Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Clodronate-containing liposomes attenuate lung injury in rats with severe acute pancreatitis*

Sheng-chun DANG[1], De-li JIANG[2], Min CHEN[2], Di LI[2], Jian-xin ZHANG[†‡1]

([1]*Department of General Surgery, the Affiliated Hospital of Jiangsu University, Zhenjiang 212001, China*)

([2]*School of Chemistry and Chemical Engineering, Jiangsu University, Zhenjiang 212013, China*)

[†]E-mail: dscgu@163.com

Received Feb. 3, 2010; Revision accepted June 7, 2010; Crosschecked Sept. 27, 2010

Abstract: Objectives: Severe acute pancreatitis (SAP) can lead to acute lung injury (ALI). The purpose of this paper is to investigate the protective effect of clodronate-containing liposomes on ALI in rats with SAP. Methods: The thin film method was used to prepare liposomes. Sprague-Dawley rats were randomly divided into three groups. After the SAP model was established by injecting 5% (w/v) sodium taurocholate (2 ml/kg body weight) into the subcapsular space of the pancreata, normal saline was administered to the control (C) group, phosphate buffer solution (PBS)-containing liposome to the P group, and clodronate-containing liposome to the T group through tail veins. Blood samples were obtained from the superior mesenteric vein at 2 and 6 h to measure the levels of amylase, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). Morphological changes in the pancreata and lung were observed using hematoxylin and eosin (H&E) staining, while cell apoptosis was detected using terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL). In addition, the macrophage marker cluster of differentiation 68 (CD68) in lung tissue was detected with immunohistochemistry. Results: Blood levels of amylase, IL-6, and TNF-α were significantly increased in the P group compared to those in the T group (P<0.05). In the T group, large numbers of TUNEL-positive cells were observed, but no or few in the C and P groups. Gross inspection and H&E staining of pancreata and lung showed dramatic tissue damage, including inflammation and necrosis in the P group. Less remarkable changes were noted in the T group, and the C group exhibited normal histology. The histological scores according to Kaiser's criteria were consistent with H&E findings. The number of CD68-positive macrophages decreased in the T group. Conclusions: Clodronate-containing liposomes have a protective effect against ALI in rats with SAP. Blockade of macrophages may represent a novel therapeutic strategy in SAP.

Key words: Pancreatitis, Clodronate disodium, Macrophage, Lung injury
doi:10.1631/jzus.B1000044 **Document code:** A **CLC number:** R56

1 Introduction

Patients with severe acute pancreatitis (SAP) may develop acute lung injury (ALI), leading to the adult respiratory distress syndrome (ARDS) (Steer, 2001). The mechanism underlying ALI induced by SAP is still not clear, but activated enzyme-induced ischemia-reperfusion of the pancreata, diverse pro-inflammatory mediators generated in the pancreata, and activated leukocytes are known to contribute to the lung complication (Weinbroum, 2009). SAP involves multiple inflammatory mediators initiating and amplifying the systemic inflammatory response syndrome (SIRS) and therefore leads to the multiple organ dysfunction syndrome (MODS) (Singh *et al*., 2009). Among the distant organ dysfunctions, both ARDS and ALI represent serious complications and are responsible for substantial mortality and morbidity

‡ Corresponding author

* Project supported by the National Natural Science Foundation of China (No. 30772117), and the Science Foundation of Jiangsu Province of China (No. BK2007096)

© Zhejiang University and Springer-Verlag Berlin Heidelberg 2010

(Chen *et al*., 2008; Pezzilli *et al*., 2009; Sha *et al*., 2009).

We have previously reported that microcirculatory derangements play an important role in the development process of ALI (Zhang and Dang, 2006). Recent research has revealed that alveolar macrophages (AMs) not only serve as phagocytes but also play an important role as secretory cells in various respiratory diseases (Serin *et al*., 2007; Bem *et al*., 2008; Traeger *et al*., 2009). Activated AMs can release cytokines and inflammatory mediators which finally result in MODS (Sugita *et al*., 1997; Viterbo *et al*., 2008).

It is speculated that depleting macrophages may reduce alveolar tissue damage in SAP. One study showed that systemic injection of liposomes containing clodronate resulted in macrophage depletion (van Rooijen and Sanders, 1994). Clodronate, a bisphosphonate (BP), is a potent inhibitor of osteoclasts. As with other BPs, the cell membrane is not readily permeable to clodronate (Roelofs *et al*., 2006). However, liposomes can be readily phagocytized by the reticuloendothelial system, especially macrophages. The liposome then kills the macrophages but is not toxic to non-phagocytic cells (Selander *et al*., 1996).

In the present experiment, we investigated the effect of clodronate delivered via liposomes on AMs in a rat model of SAP.

2 Materials and methods

2.1 Materials and instruments

Sodium taurocholate (Sigma, USA) was used to generate the SAP model. Clodronate was purchased from Shanghai Wei Jing Technology Enterprise Co., Ltd., China. The cluster of differentiation 68 (CD68) immunohistochemical kit was purchased from Fuzhou Maxim Biosciences (China). We also used the Rotary Evaporators R-200 (BUCHI Labortechnik AG, Switzerland) and an automatic biochemistry analyzer CL-7300 (Shimadzu, Japan).

2.2 Preparation and use of liposomes

Liposomes containing phosphate buffer solution (PBS) or clodronate were synthesized using an improved reverse phase evaporation method as previously described (van Rooijen and van Kesteren-Hendrikx, 2003). The liposomal suspension was then stored in a liquid nitrogen tank. It was thawed and gently shaken prior to administration to the rats.

2.3 Animal models and experimental grouping

A total of 48 healthy Sprague-Dawley rats, weighing between 350 and 400 g each, were provided by the Laboratory Animal Center of the School of Medicine of Jiangsu University (China). The rats were housed in an environmentally controlled room at 21–23 °C with a light-dark cycle of 12 h:12 h. The rats were fed the standard laboratory diet, given water ad libitum, and fasted overnight before each experiment. The experiment was performed according to the Institutional Animal Care and Use Committee of Jiangsu University, China. The rats were randomly divided into three groups with equal size: SAP injected with normal saline group (controls; C group), SAP injected with PBS-liposome group (P group), and SAP injected with clodronate-containing liposome group (T group). Each of these groups was further divided into two subgroups with eight rats in each group at 2 and 6 h. The abdominal cavities of the rats were opened after receiving anesthesia. SAP models were generated by injecting 5% (w/v) sodium taurocholate (2 ml/kg body weight) into the subcapsular space of the pancreata (Zhang *et al*., 2006). PBS-liposome (2 ml/kg body weight), clodronate-containing liposome (2 ml/kg body weight), and normal saline (2 ml/kg body weight) were then slowly injected into the tail veins of SAP rats in the P, T, and C groups, respectively. The dose of clodronate, in previous study (Zhang *et al*., 2010), was shown to be effective. At 2 and 6 h after injection, the animals were sacrificed, and then the pancreata and lungs were harvested.

2.4 Analysis of serum amylase, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) levels

The analysis methods are similar as previously described by Zhang *et al*. (2010). To assess serum amylase, TNF-α, and IL-6 levels, blood was obtained from the superior mesenteric vein, and centrifuged for 10 min at 3000 r/min. The supernatant was preserved at −20 °C. Serum amylase was measured with an automatic biochemical analyzer. Serum concentrations of TNF-α and IL-6 were measured by

enzyme-linked immunosorbent assay (ELISA; Invitrogen, USA) according to the manufacturer's protocol.

2.5 Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay of lung

The TUNEL assay (in situ Apoptosis Detection Kit; Cat. No. 11684817910; Roche, Switzerland) was performed according to the manufacturer's protocol.

2.6 Identification of macrophages in lung tissue with immunohistochemistry

A commercially acquired monoclonal CD68 antibody (Maxim Corp, Fuzhou, China) was used to detect macrophages in formalin-fixed, paraffin-embedded tissues from all the rats.

2.7 Pathological examination

Paraffin-embedded pancreata and lungs were sectioned at 5 μm, and then stained sequentially with hematoxylin and eosin (H&E). Experienced histologists blind to the treatment scored each specimen. This pathological grading of the pancreatic tissue was performed using the scoring systems proposed by Kaiser *et al*. (1995). ALI was quantified by histologic examination as previously described (Lee *et al*., 2008).

2.8 Statistical analysis

SPSS Version 18.0 was used. Statistical estimates of mean±standard deviation (SD) were used. If equal variances were assumed, one-way analysis of variance (ANOVA) was applied; otherwise a non-parametric test (Kruskal-Wallis) was used. The Mann-Whitney *U* test was used to assess the differences in grading of pancreata and lung injury. A significance level $P<0.05$ was applied.

3 Results

3.1 Changes in serum amylase, TNF-α, and IL-6 levels

As shown in Table 1, blood levels of serum amylase at 2 and 6 h among rats in the P group were significantly higher than those in the C group ($P<0.05$). In contrast, the levels of serum amylase at both time points in the T group were significantly lower than those in the P group ($P<0.05$). The amylase levels in the T group were not significantly different from those in the C group.

At both time points, higher levels of TNF-α and IL-6 were obtained in the P group than in either the C or T group ($P<0.05$).

3.2 Apoptosis ratio of lung tissue by TUNEL

In the T group, large numbers of TUNEL-positive cells were observed, but no or few in the C and P groups (Fig. 1).

3.3 Morphological and pathological changes

3.3.1 Pancreata

Gross observation: In the C group, the pancreata of rats showed no significant change. In the P group, hemorrhagic ascites were noted in the cavum abdominis, as well as pancreatic hyperemia, edema, hemorrhage, and necrosis. In the T group, pathological changes were obviously milder than those in the P group. Light microscopy: In the C group, animals showed normal pancreatic tissue. In the P group, mild edema and inflammatory cell infiltration accompanied by extensive exudation were observed at the 2 h; additionally, necrosis of adjacent adipose tissue, moderate hemorrhage, and acinar cell necrosis were observed at 6 h. The rats in the T group showed

Table 1 Levels of serum amylase, TNF-α, and IL-6 at 2 and 6 h in each group

Group	Amylase (U/L)		TNF-α (pmol/L)		IL-6 (pmol/L)	
	2 h	6 h	2 h	6 h	2 h	6 h
C	810.89±79.43	813.91±79.77	23.20±2.03	30.28±6.07	23.75±3.78	26.69±5.73
P	3370.71±332.53*	5030.59±471.29*	129.98±13.50*	234.05±17.09*	152.39±4.63*	218.02±4.68*
T	2069.28±227.89$^{*\#}$	2842.75±236.37$^{*\#}$	80.92±29.90$^{*\#}$	163.18±19.12$^{*\#}$	70.73±7.90$^{*\#}$	112.29±8.00$^{*\#}$

$^{*}P<0.05$ as compared with the C group, $^{\#}P<0.05$ as compared with the P group. Data are expressed as mean±SD (n=8)

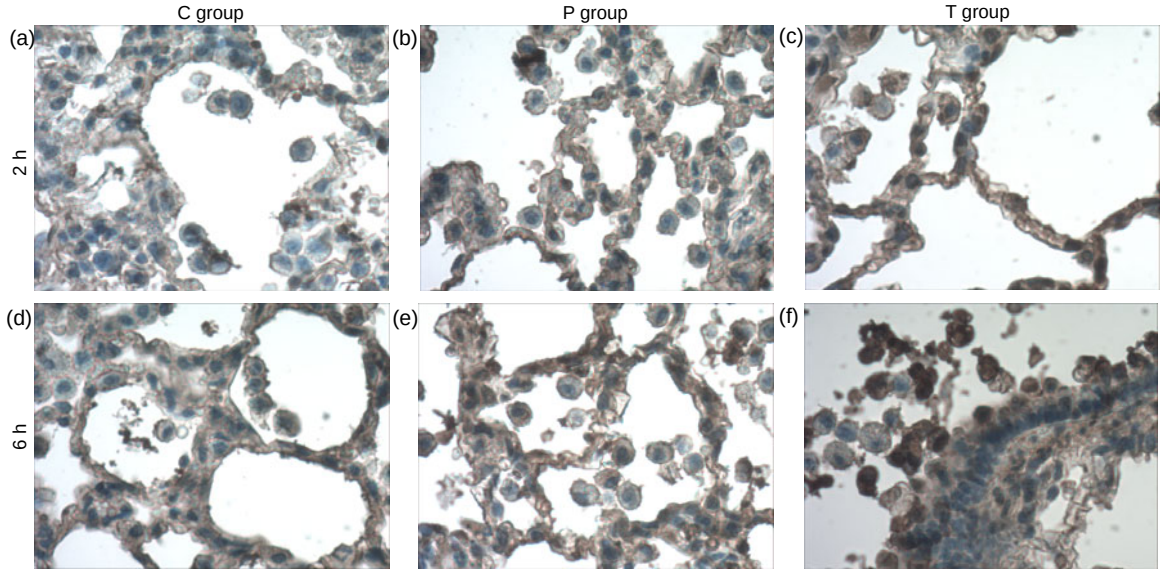


Fig. 1 TUNEL in situ detection of apoptosis of lung tissue cells

(a, d) C group at 2 and 6 h, respectively; (b, e) P group at 2 and 6 h, respectively; (c, f) T group at 2 and 6 h, respectively (all original magnification ×400). Note the presence of multiple AMs without apoptosis in the C and P groups and with apoptosis in the T group

mild interstitial edema of the pancreata, as well as infiltration by inflammatory cells; however, no pancreatic parenchymal hemorrhage or necrosis was evident. These histological changes in the T group were less marked than those in the P group. According to Kaiser's criteria, the histological scores differed significantly in the P and T groups compared with the score in the C group. Less dramatic pathological changes were observed in the T group than in the P group (P<0.05) (Fig. 2).

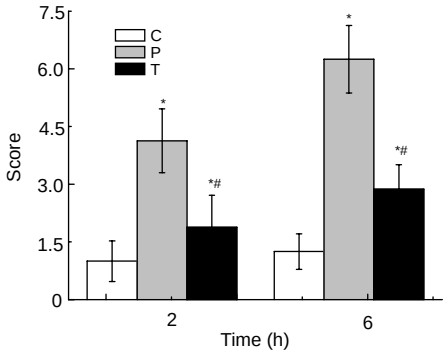


Fig. 2 Pathological changes in the pancreata

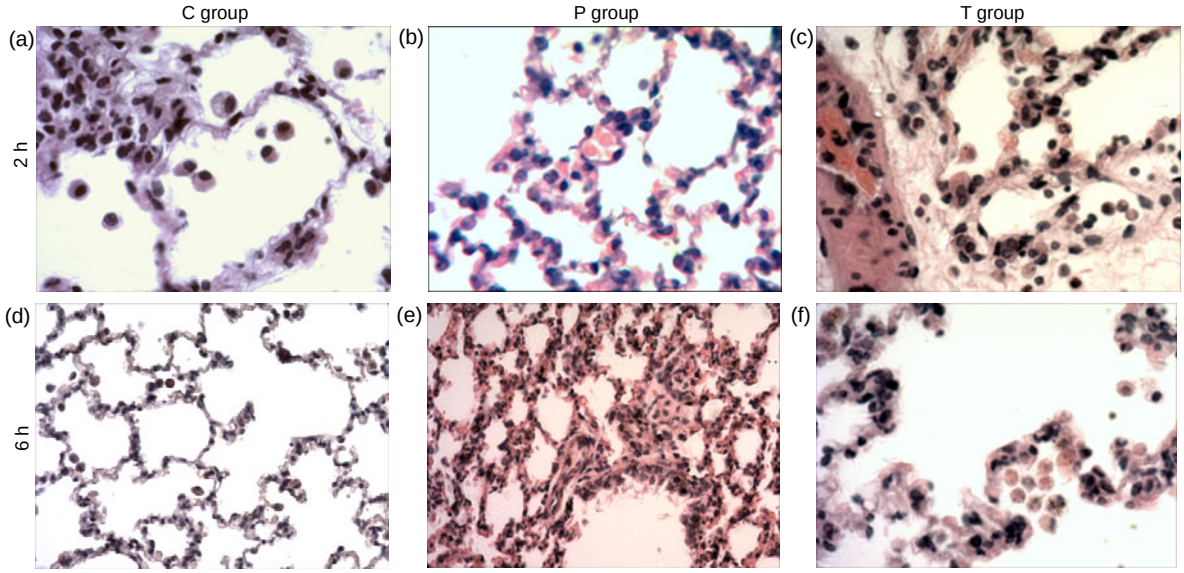
Histological scores showed that both the P and T groups differed significantly from the C group; however, pathological changes in the T group were less severe than those in the P group. * P<0.05 as compared with the C group, # P<0.05 as compared with the P group. Data are expressed as mean±SD (n=8)

3.3.2 Lung

Gross observation: In the C group, the rats' lungs showed normal morphology. In the P group, varying degrees of congestion and swelling were seen at both time points. In the T group, congestion and swelling at both time points were milder than those in the P group. Light microscopy: In the C group, the rats' lungs showed no obvious morphological or structural abnormalities. In the P group, obvious inflammatory cell infiltration was noted, predominantly distributed in the portal and necrotic areas. In the T group, the pathological changes in pulmonary tissue were significantly less severe. A quantitative score standard for severity was made based on pathological changes of the lung tissue in the various groups. The pathological severity score was significantly higher in the P and T groups than in the C group at 2 and 6 h (P<0.05), and it was significantly lower in the T group than in the P group at 2 and 6 h (P<0.05) (Fig. 3).

3.4 Pulmonary tissue macrophages stained by immunohistochemistry for CD68

Rat pulmonary tissue sections were immunostained and examined for the macrophage-specific marker CD68 under different conditions in each group (Fig. 4).



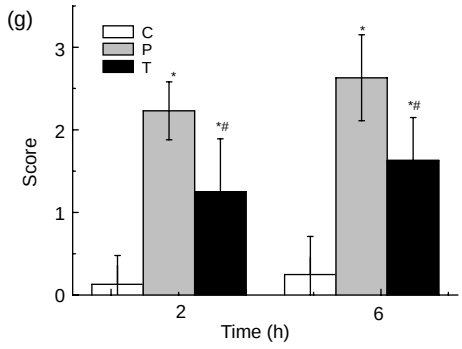


Fig. 3 Morphological and pathological changes in the lung

(a–f) Morphologic changes of the lung injury. Representative H&E-stained sections of lung were examined by light microscopy. (a, d) C group at 2 and 6 h, respectively (original magnification ×400 (a) and ×200 (d)). No histological alterations were observed; (b, e) P group at 2 and 6 h, respectively (original magnification ×200). Alveolar septum thickening and inflammatory cells penetrating into the alveolar spaces were noted; (c, f) T group at 2 and 6 h, respectively (original magnification ×400). Lung injury was attenuated to a great extent. (g) Acute lung injury score. Histopathological scores were higher in the P and T groups than in the C group, and lower in the T group than in the P group at 2 and 6 h. $^{*}P<0.05$ as compared with the C group, $^{\#}P<0.05$ as compared with the P group. Data are expressed as mean±SD (n=8)

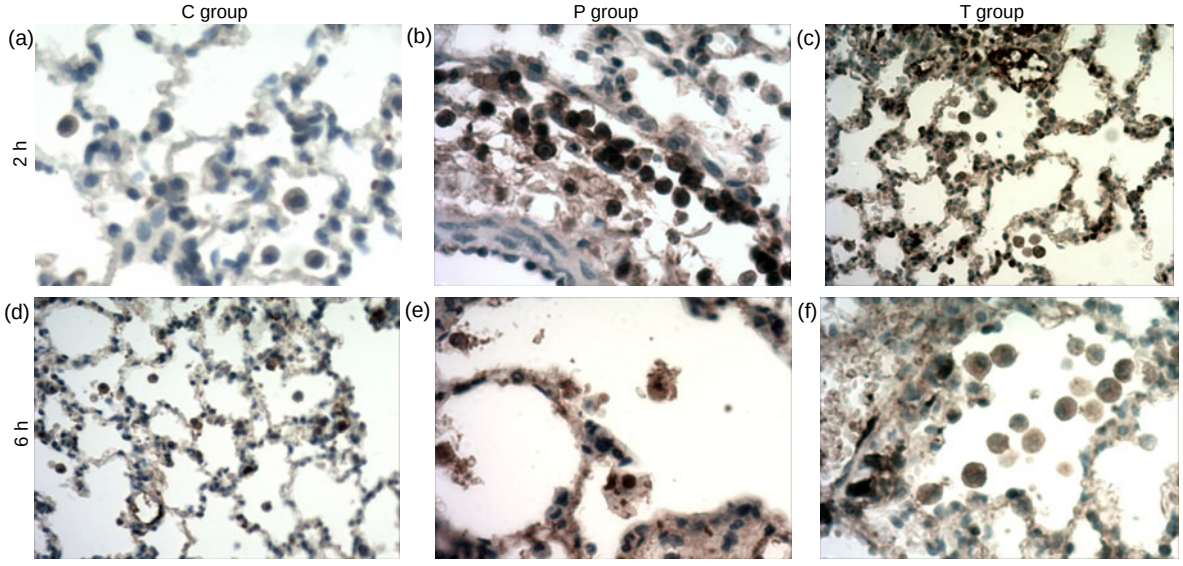


Fig. 4 Immunostaining for CD68 in rat pulmonary tissues

(a, d) C group at 2 and 6 h, respectively (original magnification ×200). At steady state, most macrophages are located in the interstitial and alveolar spaces during SAP; (b, e) P group at 2 and 6 h, respectively (original magnification ×400). The tissue architecture is massively distorted, and numerous macrophages (brown) are dispersed in the interstitial and alveolar spaces of lung segments; (c, f) T group at 2 and 6 h, respectively (original magnification ×200 (c) and ×400 (f)). Macrophages are decreased in lung tissue sections

4 Discussion

SAP is associated complications including SIRS, MODS, ALI, and local complications within the pancreata (Ammori, 2003; Lankisch and Lerch, 2006). Among the extrapancreatic complications of SAP, ALI is most frequently associated with death. The incidence of ALI approaches 70%, and when concomitant with SAP, ALI evolves into ARDS. ALI and ARDS are well-known as common causes of morbidity after SAP, with an overall mortality rate of 30% to 40%. The cellular and molecular mechanisms that contribute to ALI in SAP are not well understood (Matsuda *et al*., 2006). Oxygen free radicals and various inflammatory mediators are acknowledged as the principal mediators in the transformation of SAP from a local inflammatory process into a systemic illness. Cytokines and inflammatory mediators such as TNF-α, IL-1, and IL-6 have been shown to increase in a "cytokine storm" that resulted in an uncontrolled inflammatory process (Makhija and Kingsnorth, 2002). Activation of numerous inflammatory effector cells, such as polymorphonuclear leukocytes and macrophages, has also been found to trigger excessive release of inflammatory mediators and cytokines in ALI (Zhao *et al*., 2006).

However, the pathogenesis has yet to be fully clarified. In recent years, in-depth studies have revealed that macrophages play a major role in a course of SAP (Folch-Puy, 2007). Closa *et al.* (1999) have found that infiltration and activation of macrophages are initial factors and the most important pathophysiological process, eventually resulting in MODS. AMs are activated as a consequence of SAP; Cheng *et al*. (2002) determined that lung damage induced by experimental SAP is associated with AM activation. Inhibiting the activation of macrophages or counteracting the release of inflammatory mediators during SAP would ameliorate the degree of ALI resulting from SAP.

At present, the cellular level therapy aimed at macrophages is a field of intense activity, and finding ways to adjust the function of macrophages is a new research direction in treating SAP. Macrophage infiltration and activation are not only the trigger for the development of initial events in SAP but also an important pathophysiological step in MODS (Gutierrez *et al*., 2008). This central role in regulating inflammation makes macrophages interesting targets for designing therapeutic strategies focused on controlling the systemic effects of acute pancreatitis. These proinflammatory cytokines promote the spread of inflammation and also augment levels of neutrophil elastase to produce free radicals that damage the endothelial cells, causing endothelial swelling and circulatory stasis. In SAP, increased levels of proinflammatory cytokines and decreased levels of anti-inflammatory cytokines are crucial factors in its progression. The bisphosphonate clodronate is known to deplete monocytic cells. van Rooijen *et al*. (1996) found that intravenous injection of clodronate-containing liposomes could selectively clear macrophages in vivo. Clodronate is speculated to function mainly during induction of apoptosis, in which clodronate might compete with ATP and act as a substrate for intracellular ATPase. Free clodronate has poor permeability and a short half-life in the systemic circulation. Delivery of clodronate incorporated into liposomes can greatly enhance its uptake by phagocytes, leading to selectively targeted macrophage killing (Frith *et al*., 1997). Despite inhibiting the growth of cultured macrophages, liposomal clodronate had no effect on endothelial or smooth muscle cells (Danenberg *et al*., 2002).

Our results showed that clodronate-containing liposome could inhibit ALI by repressing IL-6 and TNF-α. Serum TNF-α and IL-6 levels were notably increased in the P group, and levels of these cytokines were lower in the T group. Less damage in the lung tissue was observed in the T group than in the P group. In the T group, large numbers of positive cells were found by TUNEL, but few or no positive cells were detected in the C and P groups. These findings suggest that administrating clodronate-containing liposome might therefore modulate the process of pulmonary injury by regulating the apoptosis of AMs and the production of inflammatory mediators. In our model of ALI, results on a standard microscopic pathological scale of lung tissue injury were nearly paralleled by CD68 expression.

5 Conclusions

The current data indicate that AMs contribute to the process of ALI associated with SAP. Liposomes

containing clodronate can induce macrophage apoptosis in a rat model of SAP to inhibit excessive release of cytokines and inflammatory mediators, thereby improving the prognosis of SAP. This study shows that systemic administration of clodronate-containing liposomes can inhibit AMs in rats with SAP. We have proved our hypothesis regarding the role of AMs in the pathogenesis of ALI. The results suggest a useful therapeutic strategy to alleviate the clinical course of ALI related to SAP.

References

Ammori, B.J., 2003. Role of the gut in the course of severe acute pancreatitis. *Pancreas*, **26**(2):122-129. [doi:10.1097/00006676-200303000-00006]

Bem, R.A., Farnand, A.W., Wong, V., Koski, A., Rosenfeld, M.E., van Rooijen, N., Frevert, C.W., Martin, T.R., Matute-Bello, G., 2008. Depletion of resident alveolar macrophages does not prevent Fas-mediated lung injury in mice. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **295**(2):L314-L325. [doi:10.1152/ajplung.00210.2007]

Chen, X., Li, S.L., Wu, T., Liu, J.D., 2008. Proteasome inhibitor ameliorates severe acute pancreatitis and associated lung injury of rats. *World J. Gastroenterol.*, **14**(20):3249-3253. [doi:10.3748/wjg.14.3249]

Cheng, S., He, S., Zhang, J., 2002. The role of alveolar macrophage activation in rats with lung injury associated with acute necrotizing pancreatitis. *Zhonghua Wai Ke Za Zhi*, **40**(8):609-612 (in Chinese).

Closa, D., Sabater, L., Fernandez-Cruz, L., Prats, N., Gelpi, E., Roselló-Catafau, J., 1999. Activation of alveolar macrophages in lung injury associated with experimental acute pancreatitis is mediated by the liver. *Ann. Surg.*, **229**(2):230-236. [doi:10.1097/00000658-199902000-00011]

Danenberg, H.D., Fishbein, I., Gao, J., Mönkkönen, J., Reich, R., Gati, I., Moerman, E., Golomb, G., 2002. Macrophage depletion by clodronate-containing liposomes reduces neointimal formation after balloon injury in rats and rabbits. *Circulation*, **106**(5):599-605. [doi:10.1161/01.CIR.0000023532.98469.48]

Folch-Puy, E., 2007. Importance of the liver in systemic complications associated with acute pancreatitis: the role of Kupffer cells. *J. Pathol.*, **211**(4):383-388. [doi:10.1002/path.2123]

Frith, J.C., Monkkonen, J., Blackburn, G.M., Russell, R.G., Rogers, M.J., 1997. Clodronate and liposome-encapsulated clodronate are metabolized to a toxic ATP analog, adenosine 5′-(β,γ-dichloromethylene) triphosphate, by mammalian cells in vitro. *J. Bone Miner. Res.*, **12**(9):1358-1367. [doi:10.1359/jbmr.1997.12.9.1358]

Gutierrez, P.T., Folch-Puy, E., Bulbena, O., Closa, D., 2008. Oxidised lipids present in ascitic fluid interfere with the regulation of the macrophages during acute pancreatitis, promoting an exacerbation of the inflammatory response. *Gut*, **57**(5):642-648. [doi:10.1136/gut.2007.127472]

Kaiser, A.M., Saluja, A.K., Sengupta, A., Saluja, M., Steer, M.L., 1995. Relationship between severity, necrosis, and apoptosis in five models of experimental acute pancreatitis. *Am. J. Physiol. Cell Physiol.*, **269** (5 Pt. 1):C1295-C1304.

Lankisch, P.G., Lerch, M.M., 2006. Pharmacological prevention and treatment of acute pancreatitis: where are we now? *Dig. Dis.*, **24**(1-2):148-159. [doi:10.1159/000090318]

Lee, J., Kwon, W., Jo, Y., Suh, G., Youn, Y., 2008. Protective effects of ethyl pyruvate treatment on paraquat-intoxicated rats. *Hum. Exp. Toxicol.*, **27**(1):49-54. [doi:10.1177/0960327108088976]

Makhija, R., Kingsnorth, A.N., 2002. Cytokine storm in acute pancreatitis. *J. Hepatobiliary Pancreat. Surg.*, **9**(4):401-410. [doi:10.1007/s005340200049]

Matsuda, N., Nishihira, J., Takahashi, Y., Kemmotsu, O., Hattori, Y., 2006. Role of macrophage migration inhibitory factor in acute lung injury in mice with acute pancreatitis complicated by endotoxemia. *Am. J. Respir. Cell Mol. Biol.*, **35**(2):198-205. [doi:10.1165/rcmb.2005-0272OC]

Pezzilli, R., Bellacosa, L., Felicani, C., 2009. Lung injury in acute pancreatitis. *JOP*, **10**(5):481-484.

Roelofs, A.J., Thompson, K., Gordon, S., Rogers, M.J., 2006. Molecular mechanisms of action of bisphosphonates: current status. *Clin. Cancer Res.*, **12**(20):6222s-6230s. [doi:10.1158/1078-0432.CCR-06-0843]

Selander, K.S., Monkkonen, J., Karhukorpi, E.K., Harkonen, P., Hannuniemi, R., Vaananen, H.K., 1996. Characteristics of clodronate-induced apoptosis in osteoclasts and macrophages. *Mol. Pharmacol.*, **50**(5):1127-1138.

Serin, M., Gulbas, H., Gurses, I., Erkal, H.S., Yucel, N., 2007. The histopathological evaluation of the effectiveness of melatonin as a protectant against acute lung injury induced by radiation therapy in a rat model. *Int. J. Radiat. Biol.*, **83**(3):187-193. [doi:10.1080/09553000601129093]

Sha, H., Ma, Q., Jha, R.K., Wang, Z., 2009. Resveratrol ameliorates lung injury via inhibition of apoptosis in rats with severe acute pancreatitis. *Exp. Lung Res.*, **35**(4):344-358. [doi:10.1080/01902140902718197]

Singh, V.K., Wu, B.U., Bollen, T.L., Repas, K., Maurer, R., Mortele, K.J., Banks, P.A., 2009. Early systemic inflammatory response syndrome is associated with severe acute pancreatitis. *Clin. Gastroenterol. Hepatol.*, **7**(11):1247-1251. [doi:10.1016/j.cgh.2009.08.012]

Steer, M.L., 2001. Relationship between pancreatitis and lung diseases. *Respir. Physiol.*, **128**(1):13-16. [doi:10.1016/S0034-5687(01)00259-6]

Sugita, H., Yamaguchi, Y., Ikei, S., Yamada, S., Ogawa, M., 1997. Enhanced expression of cytokine-induced neutrophil chemoattractant (CINC) by bronchoalveolar macrophages in cerulein-induced pancreatitis rats. *Dig. Dis. Sci.*, **42**(1):154-160. [doi:10.1023/A:1018809810561]

Traeger, T., Kessler, W., Hilpert, A., Mikulcak, M., Entleutner, M., Koerner, P., Westerholt, A., Cziupka, K., van Rooijen, N., Heidecke, C.D., *et al.*, 2009. Selective depletion of alveolar macrophages in polymicrobial sepsis increases

lung injury, bacterial load and mortality but does not affect cytokine release. *Respiration*, **77**(2):203-213. [doi:10.1159/000160953]

van Rooijen, N., Sanders, A., 1994. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. *J. Immunol. Methods*, **174**(1-2):83-93. [doi:10.1016/0022-1759(94)90012-4]

van Rooijen, N., van Kesteren-Hendrikx, E., 2003. "In vivo" depletion of macrophages by liposome-mediated "suicide". *Methods Enzymol.*, **373**:3-16. [doi:10.1016/S0076-6879(03)73001-8]

van Rooijen, N., Sanders, A., van den Berg, T.K., 1996. Apoptosis of macrophages induced by liposome-mediated intracellular delivery of clodronate and propamidine. *J. Immunol. Methods*, **193**(1):93-99. [doi:10.1016/0022-1759(96)00056-7]

Viterbo, D., Bluth, M.H., Mueller, C.M., Zenilman, M.E., 2008. Mutational characterization of pancreatitis-associated protein 2 domains involved in mediating cytokine secretion in macrophages and the NF-κB pathway. *J. Immunol.*, **181**(3):1959-1968.

Weinbroum, A.A., 2009. Mannitol prevents acute lung injury after pancreas ischemia-reperfusion: a dose-response, ex vivo study. *Lung*, **187**(4):215-224. [doi:10.1007/s00408-009-9154-6]

Zhang, J.X., Dang, S.C., 2006. Ligustrazine alleviates acute lung injury in a rat model of acute necrotizing pancreatitis. *Hepatobiliary Pancreat. Dis. Int.*, **5**(4):605-609.

Zhang, J.X., Dang, S.C., Qu, J.G., Wang, X.Q., 2006. Preventive effect of tetramethylpyrazine on intestinal mucosal injury in rats with acute necrotizing pancreatitis. *World J. Gastroenterol.*, **12**(39):6386-6390.

Zhang, J.X., Dang, S.C., Zhang, Y., Sha, X., Zhang, L.R., Wei, C.S., Chen, M., Jiang, D.L., 2010. MRI shows clodronate-liposomes attenuating liver injury in rats with severe acute pancreatitis. *Hepatobiliary Pancreat. Dis. Int.*, **9**(2):192-200.

Zhao, M.Q., Fernandez, L.G., Doctor, A., Sharma, A.K., Zarbock, A., Tribble, C.G., Kron, I.L., Laubach, V.E., 2006. Alveolar macrophage activation is a key initiation signal for acute lung ischemia-reperfusion injury. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **291**(5):L1018-L1026. [doi:10.1152/ajplung.00086.2006]

New Information on *JZUS(A/B/C)*

(http://www.zju.edu.cn/jzus)

In 2010, we have updated the website and opened a few active topics:

- **The top 10 most recently cited papers in parts A, B, C;**
- **The newest cited papers in parts A, B, C;**
- **The top 10 DOIs monthly;**
- **The top 10 most recently commented papers in parts A, B, C.**
  (Welcome your comment and opinion!)

We also list the International Reviewers to express our deep appreciation and Crosscheck information etc.

If you would like to allot a little time to open http://www.zju.edu.cn/jzus, you will find more interesting information. Many thanks for your interest in our journals' publishing change and development in the past, present and future!

Welcome to comment on what you would like to discuss. And also welcome your interesting/high-quality paper to *JZUS(A/B/C)* soon.