

The role of inflammatory mediators in severe acute pancreatitis and regulation of glucocorticoids

Zi-Fa Wang, Cheng-En Pan, Yi Lu, Shao-Gao Liu,
Guan-Jun Zhang and Xue-Bin Zhang

Xi'an, China

OBJECTIVE: To investigate the effect of glucocorticoids on systemic inflammatory mediator release in rats with acute pancreatitis and the outcome of dexamethasone in treatment of acute pancreatitis.

METHODS: Sixty-eight Wistar rats were divided into sham, acute pancreatitis, and treatment (intravenous dexamethasone 0.5 mg/kg) groups. Experimental acute pancreatitis was induced by the injection of 5% sodium taurocholate (0.1 ml/100 mg body weight) into the pancreatic-biliary duct. The blood samples were obtained and examined for 6-keto-PGI₁α, TXB₂ and IL-6 postoperatively at 3, 6 and 12 hours, respectively. The pancreatic samples were evaluated by a blinded method. Twelve-hour survival rate was determined and compared between the groups.

RESULTS: The high serum concentrations of 6-keto-PGI₁α, TXB₂ and IL-6 were noted in the rats with acute pancreatitis associated with pancreatic hemorrhage and necrosis. Their 12-hour survival rate was 42.9%. The rats in the treatment group survived with significantly reduced serum concentrations of 6-keto-PGI₁α, TXB₂ and IL-6 ($P < 0.05$). Their pancreatic morphology was normal.

CONCLUSION: Dexamethasone may reduce the serum concentration of 6-keto-PGI₁α, TXB₂, and IL-6, and the severity of acute pancreatitis while increasing the survival rate of rats with acute pancreatitis.

(*HBPD Int* 2003; 2: 458-462)

Key words: acute pancreatitis; inflammatory mediators; dexamethasone

Introduction

Acute pancreatitis (AP) is usually mild and self-limited. However, 15%–20% of patients

with AP may develop organ failure or local complications including necrosis, pseudocyst and abscess. The patients with severe AP who develop organ failure during the first few days of illness account for the majority of early deaths. Although a number of treatments are currently available for the treatment of AP, they have failed to significantly affect the disease. It is known that the activation of trypsin is the trigger of AP. The key to understanding the pathophysiology of AP lies in discovering why a proportion of patients develop from a limited local inflammation to a potentially dangerous systemic inflammatory response. It has been proposed that the systemic sequelae of AP arise from excessive leukocyte activation with the release of secondary inflammatory mediators including interleukin (IL)-1α, IL-6, IL-8, IL-10; tumor necrosis

From the Departments of Surgery (Wang ZF, Pan CE, Lu Y and Liu SG); and Pathology (Zhang GJ and Zhang XB), First Hospital, Xi'an Jiaotong University, Xi'an 710061, China; and the Department of Surgery, University of Pittsburgh, NW607, Montefiore University Hospital, 3459 Fifth Avenue, Pittsburgh, PA 15213, USA (Wang ZF)

Correspondence: Zi-Fa Wang, MD, PhD, Department of Surgery, University of Pittsburgh, NW607, Montefiore University Hospital, 3459 Fifth Avenue, Pittsburgh, PA 15213, USA (Tel: 1-412-647-5696; Fax: 1-412-647-5959; Email: ziw3@pitt.edu)

factor- α (TNF- α); platelet-activating factor (PAF); nitric oxide (NO); and arachidonic acid metabolites.^[1-7] Excessive production of these mediators contributes to the induction of the systemic inflammatory response syndrome (SIRS), acute phase response, and multiple organ failure.^[8-10] The inflammatory mediators may mediate the key to the severity of AP. Recent advances in understanding pathophysiology of the early systemic illness have led to the development of a new therapeutic approach to AP. A number of new therapeutic approaches to the disease are in various stages of development.^[11-16] Because the degree and duration of inflammatory mediators are associated with the severity of AP, many investigators speculate that inhibitors of these mediators are beneficially effective.^[14-17] A randomized controlled study, however, showed that the antagonistic activity of PAF on its own is not sufficient to ameliorate SIRS in severe AP.^[18] Multiple inflammatory mediators may be involved in the pathophysiology of AP, and one specific antagonist may not be able to successfully downregulate this inflammatory response, the systemic effects, and multiple organ failure.

Glucocorticoids as non-specific anti-inflammatory agents inhibit or block several inflammatory mediator production, including inhibition of synthesis of TNF- α , PAF, IL-1 α , IL-6, IL-8, and prostaglandins.^[19-23] In this study, we investigated the effect of dexamethasone on breakdown products of arachidonic acid (6-keto-PGI α , TXB $_2$), cytokine release (IL-6), and the outcome in experimental AP. We hypothesized that if glucocorticoids could be given in the early stage of pancreatitis, the long-term effects might be ameliorated.

Methods

Animal models and study design

Sixty-eight male Wistar rats weighing 350–500 g were randomly divided into sham group, AP group, and dexamethasone (Dex) group. In the sham group, laparotomy was performed. In the AP and Dex groups, experimental AP was induced by the injection of 5% sodium taurocholate (0.1 ml/100 mg body weight) into the pancreatic-biliary duct

Table. Group descriptions and number of animals

Group	Intervention	No. of animals
Sham	Laparotomy	18
AP	Taurocholate	25
Dex	Taurocholate + dexamethasone	25

after clipping of the proximal duct for full injection of sodium taurocholate into the pancreas.^[24] In the Dex group, 0.5 mg/kg dexamethasone was administered into the dorsal vein of the penis 5 minutes after induction of AP. Six rats were sacrificed in each group post-operatively at 3 and 6 hours. Thirteen rats were observed for 12-hour survival in both AP and Dex groups. If survived 12 hours, they were sacrificed. Group descriptions and the number of rats are shown in Table.

Chemical analysis

At 3, 6, and 12 hours after operation, venous blood of the rats was obtained for immediate centrifugation and serum was stored at -80°C for further assay. The serum concentrations of 6-keto-PGI α , TXB $_2$, and IL-6 were determined by radioimmunoassay.

Tissue analysis

The pancreas of rats was removed immediately after their death, and fixed in 10% buffered-formalin for histopathological analysis. Specimens of the pancreas were stained with hematoxylin-eosin. Histological changes (edema, acinar necrosis, inflammation, and hemorrhage) were graded in a blind manner by two pathologists as described previously.^[9]

Statistical analysis

The values of 6-keto-PGI α , TXB $_2$, and IL-6 were expressed as mean \pm standard error. Differences between the groups were compared with Student's *t* test. A $P < 0.05$ was considered statistically significant.

Results

Arachidonic acid metabolites

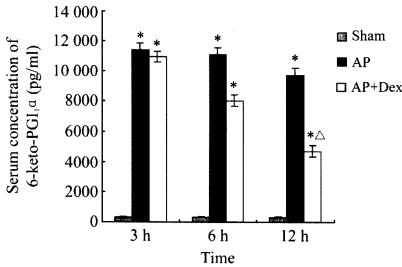


Fig. 1. The serum concentration of 6-keto-PGI₁α was higher in the AP and Dex groups than in the sham group. At 12 hours, the concentration decreased more significantly in the Dex group than in the AP group. * $P < 0.01$ versus the sham group; Δ $P < 0.05$ versus the AP group.

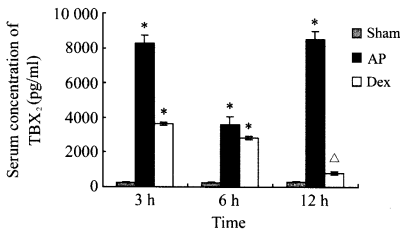


Fig. 2. The serum concentration of TXB₂ was higher in the AP and Dex groups than in the sham group. At 12 hours, the concentration decreased more significantly in the Dex group than in the AP group. * $P < 0.01$ versus the sham group; Δ $P < 0.05$ versus the AP group.

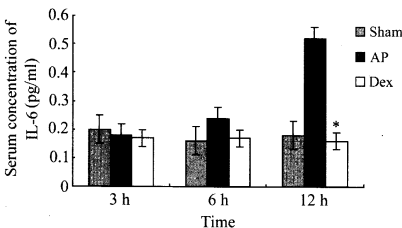


Fig. 3. The serum concentration of IL-6 in the Dex group decreased more significantly than in the AP group. * $P < 0.05$ versus the AP group.

The serum concentrations of 6-keto-PGI₁α and TXB₂ were higher postoperatively in both AP and Dex groups at 3, 6, and 12 hours than in the sham group (Figs. 1 and 2). The serum concentrations of 6-keto-PGI₁α and TXB₂ at 12 hours were reduced more significantly in the Dex group than in the AP group ($P < 0.05$). The effects of Dex were not significant at 3 and 6 hours after operation.

Interleukins

At 3 and 6 hours the serum concentration of IL-6 was not different in the 3 groups. The serum concentration of IL-6 in rats with AP was higher at 12 hours postoperation than in the Dex group (Fig. 3).

Histological characteristics

Severe pancreatic hemorrhage and necrosis were observed in rats with AP. According to the grade of histological changes (edema, acinar necrosis, inflammation and hemorrhage), hemorrhage and acinar necrosis were improved more significantly in the Dex group than in the AP group ($P < 0.05$).

Survival rate

In the AP group, 6 (42.9%) of 13 rats survived more than 12 hours. In the Dex group, all rats survived more than 12 hours ($P < 0.01$).

Discussion

Recent studies^[2-7] have shown that inflammatory mediators including IL-1, IL-6, PAF, and arachidonic acid metabolites are excessively produced during AP. These mediators play roles in initiating or amplifying the cascade of cytokines,^[4] and in the development of AP from a local to a systemic disease.^[6,23,25] The cumulative effect of these mediators eventually leads to vascular leakage, hypovolemia, SIRS, shock, and organ failure.^[26,27] The severity of pancreatitis is manifested by the serum concentration of IL-6. Patients with complicated or lethal pancreatitis may have a significantly higher serum concentration of IL-6 than those with simple or mild pancreatitis. Therefore, physicians try to predict the severity of AP during the first 24 hours after AP onset or so as to decide whether aggressive

resuscitation and monitoring techniques are needed.^[28,29] It has been better understood that these mediators play a dominant role in the pathogenesis of SIRS and organ dysfunction after AP. There is little doubt that inhibiting or blocking the effect of PAF, IL-1 or TNF may dramatically alter the expected course of experimental pancreatitis.^[30-34] In the present study, dexamethasone attenuated the inflammatory mediators, 6-keto-PGI₁α, TXB₂, and cytokine IL-6, and then improved the survival rate of rats with AP. As a non-specific anti-inflammatory drug, dexamethasone inhibits such inflammatory mediators as IL-1, IL-6, and TNF-α, and blocks the production of arachidonic acid metabolites like 6-keto-PGI₁α and TXB₂.^[20,25] The mechanism by which glucocorticoids, and dexamethasone inhibit arachidonic acid and its metabolites is that glucocorticoids induce PLA₂ inhibitor lipocortin.

The adrenocortical function is stimulated during AP, and the serum levels of corticosterone were significantly higher than basal levels.^[35] But endogenous glucocorticoids did not meet the need of the body. This is why some AP rats deteriorated and progressed to severe AP. The administration of exogenous hydrocortisone suppressed the elevation of serum concentration of IL-8 and decreased both severity and mortality of pancreatitis.^[35] Despite exogenous glucocorticoids have beneficial effects on experimental AP,^[36,37] some research results of AP remain controversial. The crucial reasons may be different administration time and dose of glucocorticoids. It is clear that the earlier glucocorticoids administered, the better results obtained, but the optimal dose of glucocorticoids is not known. In our study a high dose of glucocorticoids and short time of administration were prescribed to treat this model of AP, and significant results were obtained. Although glucocorticoids have a positive impact on AP, Gomez et al^[38] found that the survival rate decreased and pancreatic necrosis increased in mice after seven days of pretreatment with hydrocortisone.

In summary, this study provides an evidence that glucocorticoids or dexamethasone attenuates 6-keto-PGI₁α, TXB₂, and IL-6 in rats with experimentally induced AP. Apart from less pancreatic damage, the treatment with glucocorticoids or dexamethasone may improve the survival of rats.

Acknowledgement

We thank Dr. David Blumberg and Dr. Yue Zhu for their critical review of the manuscript.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

- 1 Kingsnorth A. Role of cytokines and their inhibitors in acute pancreatitis. *Gut* 1997;40:1-4.
- 2 Kusske AM, Rongione AJ, Reder HA. Cytokines and acute pancreatitis. *Gastroenterology* 1996;110:639-642.
- 3 Norman J, Fink G, Franz M, et al. Systemic cytokines gene expression induced by acute pancreatitis. *Gastroenterology* 1995;108(Suppl a):1236.
- 4 McKay CJ, Gallagher G, Brooks B, et al. Increased monocyte cytokine production in association with systemic complication in acute pancreatitis. *Br J Surg* 1996;83:919-923.
- 5 Uhl W, Schrag HJ, Schmitter N, et al. Experimental study of a novel phospholipase A2 inhibitor in acute pancreatitis. *Br J Surg* 1998;85:618-623.
- 6 Uhl W, Schrag HJ, Schmitter N, et al. Pathophysiological role of secretory type I and II phospholipase A2 in acute pancreatitis: an experimental study in rats. *Gut* 1997;40:386-392.
- 7 Abe T, Shimsegawa T, Satoh A, et al. Nitric oxide modulate pancreatic edema formation in rat caerulein-induced pancreatitis. *J Gastroenterol* 1995;30:636-642.
- 8 de Beaux AC, Goldie AS, Ross JA, et al. Serum concentrations of inflammatory mediators related to organ failure in patients with acute pancreatitis. *Br J Surg* 1996;83:349-353.
- 9 Schmidt J, Lewandrowski K, Warshaw A, et al. Morphometrics characteristics and homogeneity of a new model of acute pancreatitis in the rats. *Int J Pancreatol* 1992;12:41-51.
- 10 Closa D, Rossell CJ, Martrat C, et al. Changes of systemic procacyclins and thromboxaneA2 in sodium taurocholate and cerulein-induced pancreatitis in rats. *Dig Dis Sci* 1993;38:33-38.
- 11 Osman MO, Kristensen JU, Jacobsen NO, et al. A monoclonal anti-interleukin 8 antibody (WS-4) inhibits cytokine response and acute lung injury in experimental severe acute necrotizing pancreatitis in rabbits.

- Gut 1998;42:232-239.
- 12 McKay CJ, Curran F, Sharples C, et al. Prospective placebo-controlled randomized trial of lixipafant in predicted severe acute pancreatitis. *Br J Surg* 1997; 84:1239-1243.
 - 13 Kusske AM, Rongione AJ, Ashley SW, et al. Interleukin-10 prevents death in lethal necrotizing pancreatitis in mice. *Surgery* 1996;120:284-288.
 - 14 Norman J, Franz M, Fink G, et al. Decreased mortality of severe acute pancreatitis following proximal cytokines blockade. *Ann Surg* 1995;221:625-631.
 - 15 Hughes CB, Grewal HP, Gaber LW, et al. Anti-TNF- α therapy improves survival and ameliorates the pathophysiology sequelae in acute pancreatitis in the rat. *Am J Surg* 1996;171:274-280.
 - 16 Kingsnorth AN, Galloway SW, Formela PJ, et al. Randomized double blind phase trial of lixipafant, a platelet-activating factor antagonist, in human acute pancreatitis. *Br J Surg* 1995;82:1414-1420.
 - 17 Grewal HP, Mohey EL, Din A, et al. Amelioration of the physiologic and biochemical changes of acute pancreatitis using an anti-TNF-alpha polyclonal antibody. *Am J Surg* 1994;167:214-218.
 - 18 Johnson CD, Kingsnorth AN, Imrie CW, et al. Double blind, randomized, placebo controlled study of a platelet activating factor antagonist, lixipafant, in the treatment and prevention of organ failure in predicted severe acute pancreatitis. *Gut* 2001;48:62-69.
 - 19 Santos AA, Scheltinga MR, Lynch E, et al. Elaboration of interleukin 1-receptor antagonist is not attenuated by glucocorticoids after endotoxemia. *Arch Surg* 1993;128:138-143.
 - 20 Zanker B, Walz G, Wieder KJ, et al. Evidence that glucocorticosteroids block expression of the human interleukin-6 gene by accessory cells. *Transplantation* 1990;49:183-185.
 - 21 Yao XL, Cowan MJ, Gladwin MT, et al. Dexamethasone alters arachidonate release from human epithelial cells by induction of p11 protein synthesis and inhibition of phospholipase A2 activity. *J Biol Chem* 1999; 274:17202-17208.
 - 22 Dolan-O'keefe M, Nick HS. Inhibition of cytoplasmic phospholipase A2 expression by glucocorticoids in rat intestinal epithelial cells. *Gastroenterology* 1999;116: 855-864.
 - 23 Hardman JG, Linbird LE. Goodman and Gilman's the pharmacological basis of therapeutics. Ninth edition. New York: McGraw-Hill. 1996,1465-1481.
 - 24 Uhl W, Schrag HJ, Schmitter N, et al. Experimental study of a novel phospholipase A2 inhibitor in acute pancreatitis. *Br J Surg* 1998;85:618-623.
 - 25 Folch E, Prats N, Hotter G, et al. P-selectin expression and Kupffer cell activation in rat acute pancreatitis. *Dig Dis Sci* 2000;45:1535-1544.
 - 26 Norman J. The role of cytokines in the pathogenesis of acute pancreatitis. *Am J Surg* 1998;175:76-83.
 - 27 Sandoval D, Gukovskaya A, Reavey P, et al. The role of neutrophils and platelet-activating factor in mediating experimental pancreatitis. *Gastroenterology* 1996; 111:1081-1091.
 - 28 Pezilli R, Billi P, Miniero R, et al. Serum interleukin-6, interleukin-8, and beta 2-microglobulin in early assessment of severity of acute pancreatitis. Comparison with serum C-reactive protein. *Dig Dis Sci* 1995; 40:2341-2348.
 - 29 Inagaki T, Hoshino M, Hayakawa T, et al. Interleukin-6 is a useful marker for early prediction of the severity of acute pancreatitis. *Pancreas* 1997;14:1-8.
 - 30 Hughes CB, Gaber LW, Mohey el-Din AB, et al. Inhibition of TNF alpha improves survival in an experimental model of acute pancreatitis. *Am Surg* 1996;62: 8-13.
 - 31 Windsor ACJ, Mullen PG, Walsh CJ, et al. Delayed tumor necrosis factor a blockade attenuates pulmonary dysfunction and metabolic acidosis associated with experimental gram-negative sepsis. *Arch Surg* 1994;129: 80-89.
 - 32 Norman JG, Franz M, Messina J, et al. Interleukin-1 receptor antagonist decreases severity of experimental acute pancreatitis. *Surgery* 1995;117:648-655.
 - 33 Guice K, Oldham KT, Remick DG, et al. Anti-tumor necrosis factor antibody augments edema formation in caerulein-induced acute pancreatitis. *J Surg Res* 1991; 51:495-499.
 - 34 Norman JG, Fink GW, Denham W, et al. Tissue-specific cytokine production during experimental acute pancreatitis: a probable mechanism for distant organ dysfunction. *Dig Dis Sci* 1997;42:1783-1788.
 - 35 Abe R, Shimosegawa T, Kimura K, et al. The role of endogenous glucocorticoids in rat experimental models of acute pancreatitis. *Gastroenterology* 1995;109:933-943.
 - 36 Osman MO, Jacobsen NO, Kristensen JU, et al. Beneficial effects of hydrocortisone in a model of experimental acute pancreatitis. *Dig Surg* 1999; 16: 214-221.
 - 37 Gloor B, Uhl W, Tcholakov O, et al. Hydrocortisone treatment of early SIRS in acute experimental pancreatitis. *Dig Dis Sci* 2001;46:2154-2161.
 - 38 Gomez G, Townsend CMJ, Green D, et al. Involvement of cholecystokinin receptors in the adverse effect of glucocorticoids on diet-induced necrotizing pancreatitis. *Surgery* 1989;106:230-238.

Received December 28, 2002

Accepted after revision February 15, 2003