 observers (M.A., DF.) who were completely blind to the experimental data. Apoptosis was expressed as an apoptotic index (AI), the ratio of positively stained apoptotic cells to all acinar cells.

Results are presented as mean ± (SEM). The significance of the differences in survival rates was assessed by Fisher's exact test while histopathological results and enzyme activities were assessed by the Kruskal-Wallis and Mann-Whitney U tests. Differences were considered significant at $P < 0.05$.

Results

The mortality rate was found to be 0% in the sham+saline group, 0% in the sham+PFA group, 31% in the ANP+saline group, and 37.5% in the ANP+PFA group. There was a significant difference between the pancreatitis and sham groups ($P < 0.05$, Table 1).

The induction of ANP led to a significant decrease in MAP, an increase in HR, and a reduction of urine volume, pO_2, and FCD. The administration of PFA did not change these alterations (Table 1).

The induction of pancreatitis resulted in a significant increase in the serum activity of amylase, ALT, IL-6, and serum urea (Table 2), and a decrease in serum calcium concentration in the pancreatitis groups (Table 2, $P < 0.05$). Serum glucose and creatinine values showed no changes in any group (Table 2).

A significant increase in LDH in BAL was observed in the pancreatitis groups ($P < 0.05$, Table 2). Considerable increases in MPO and MDA activities in the lung and pancreatic tissues occurred after the induction of acute pancreatitis in the pancreatitis groups (Table 2).

According to the histological examination, the ANP groups had greater edema, necrosis, and

Table 1. Systemic hemodynamic variables, blood gas analysis, mortality, and functional capillary density (FCD) at 24 h; data are given as mean ± SEM.

	Sham+saline (n = 10)	Sham+PAF (n = 10)	ANP+saline (n = 11)	ANP+PFA (n = 10)
MAP (mmHg)	133 ± 8	129 ± 7	83 ± 4*	85 ± 4*
HR (beats/min)	322 ± 21	325 ± 20	392 ± 17*	398 ± 21*
pH	7.36 ± 0.04	7.35 ± 0.05	7.3 ± 0.04	7.31 ± 0.04
pO_2 (mmHg)	110 ± 4	108 ± 3	82 ± 5*	84 ± 4*
pCO_2 (mmHg)	33 ± 3	34 ± 4	38 ± 4	39 ± 5
Urine (mL/h)	0.7 ± 0.1	0.8 ± 0.1	0.35 ± 0.1**	0.26 ± 0.1**
Death	0/10	0/10	5/16*	6/16*
FCD cm/cm^2	32 ± 2	33 ± 2.4	20.2 ± 2.1*	22 ± 1.8*

MAP: mean arterial pressure; HR: heart rate.
*P < 0.05, **P < 0.01, when compared to the sham groups

Table 2. Serum IL-6, tissue concentrations of MPO and MDA, LDH levels in BAL, serum levels of amylase, and other biochemical parameters among all groups at 24 h; data are given as mean ± SEM.

	Sham+saline (n = 10)	Sham+PAF (n = 10)	ANP+saline (n = 11)	ANP+PFA (n = 10)
IL-6 (pg/mL)	53 ± 4	51 ± 5	103 ± 11*	83 ± 9*
Amylase (U/L)	1632 ± 16	1480 ± 33	12,369 ± 301**	14330 ± 420**
Glucose (mg %)	76 ± 4	79 ± 5	107 ± 5	89 ± 8
Urea (mg %)	19 ± 2	18 ± 3	45 ± 7*	49 ± 8*
Creatinine (mg %)	0.47 ± 0.1	0.43 ± 0.1	0.45 ± 0.1	0.48 ± 0.1
ALT (U/dL)	68 ± 7	66 ± 8	220 ± 15*	215 ± 12*
Calcium (mg %)	8.9 ± 0.2	9.1 ± 0.2	8.0 ± 0.2*	8.1 ± 0.2
BAL LDH (U/dL)	353 ± 25	380 ± 20	789 ± 92*	1090 ± 112*
Lung MPO (U/mg protein)	3.3 ± 0.3	3.1 ± 0.3	6.3 ± 0.3*	6.6 ± 1.2*
Lung MDA (nmol/mg protein)	0.26 ± 0.02	0.27 ± 0.03	0.4 ± 0.03*	0.43 ± 0.03*
Pancreatic MPO (U/mg protein)	0.35 ± 03	0.36 ± 0.05	0.81 ± 0.0.6*	0.96 ± 0.07*
Pancreatic MDA (nmol/mg protein)	0.24 ± 0.03	0.26 ± 0.03	0.51 ± 0.15*	0.45 ± 0..03*

*P < 0.05, **P < 0.01, when compared to the sham groups.

MPO: myeloperoxidase, **MDA:** malondialdehyde, **LDH:** lactate dehydrogenase, **BAL:** bronchoalveolar lavage, **ANP:** acute necrotizing pancreatitis, **IL:** interleukin, **ALT:** alanine aminotransferase.

leukocyte infiltration than the sham groups (Table 3). Similar necrosis was observed in the ANP+saline group and the ANP+PFA group (Table 3, Figure 2). The induction of acute pancreatitis resulted in increased AI in the pancreatitis groups (Table 3, Figure 3). The highest AI was observed in the ANP+PFA group, but there was no significant difference between the pancreatitis groups.

Discussion

The ANP model described by Schmidt et al. (28) was used in this study. This model provides a superior opportunity to study an innovative treatment by standard processes. ANP in small animals is 4-6 times faster than in humans and most patients with acute pancreatitis are admitted 24-36 h after its onset (31). Therefore, we administered PFA 6 h after the induction of pancreatitis. We chose severe forms of acute pancreatitis as an induction method for pancreatitis because mild or moderate forms of acute pancreatitis can be treated with minimal morbidity and mortality (2). The first step in the treatment of acute pancreatitis is appropriate fluid replacement, so we used aggressive fluid replacement to maximize the organ function (6 mL/kg, hourly) after the induction of ANP.

In 1999, PFA was shown to selectively inhibit p53 transcriptional activity in various mouse cell lines and to prevent DNA damage-induced apoptosis in those cells (26). This small molecule has been further demonstrated to protect normal cells, but it has not successfully been used on cells from p53-null mice, or in vivo against death induced by anticancer treatment (26). The dose and route of application of PFA (2.2 mg/kg intraperitoneally) were chosen in accordance with the above-referenced report (26). The ideal log P value of PFA at a physiological pH allows us to predict at high permeability, not only at the blood-brain barrier (26) but also at the gastrointestinal tract (26,27). The same dose and route were used in heart tissue by Liu et al. (34) and in liver tissue by Schafer et al. (27). Therefore, we administered the same 2.2 mg/kg intraperitoneal dose of PFA in our study.

Table 3. Histological assessment of edema, necrosis, inflammation, and apoptosis (values are given as mean ± SEM).

	Sham+saline (n = 10)	Sham+PAF (n = 10)	ANP+saline (n = 11)	ANP+PFA (n = 10)
Edema	0.5 ± 0.1	0.4 ± 0.2	0.82 ± 0.1*	0.72 ± 0.1*
Necrosis	0.2 ± 0.2	0.2 ± 0.2	1.45 ± 0.2**	1.54 ± 0.2**
Inflammation	0.2 ± 0.2	0.2 ± 0.2	1.19 ± 0.3**	1.12 ± 0.2*
Apoptotic index (%)	0.2 ± 0.2	0.2 ± 0.2	1.42 ± 0.4**	1.51 ± 0.4**

*P < 0.05, **P < 0.01, when compared to the sham groups.

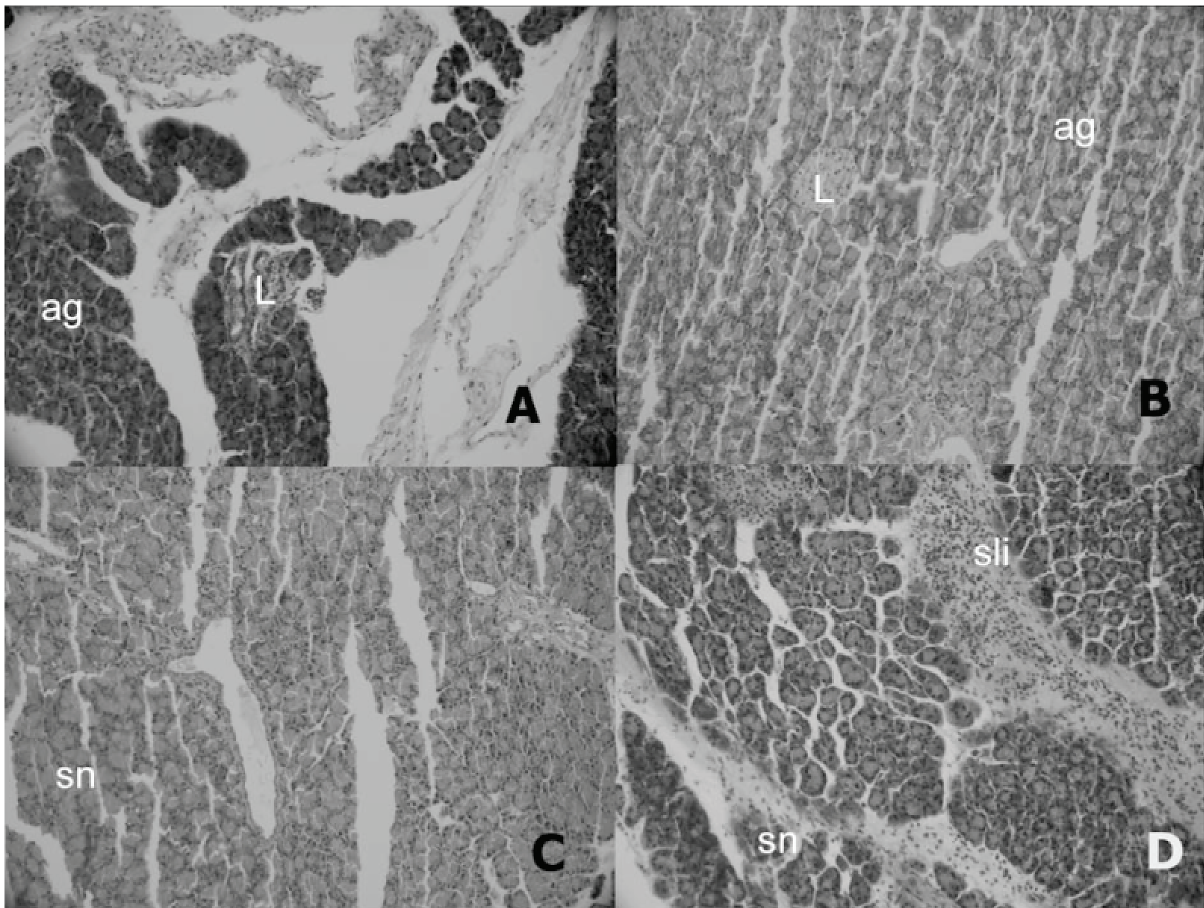

Figure 2. A) Dual composition of normal pancreas in sham+saline group (hematoxylin-eosin [HE], ×200 magnification; **ag**: lobular units of acini of the exocrine pancreas, **L**: Langerhans islets of the endocrine component), B) a similar pattern in the sham+PAF group (HE, ×200), C) severe acinar necrosis in the ANP+saline group (HE, ×200; **sn**: severe necrosis), D) severe necrosis and leukocyte infiltration in the ANP+PAF group (HE, ×200; **sli**: severe leukocyte infiltration).

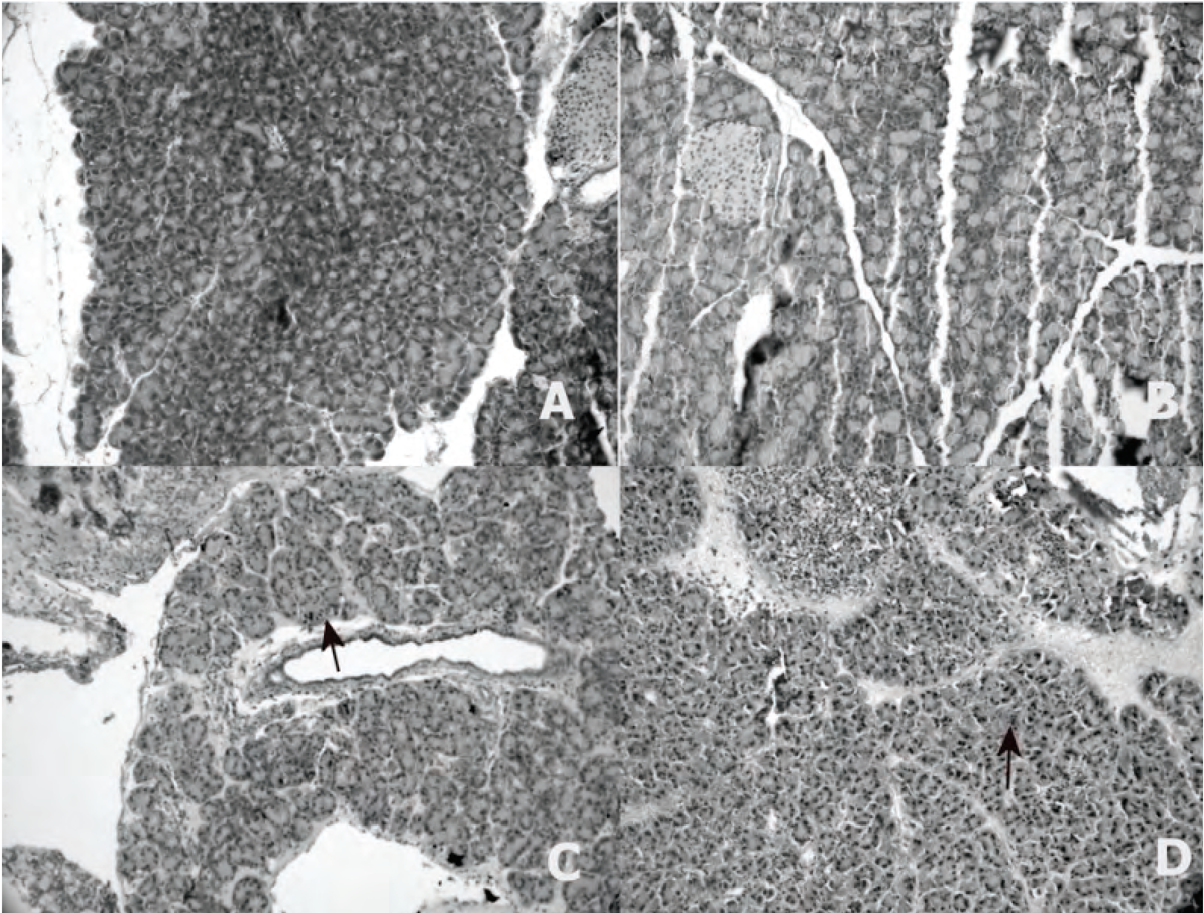

Figure 3. A) No CD95 Fas staining in pancreatic acinar cells in the sham+saline group (FAS, ×200), B) similar pattern in the sham+PAF group (FAS, ×200), C) slight CD95 Fas staining in the ANP+saline group (FAS, ×200; the arrow (↑) indicates Fas staining in the membrane of the acinar cell), D) slight CD95 Fas staining in the ANP+PAF group (FAS, ×200).

Pancreatic necrosis is the hallmark of severe pancreatitis (8). Autodigestion of the pancreas by activated digestive enzymes, ischemia of the pancreas, and ischemia-related reactive oxygen species (ROS)-associated lipid peroxidation cause pancreatic damage during acute pancreatitis (2-4,8,9). Microcirculation disturbance is an important pathogenetic factor during acute pancreatitis (3-5). Microcirculatory perfusion of the pancreas depends on many factors, including the systemic oxygenation and blood flow of the pancreas, the distribution of flow within the pancreas, and oxygen consumption by the pancreas. Blood flow is a function of inflow, arteriolar regulation, congestion, shunting, and opening or closing capillary beds (3,4). The microcirculation of the pancreas can be measured by diffuse reflectance spectroscopy, intravital microscopy, multiple indicator dilution technique, and orthogonal polarization imaging (3,4,29). We first measured pancreatic microcirculation by orthogonal polarization imaging in this study. The induction of pancreatitis resulted in a decrease in FCD. Many authors have reported similar results using intravital microscopy, diffuse reflectance spectroscopy, or orthogonal polarization imaging during acute pancreatitis (3,4,29). The use of PFA did not change FCD in our study, however. Schafer et al. (27) reported that PFA protected liver microcirculation after endotoxin-induced injury. While they gave PFA a short time after the injury, we administered PFA 6 h after the induction of acute pancreatitis. In this study, MPO and MDA in pancreatic tissue as ischemia

mediators increased in the pancreatitis groups. These results show a parallelism with FCD findings.

Apoptosis is another factor that results in acinar cell death. Caspase 8 activation, oxidative stress, the Bcl-2 family, stress-induced protein genes, transcription factor p53, nuclear factor KB, neutrophil activation, and TNF-α may be involved, leading to apoptosis during acute experimental pancreatitis (9,19-24). Recent studies noted that the form of acinar cell death itself may be an important determinant of the severity of acute pancreatitis. Bhatia (9,35) suggested that there is an inverse correlation between the extent of apoptosis on the one hand and necrosis and the severity of disease on the other, as observed in experimental models of pancreatitis. In these studies, mild acute pancreatitis was found to be associated with extensive apoptotic acinar cell death while severe acute pancreatitis was found to involve extensive acinar cell necrosis but very little acinar cell apoptosis (9,35). As we selected the severe form of acute pancreatitis as an induction method for pancreatitis, we did not observe extensive apoptosis in the pancreatitis groups.

We found increased apoptosis after the induction of pancreatitis, when compared to sham groups. Many authors reported similar results (9). Despite our hypothesis that the use of PFA would decrease apoptosis, we found no differences between the pancreatitis groups in our study. We administered PFA 6 h after the induction of ANP, perhaps explaining the results in this study. Recently, Yuan et al. (16,36) reported that pancreatic acinar cell apoptosis correlated with the expression of the apoptosis-regulated gene Bax but had no relationship with the expression of p53. These findings may support our results. On the other hand, oxidative stress is regarded as a major pathogenetic factor in acute pancreatitis and it induces apoptosis of acinar cells involving activation of caspase-3, which degrades the DNA repair proteins Ku 70 and Ku 80 (20). The similar MPO and MDA levels in pancreatic tissue in the pancreatitis groups may have caused the same AI shared by the pancreatitis groups in this study.

Acute respiratory distress syndrome (ARDS) occurs often in the early stage of severe pancreatitis and may be related to early death (37). The activation of leukocytes and increased capillary permeability both play a major role in the pathogenesis of ARDS. Activated leukocytes damaged pulmonary basement membranes by generating ROS and increased capillary permeability (36). We used LDH levels in BAL fluid for the assessment of capillary permeability (30). The induction of ANP in our study resulted in the increased activities of MPO and MDA in lung tissue, LDH in BAL fluid, and decreased pO_2. The use of PFA did not reverse these changes in the present study. To our knowledge, there is no study in the literature with which we can compare our results.

During the course of acute pancreatitis, several biochemical abnormalities develop and reflect alterations in various organs and metabolic pathways within the affected cells. Severe parenchymal damage has been reported in the liver, lungs, kidneys, and heart in bile-induced pancreatitis (2,6). Patients and animals with pancreatitis die due to multiorgan failure through the development of systemic inflammatory response to pancreatic injury by the activation of various enzymes, cytokines, and vasoactive substances (38). After the induction of ANP in our study, hypotension occurred, together with an increased concentration of serum urea and ALT activity and decreased concentration of serum calcium and urine volume. The use of PFA did not alter these changes. However, we were unable to find any study in the literature with which to compare our results.

Some markers, such as trypsinogen-activated peptide, C-reactive protein, TNF-α, IL-6, and IL-10, can be used as an index of the severity and outcome of the disease (39). We measured serum IL-6 as a marker and found that the use of PFA did not improve serum IL-6 levels in our study. This finding correlated with the pancreatic damage and mortality rate. Despite our expectation of an inhibitory effect of PFA on cytokine release, we observed no effect on the serum IL-6. This may be explained by the fact that we administered PFA 6 h after the induction of ANP.

We concluded that ischemia of the pancreas and apoptosis are major causes of pancreatic necrosis. Multiorgan failure induced through ischemia-related ROS, which was produced by activated leukocytes, has an important role in the progression of acute pancreatitis, and the use of PFA did not improve

cardiovascular, hepatic/lung/renal functions, serum IL-6, or pancreatic microcirculation. It was not found to reduce pancreatic apoptosis, mortality, or pancreatic damage during acute pancreatitis. Moreover, PFA has limited effects during the course of ANP in rats; therefore, one can speculate that p53 is not playing a role in the late phase of acute experimental pancreatitis in rats. However, further studies on this subject are required.

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