PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 June 7; 13(21): 2932-2938
World Journal of Gastroenterology ISSN 1007-9327

BASIC RESEARCH

Leptin treatment ameliorates acute lung injury in rats with cerulein-induced acute pancreatitis

Fatma Ayca Gultekin, Mustafa Kerem, Ertan Tatlicioglu, Aysel Aricioglu, Cigdem Unsal, Neslihan Bukan

Fatma Ayca Gultekin, Mustafa Kerem, Ertan Tatlicioglu, Gazi University, School of Medicine, Department of General Surgery, Besevler 06510, Ankara, Turkey
Aysel Aricioglu, Neslihan Bukan, Gazi University, School of Medicine, Department of Biochemistry, Besevler 06510, Ankara, Turkey
Cigdem Unsal, Gazi University, School of Medicine, Department of Pathology, Besevler 06510, Ankara, Turkey
Supported by a grant from The Scientific and Technical Research Council of Turkey, TUBITAK, project no: 105S018 (SBAG-HD-5), and Gazi University, Scientific Research Projects Unit, No. G.U.ET-05.013
Correspondence to: Dr. Fatma Ayca Gultekin,Gazi University, School of Medicine, Department of General Surgery, 42. Cadde 451 Sokak Konak Apt. No:2/2 Cukurambar 06520 Ankara, Turkey. faycagultekin@yahoo.com
Telephone: +90-312-2857962 Fax: +90-312-2230528
Received: 2007-02-11 Accepted: 2007-04-16

Abstract

AIM: To determine the effect of exogenous leptin on acute lung injury (ALI) in cerulein-induced acute pancreatitis (AP).

METHODS: Forty-eight rats were randomly divided into 3 groups. AP was induced by intraperitoneal (i.p.) injection of cerulein (50 μg/kg) four times, at 1 h intervals. The rats received a single i.p. injection of 10 μg/kg leptin (leptin group) or 2 mL saline (AP group) after cerulein injections. In the sham group, animals were given a single i.p. injection of 2 mL saline. Experimental samples were collected for biochemical and histological evaluations at 24 h and 48 h after the induction of AP or saline administration. Blood samples were obtained for the determination of amylase, lipase, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, macrophage inflammatory peptide (MIP)-2 and soluble intercellular adhesion molecule (sICAM)-1 levels, while pancreatic and lung tissues were removed for myeloperoxidase (MPO) activity, nitric oxide (NOx) level, CD40 expression and histological evaluation.

RESULTS: Cerulein injection caused severe AP, confirmed by an increase in serum amylase and lipase levels, histopathological findings of severe AP, and pancreatic MPO activity, compared to the values obtained in the sham group. In the leptin group, serum levels of MIP-2, sICMA-1, TNF-α, and IL-1β, pancreatic MPO activity, CD40 expression in pancreas and lung tissues, and NOx level in the lung tissue were lower compared to those in the AP group. Histologically, pancreatic and lung damage was less severe following leptin administration.

CONCLUSION: Exogenous leptin attenuates inflammatory changes, and reduces pro-inflammatory cytokines, nitric oxide levels, and CD40 expression in cerulein-induced AP and may be protective in AP associated ALI.

Key words: Leptin; Acute pancreatitis; Lung injury; CD40; Cytokines

Gultekin FA, Kerem M, Tatlicioglu E, Aricioglu A, Unsal C, Bukan N. Leptin treatment ameliorates acute lung injury in rats with cerulein-induced acute pancreatitis. *World J Gastroenterol* 2007; 13(21): 2932-2938

http://www.wjgnet.com/1007-9327/13/2932.asp

INTRODUCTION

Acute pancreatitis (AP) is a non-infectious inflammatory reaction of the pancreas, associated with autodigestion of the organ[1]. Lung injury is the most important manifestation of extra abdominal organ dysfunction in acute pancreatitis[2]. Approximately one-third of patients develop acute lung injury (ALI), and acute respiratory distress syndrome (ARDS) accounts for 60% of all deaths in the first week[3]. Apart from mechanical ventilatory support, few therapies have shown any clinical benefit. New agents such as anti-cytokines and anti-nuclear factor kappa are still being evaluated in experimental models of pancreatitis[1,4]. ALI is characterized by an increase in pulmonary microvascular permeability, with protein-rich transudate spilling into the alveolar spaces, resulting in decreased lung compliance. Such physicochemical alterations are mediated by the local release of cytotoxic and vasoactive substances. Recent studies have shown that cytokines and adhesion molecules such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), macrophage inflammatory peptide-2 (MIP-2), intercellular adhesion molecule-1 (ICAM-1) and CD40 (50-kDa protein expressed on the membranes of B lymphocytes, monocytes, dendritic cells, and biliary epithelial cells) as well as neutrophil activation and adhesion contribute to the development and severity of AP and ALI[2,5-7].

Leptin is a peptide hormone that is produced

predominantly by white adipose cells[8]. The mature protein, encoded by the obese (*ob*) gene, is localized on human chromosome 7 and mouse chromosome 6[9]. In addition to metabolic and endocrine functions, leptin also plays a regulatory role in hematopoiesis, immunity and inflammation[10,11]. Alterations in immune and inflammatory responses are present in leptin or leptin-receptor-deficient animals, as well as during starvation and malnutrition. Both conditions are characterized by low levels of circulating leptin. Leptin is believed to play a role in immune function, as it is a member of the helical cytokine family with a structure resembling the hematopoietic cytokines IL-2, IL-6 and IL-15[10]. The leptin receptors (Ob-R) show amino acid sequence homology to hematopoietic (class 1) cytokine receptors[12]. Besides fat tissues, Ob-R is also present in other tissues such as liver, pancreas, lung, and kidney[13]. Leptin exerts proliferative and anti-apoptotic activity in a variety of cell types including T lymphocytes, leukemia cells, and hematopoietic progenitors. It also affects cytokine production, the activation of monocytes/macrophages, wound healing and angiogenesis. Moreover, leptin production increases acutely during infection, inflammation, and pancreatitis[10,14]. Recent studies have demonstrated that AP in rats and humans is accompanied by an increase in plasma levels of leptin[15,16]. Furthermore, the elevation of leptin mRNA expression in the pancreas during AP suggests that the increased level of this hormone in serum originates from the pancreas[16]. Another study showed that exogenous leptin administration was associated with a reduction in the severity of AP, presumably through a decrease in the production of plasma pro-inflammatory cytokines and NO[17]. Whether exogenous leptin has any effect on AP-associated lung injury remains unclear, however, its anti-inflammatory and anti-apoptotic actions would suggest that it may reduce the occurrence of extra-abdominal organ dysfunctions such as ALI. In the present study, we examined the effects of exogenous leptin on lung injury in a cerulein model of AP.

MATERIALS AND METHODS

Experimental animal models and Study groups

Forty eight female Wistar rats weighing 230-260 g were used in the study. The experiments were performed in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals, and was approved by the Committee for Research and Animal Ethics of Gazi University. The rats were maintained at 23℃ in a 12 h light dark cycle, with free access to water and standard rat chow. Prior to the start of the experiments, the rats were deprived of food while drinking water was available ad libitum. Three experimental groups were established: sham group (n = 16), acute pancreatitis (AP) group (n = 16) and leptin group (n = 16). AP was induced by intraperitoneal (i.p.) injection of cerulein (Sigma-Aldrich Chemical, Steinheim, Germany) diluted in saline and administered at the dose of 50 μg/kg, four times, at 1h intervals[18]. At the end of cerulein injections the rats were given 2 mL i.p. saline in the AP group. The leptin group, received leptin (mouse recombinant leptin, Sigma, Saint-Louis, Missouri, USA) at a dose of 10 μg/kg i.p. dissolved in 1 mL of saline followed by cerulein injections. The sham group was injected with 2 mL 0.9% NaCl solution i.p. All groups were randomly subdivided into 24 h and 48 h groups, each group containing 8 rats. Frossard *et al*[2] have demonstrated that administration of supramaximal dose of cerulein to rats led to AP associated ALI; the severity of ALI was time-dependent, with maximal injury occurring at 24 h and 48 h after the start of cerulein injections. Therefore, in our study, the animals were sacrificed at 24 h and 48 h after the cerulein or saline administration.

Following cessation of treatment, animals were anesthetized by an intramuscular injection of 40 mg/kg ketamine (Ketalar®, Parke Davis, Eczacıbasi, Istanbul, Turkey) and 5 mg/kg xylazine (Rompum®, Bayer AG, Leverkusen, Germany). The abdominal and thoracic cavities were opened and blood, pancreatic and lung samples were obtained. The blood samples were stored at -80℃ for biochemical analysis, which was run in duplicates. Random cross-sections of the pancreatic head, body and tail, and of the right lung were fixed in a 40 g/L solution of formaldehyde, in 0.1 mol/L phosphate-buffed saline (pH 7.4), and embedded in paraffin. Samples of pancreas and lung tissues were stored at -70℃ for subsequent biochemical measurements.

Serum amylase and lipase measurements

Serum amylase and lipase were determined with a Beckman Coulter LX-20® System analyzer (Fullerton, CA, USA) using Beckman kits (Fullerton, CA, USA), according to the supplier's specifications.

Serum TNF-α, IL-1β, MIP-2 and Soluble ICAM-1 measurements

Serum levels of TNF-α, IL-1β, MIP-2 (Biosource International, Camarillo, CA, USA) and soluble ICAM-1 (sICAM-1, Quantikine, R&D Systems, MN, USA) were determined by using a commercially available enzyme-linked immunosorbent assay (ELISA). TNF-α, IL-1β, MIP-2 and sICAM-1 levels were determined from a standard curve for the combination of these cytokines. The concentrations were expressed as pg/mL.

Measurement of tissue myeloperoxidase activity

Pancreas and lung tissues were homogenized in TRIS buffer. Sequestration of neutrophils within the pancreas and lung was evaluated by quantitation of tissue myeloperoxidase (MPO) activity by the ELISA method (Hbt Hycult Mouse MPO ELISA kit, Uden, Netherlands).

Measurement of NOx level

Total nitrate/nitrite (NOx) concentration was measured in a simple two-step process by using the Cayman Chemical NOx Assay Kit. The first step was the conversion of nitrate to nitrite, utilizing nitrate reductase. The second step was the addition of the Griess reagents, which converted nitrite into a deep-purple azo-compound. Photometric measurement of the absorbance due to this azo-chromophore accurately determined nitrite

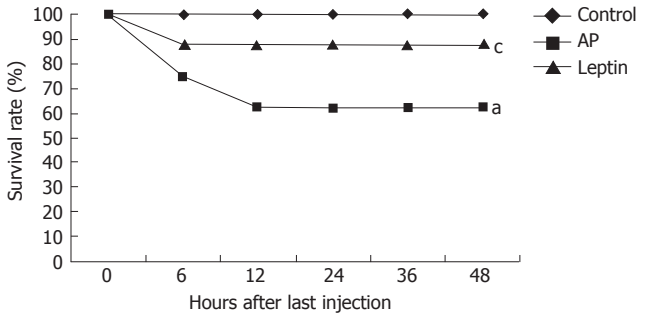

Figure 1 Effects of leptin treatment on survival rate. ($^aP < 0.001$ *vs* sham group, $^cP < 0.05$ *vs* AP group).

concentration. Concentrations were expressed as nmol/mg protein[19].

Histological examination

Sections of the pancreas and lung (4 μm thick) were stained with hematoxylin-eosin (HE) and examined with a light microscope as described previously[20,21].

Immunohistochemical assessment of CD40

Specimens were fixed in a 40 g/L solution of formaldehyde in 0.1 mol/L phosphate-buffed saline (pH 7.4), and embedded in paraffin wax from which four-μm-thick sections were taken on a slide coated with poly-L-lysine in order to be de-waxed for immunohistochemical assessment. The specimens were treated with 3% hydrogen peroxidase, following 10 min of endogenous peroxidase blockage. Antigen retrieval was performed with EDTA. The samples were incubated at room temperature for 45 min, with a 1:50 dilution of anti-CD40 primary antibody (clone: 3/23, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Following the processing of the samples with a streptavidin biotin peroxidase complex, 3-amino-9-ethylcarbazole was applied as a chromogen, and hematoxylin was used for floor staining. CD40 is expressed on the surface of B lymphocytes. Tonsil tissue was used as a positive control since the tonsils are lymphoid organs where B lymphocytes are commonly present in the lymphoid follicles[22]. CD40 staining was graded from 0 to 4; the number of cells which revealed CD40-positive staining obtained from five × 400 magnified fields was divided by the total cell number. According to these observations, less than 50% of the stained cell population was considered focally stained, and more than 50% of the stained cell population was considered diffusely stained. Grade 0: no staining; grade 1: slight cytoplasmic or membranous staining in < 50% of the cell population (focal); grade 2: slight cytoplasmic or membranous staining in > 50% of the cell population (diffuse); grade 3: strong cytoplasmic or membranous staining in < 50% of the cell population (focal); grade 4: strong cytoplasmic or membranous staining in > 50% of the cell population (diffuse).

Statistical analysis

All values were expressed as mean ± SE and the results were compared by analysis of variance (ANOVA) with post hoc analysis using the Bonferroni test. The log rank test was used for survival analysis. $P < 0.05$ was considered statistically significant. Statistical evaluation was carried out using the SPSS 12.0 software (SPSS, Chicago, IL, USA).

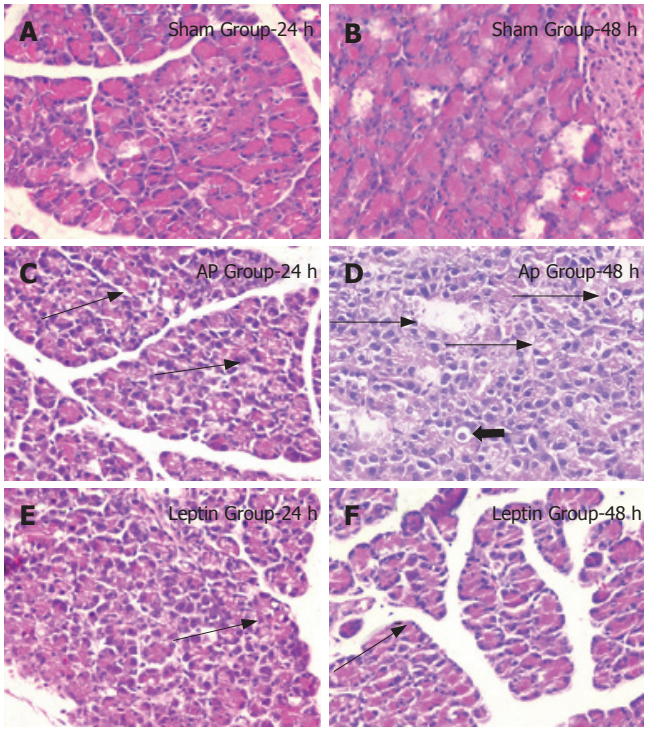

Figure 2 Light microscopy showing normal pancreatic tissue in the sham group (**A**, **B**) (HE, × 200), broad single cell necrosis (arrow) and significant increase in vacuolization (bold arrow) in the AP group at 24 h and 48 h (**C**, **D**) (HE, × 400), attenuated necrosis and vacuolization after leptin treatment at 24 h (**E**) (HE, × 400). Mild edema was observed at 48 h in leptin-treated rats (**F**, arrow).

RESULTS

Kaplan-Meier survival curves (Figure 1) showed absence of mortality after the sham operation (100% survival), while the survival rate was reduced to 62.5% in the AP group ($P < 0.001$ *vs* sham). Treatment with leptin was associated with significant improvement in the survival rate (87.5%) throughout the 48 h observation period ($P < 0.05$).

Effects of exogenous leptin on pancreatic injury in AP

Supramaximal doses of cerulein injections resulted in severe AP in the animals. This was confirmed by an increase in serum amylase and lipase levels ($P < 0.001$, Tables 1 and 2), histopathological findings of severe AP ($P < 0.05$, Table 3, Figures 2C and D), and pancreatic MPO activity, as a measurement of neutrophil infiltration ($P = 0.004$, Tables 1 and 2) in the cerulein injected rats compared to saline administered rats. Serum amylase levels and pancreatic MPO activity in rats treated with leptin were markedly lower at 24 h compared to cerulein-only injected rats ($P < 0.001$, Tables 1 and 2). However, there was no significant difference in serum lipase levels between the AP group and the leptin treated group (Tables 1 and 2). Histopathological examination of the pancreas confirmed the beneficial effect of exogenous leptin treatment on AP (Table 3). In the pancreatic tissue from the AP group,

Table 1 Biochemical markers in the blood, and pancreatic and lung tissues at 24 h mean ± SE

Groups	Amylase (U/L)	Lipase (U/L)	TNF-α (pg/mL)	IL-1b (pg/mL)	MIP (pg/mL)	sICAM (pg/mL)	Pancreas MPO (U/gr)	Lung MPO (U/gr)	Lung NOx (nmol/mg protein)
Sham Group	44.2 ± 5.8	17.2 ± 1.0	9.5 ± 0.7	6.4 ± 0.8	5.7 ± 8.5	11132 ± 626	0.17 ± 0.02	2.5 ± 0.6	8.1 ± 1.1
AP Group	1913 ± 205[a]	47.1 ± 9.0	114.6 ± 16.5[a]	107.9 ± 5.1[a]	128.3 ±5.1[a]	27828 ± 1483[a]	25.0 ± 11.1[a]	95.8 ± 17.0[a]	26.6 ± 0.8[a]
Leptin Group	1419 ± 87	39.4 ± 36.3	67.2 ± 7.0	67.2 ± 7.0	45.0 ± 10.2	21042 ± 1676	4.1 ± 1.6	38.2 ± 6.3	19.3 ± 0.7

[a]$P < 0.05$ *vs* the leptin group.

Table 2 Biochemical markers in the blood, and pancreatic and lung tissues at 48h mean ± SE

Group	Amylase (U/L)	Lipase (U/L)	TNF-α (pg/mL)	IL-1b (pg/mL)	MIP (pg/mL)	sICAM (pg/mL)	Pancreas MPO (U/gr)	Lung MPO (U/gr)	Lung NOx (nmol/mg protein)
Sham group	37.3 ± 14.3	15.3 ± 2.1	5.5 ± 0.8	8.6 ± 3.7	8.5 ±3.8	14216 ± 1148	0.51 ± 0.29	6.2 ± 2.3	6.1 ± 0.8
AP group	1078 ± 77	44.2 ± 4.5	91.2 ± 2.2[a]	94.8 ± 7.5[a]	78.1 ± 9.7[a]	28416 ± 1321[a]	11.4 ± 2.5	97.6 ± 15.1[a]	21.9 ± 0.7
Leptin group	1233 ± 128	36.3 ± 1.7	53.6 ± 3.8	37.0 ± 6.1	28.9 ± 2.3	16712 ± 1054	5.0 ± 1.7	29.7 ± 2.9	15.3 ± 0.8

[a]$P < 0.05$ *vs* the leptin group.

Table 3 Histological scores of pancreatic injury mean ± SE

Group	Edema		Inflammation		Vacuolization		Necrosis	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Sham group	0	0	0	0	0	0	0	0
AP group	2.57 ± 0.20[a]	2.37 ± 0.18[a]	1.28 ± 0.28	1.75 ± 0.46a	2.28 ± 0.35[a]	1.50 ± 0.16[a]	0.71 ± 0.18	1.00 ± 0.00[a]
Leptin group	1.14 ± 0.14	1.12 ± 0.12	0.57 ± 0.20	0.50 ± 0.18	0.42 ± 0.20	0.50 ± 0.18	0.28 ± 0.18	0.50 ± 0.14

[a]$P < 0.05$ *vs* the leptin group.

Table 4 Histological scores of pulmonary injury mean ± SE

Group	Alveolar edema		Alveolar distention		PMNC infiltration		Alveolar wall thickening	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Sham group	0	0	0	0	0	0	0	0
AP group	2.28 ± 0.35[a]	1.62 ± 0.18[a]	1.42 ± 0.36	1.25 ± 0.16	2.71 ± 0.18[a]	2.21 ± 0.12[a]	0.85 ± 0.40	2.00 ± 0.18[a]
Leptin group	1.28 ± 0.18	0.37 ± 0.18	1.25 ± 0.16	0.62 ± 0.51	0.57 ± 0.29	0.75 ± 0.16	0.42 ± 0.29	0.87 ± 0.22

[a]$P < 0.05$ *vs* the leptin group.

pancreatic acinar and inflammatory cells stained strongly with CD40 (Figure 3C and D). However, there was no such staining in the pancreatic tissue of leptin treated (Figure 3E and F) and sham treated rats (Figure 3A and B). The leptin treated group showed a significant reduction in CD40 expression ($P < 0.001$, Table 5).

Effects of exogenous leptin on ALI associated with AP

Tissue MPO activities and NOx levels in lung tissues increased significantly in cerulein administered rats compared with rats that received saline, indicating that increased neutrophil infiltration and microvascular permeability were the result of pancreatitis ($P < 0.001$, Tables 1 and 2). Treatment of exogenous leptin significantly reduced lung MPO activities and NOx levels in cerulein injected animals ($P < 0.001$, Tables 1 and 2). Histopathologically, leptin treatment significantly decreased alveolar edema, PMNC infiltration and alveolar wall thickening in ALI associated with cerulein-induced AP (Figure 2). Exogenous leptin attenuated the lung injury in cerulein injected animals ($P < 0.001$, Table 4). There was more intense cytoplasmic staining with CD40 in fibroblasts in the alveolar wall and inflammatory cells in the AP group (Figures 4C and D) compared with the sham group ($P < 0.001$, Figure 4A and B). In rats treated with leptin, there was a significant decrease in the lung CD40 expression at 24h and 48h compared with the AP group ($P < 0.001$, Table 5, Figure 4E and F).

Effects of exogenous leptin on cytokines in AP

Serum cytokines and sICAM levels were markedly higher in both AP and leptin groups compared to the sham group ($P < 0.001$, Tables 1 and 2). Serum TNF-α, IL-1β and MIP-2 levels increased at 24 h after cerulein injection and tended to decrease at 48 h. sICAM levels remained high during the 48 h study period. Treatment with leptin decreased TNF-α, IL-1β, MIP-2, and sICAM levels in cerulein administered rats ($P < 0.001$, Tables 1 and 2). The

Table 5 Comparison of pancreatic and pulmonary CD40 staining levels in different study groups

Group	Pancreas		Lung	
	24 h	48h	24 h	48 h
Sham group	0	0	0	0
AP group	2.6 ± 0.8[a]	3.4 ± 0.4[a]	2.6 ± 0.8[a]	3.1 ± 0.6[a]
Leptin group	1.5 ± 0.6	2.1 ± 0.3	1.5 ± 0.6	1.9 ± 0.5

[a]$P < 0.05$ *vs* the leptin group.

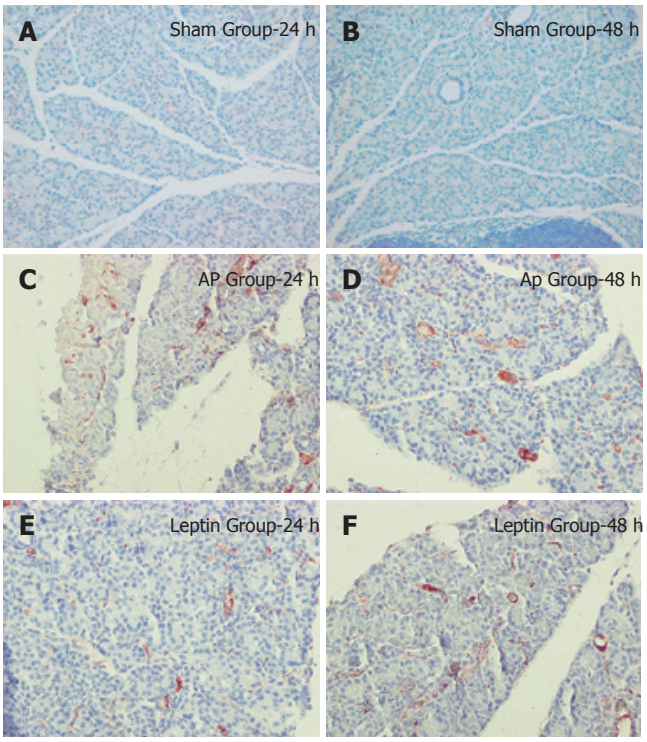

Figure 3 Immunohistochemical localization of CD40 expression in the pancreas in the sham group (**A**, **B**), AP group (**C**, **D**) and leptin group (**E**, **F**). (Divisions **A**, **C**, **E** on the figure represent pulmonary sections at 24 h and **B**, **D**, **F** represent pulmonary sections at 48 h).

decrease in cytokines and sICAM levels in the leptin group may contribute to the attenuation of lung injury associated with cerulean-induced AP.

DISCUSSION

To our knowledge, the present study is the first to demonstrate that exogenous leptin administration significantly reduces ALI in rats with cerulein induced AP. The exogenous leptin treatment reduced cerulein induced pancreatitis, and pancreatitis-associated lung injury, with reduction in the serum concentrations of TNF-α, IL-1β, MIP-2 and sICAM-1, decrease in the pancreatic and pulmonary neutrophil activation, and in MPO activity, and NOx, and CD40 levels. In addition, the exogenous leptin treatment significantly reduced mortality rates in rats with AP induced by supramaximal doses of cerulein.

Histological findings of ALI were more prominent after injection of supramaximal doses of cerulein compared to those seen in the sham group at 24 h and

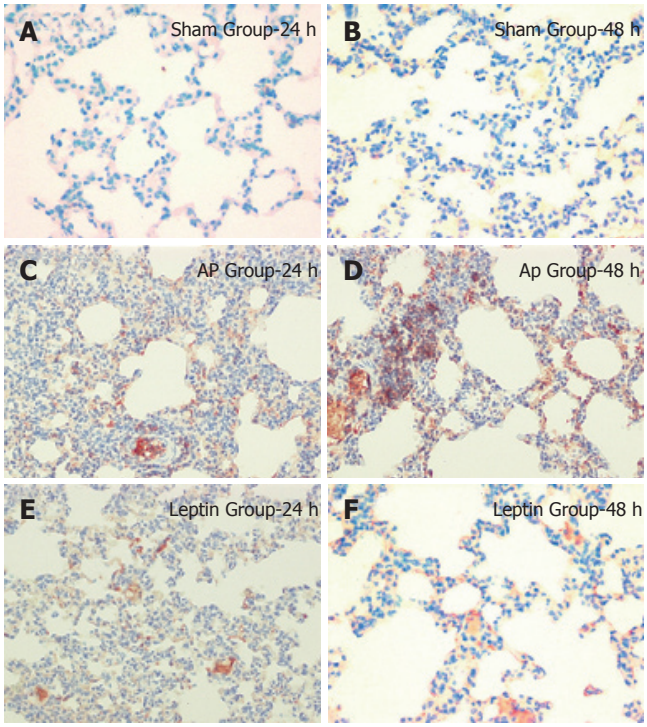

Figure 4 Immunohistochemical localization of CD40 expression in lung tissues. There was no staining in the sham group (**A**, **B**), whereas marked staining was observed in the AP group (**C**, **D**). CD40 staining was significantly reduced in leptin-treated rats (**E**, **F**). (Divisions **A**, **C**, **E** on the figure represent pulmonary sections at 24 h and **B**, **D**, **F** represent pulmonary sections at 48 h).

48 h; maximum improvements in histological features of ALI were observed at 48 h after leptin administration. During AP, lung injury was associated with accumulation of neutrophils in the interstitial and alveolar spaces, a common finding in both clinical and experimental studies[1,2,5,6]. It has been shown that reduction in circulating serum neutrophils, and strategies which interfere with neutrophil recruitment, such as ICAM-1 blocking antibodies are accompanied with decrease in neutrophil infiltration and in pancreatic and distant organ damage in AP[3,23]. In our study, induction of AP was associated with a significant increase in pancreatic and lung MPO activity, indicating that neutrophils were sequestered in both organs. Exogenous leptin treatment markedly decreased the MPO activity. Jaworek *et al*[15] have shown that exogenous leptin treatment reduced neutrophil infiltration. However, an exact explanation of the effects of leptin on neutrophil activation remains unknown. ICAM-1 and MIP-2 play an important role in the activation and the adhesion of neutrophils[23,24]. A better understanding of the effects of leptin on these factors may help in defining their effects on neutrophil activation.

ICAM-1 is an inducible protein expressed on the surface of endothelial cells. ICAM-1 plays an important role in neutrophil adhesion and neutrophil mediated lung injury during AP. Serum ICAM-1 concentrations peak at 24 h after cerulein administration[23]. In the present study, exogenous leptin significantly reduced serum ICAM-1 concentrations compared with the sham group. These findings suggest that neutrophil adhesion in the lung may be ameliorated by leptin administration.

During AP, MIP-2 is involved in neutrophil activation and sequestration in the pancreas and lungs[25]. MIP-2 is a potent rodent chemokine, homologous to GRO-β, which binds to the C-X-C chemokine receptor-2[26]. We found that cerulein induced AP was associated with a significant increase in serum MIP-2 concentrations, and that leptin treatment substantially decreased the MIP-2 concentration. The effect of leptin administration on serum MIP-2 concentration is not clearly established. To the best of our knowledge, this is the first study demonstrating the effect of leptin on serum MIP-2 concentration. A decrease in the concentration of MIP-2 may therefore play an important role in reducing neutrophil adhesion and sequestration. Moreover, Ob-R is present in the pancreas and lungs[12,13]. Therefore, leptin may also reduce pancreatic neutrophil activation by affecting its lung receptors. Several *in vivo* studies have shown that endogenous leptin increases neutrophil activation[27,28]. However, administration of a higher dose of exogenous leptin compared to the baseline circulating level may result in neutrophil inhibition.

Since leptin is a pleiotropic hormone, it is expected that its effects at supraphysiological dose may be similar to the effects in normal doses. In contrast, Konturek *et al*[16] and Warzecha *et al*[17] indicated that high doses of exogenous leptin had greater effect, and markedly attenuated pancreatic damage in both cerulein-induced and ischemia/reperfusion-induced pancreatitis models. We found similar effects with exogenous leptin in this study, where high doses of leptin exhibited the protective effects in AP and in AP associated lung injury. Nevertheless, these dichotomous effects arising from differing doses of leptin remain poorly understood and require further investigation.

TNF-α, and IL-1β are derived predominantly from activated macrophages and act via specific cell membrane-bound receptors in inflammatory situations such as AP and AP associated ALI. The severity of pancreatitis has been shown to correlate with TNF-α and IL-1β levels[2,5,7]. After the onset of pancreatitis, cytokine production from lung parenchyma increases significantly, and large quantities of chemokines, including TNF-α and IL-1β are released from macrophages via the p38 mitogen activated protein-kinase pathway[1,2,5]. Specific treatments that target the reduction of TNF-α and IL-1β levels reduce the severity of ALI in AP. Konturek *et al*[16] have shown that exogenous leptin treatment reduced plasma TNF-α level and pancreatic IL-4 expression in rats with AP. In another study, Warzecha *et al*[17] concluded that leptin treatment reduced plasma IL-1β levels. *Ob-R* is a member of the class I cytokine receptor superfamily. These receptors are membrane-spanning glycoproteins[13]. Leptin is recognized as a pro-inflammatory hormone, and it shares structural similarities to cytokines IL-6, IL-15 and granulocyte colony-stimulating factor[10]. However, our understanding of the role of leptin in inflammation is incomplete. Endogenous leptin protects against TNF-mediated toxicity. *Ob/ob* mice and *db/db* mice, as well as mice treated with leptin-receptor antagonist displayed increased sensitivity to the lethal effects of TNF. The addition of exogenous leptin protected against TNF-mediated toxicity in *ob/ob* mice, but did not increase the protective effect of endogenous leptin in wild-type mice[29]. Our study showed that AP was accompanied with increased plasma levels of TNF-α and IL-1β compared to the sham group, and exogenous leptin reduced these cytokine levels at 24 h and 48 h following cerulein injection. Overstimulation of *Ob-R* might also result in a decrease in cytokine levels. Taken together, exogenous leptin treatment appears to play an important role in the reduction of pancreatic damage during AP, through a reduction in the pro-inflammatory cytokines.

Leinder *et al*[7] have shown that the expression of iNOS correlated with apoptotic changes in the lung, suggesting that NO overproduction is an important factor in the development of a systemic inflammatory reaction in response to severe pancreatitis. In the present study, the NOx concentration in lung tissues was considerably higher in rats with cerulein-induced AP, compared with controls. However, a significant decrease in the NOx level was observed with leptin treatment. Leptin was able to reduce ALI by decreasing the NOx level, which resulted in the reduction of inflammation and neutrophil activation.

CD40, a protein member of the TNF receptor superfamily, is expressed on the membrane of a variety of cells, including B lymphocytes, monocytes, biliary and acinar cells[30,31]. CD40 binds to its ligand CD40L, a membrane glycoprotein, and mediates major immunoregulatory signals involved in auto-immune disease, inflammatory bowel disease and acute experimental pancreatitis[32]. Daoussis *et al*[32] have demonstrated that the CD40 ligation on antigen-presenting cells is associated with the following: (1) enhanced cell survival; (2) secretion of cytokines (TNF-α, IL-1β, IL-6, MIP); (3) enhanced monocyte activity, and (4) NO synthesis. In a recent study, Frossard *et al*[31] concluded that elevation of serum CD40 levels is a prognostic factor for AP. In our study, pancreatic and pulmonary CD40 staining level in the AP group was significantly higher than the sham group, and exogenous leptin treatment reduced both pancreatic and pulmonary CD40 staining. Exogenous leptin may contribute to the reduction in MPO activity by decreasing lung CD40 levels. However, whether lung CD40 levels are suppressed by leptin is unclear. Leptin directly reduces pancreatic CD40 levels and inhibits monocyte activation, which plays an important role in acute lung injury.

In conclusion, supramaximal doses of exogenous leptin treatment significantly abrogated ALI in cerulein-induced AP. Exogenous leptin markedly reduced both neutrophil activation and the levels of soluble pro-inflammatory factors such as cytokines, chemokines, NOx and CD40 that are known to be involved in the development of ALI. However, the pathophysiological role of exogenous leptin in AP and pancreatitis-associated lung injury requires further investigation.

ACKNOWLEDGMENTS

This study was presented at the 41st Congress of The European Society for Surgical Research, in Rostock, Germany, 17-21 May 2006. The authors would like to thank Syn WK. MD for excellent technical assistance.

REFERENCES

1 **Klöppel G**. Acute pancreatitis. *Semin Diagn Pathol* 2004; **21**:

221-226
2 **Frossard JL**, Hadengue A, Spahr L, Morel P, Pastor CM. Natural history of long-term lung injury in mouse experimental pancreatitis. *Crit Care Med* 2002; **30**: 1541-1546
3 **Bhatia M**. Novel therapeutic targets for acute pancreatitis and associated multiple organ dysfunction syndrome. *Curr Drug Targets Inflamm Allergy* 2002; **1**: 343-351
4 **Matsuda K**, Mikami Y, Takeda K, Fukuyama S, Egawa S, Sunamura M, Maruyama I, Matsuno S. The cannabinoid 1 receptor antagonist, AM251, prolongs the survival of rats with severe acute pancreatitis. *Tohoku J Exp Med* 2005; **207**: 99-107
5 **Frossard JL**, Kwak B, Chanson M, Morel P, Hadengue A, Mach F. Cd40 ligand-deficient mice are protected against cerulein-induced acute pancreatitis and pancreatitis-associated lung injury. *Gastroenterology* 2001; **121**: 184-194
6 **Pastor CM**, Matthay MA, Frossard JL. Pancreatitis-associated acute lung injury: new insights. *Chest* 2003; **124**: 2341-2351
7 **Leindler L**, Morschl E, László F, Mándi Y, Takács T, Jármai K, Farkas G. Importance of cytokines, nitric oxide, and apoptosis in the pathological process of necrotizing pancreatitis in rats. *Pancreas* 2004; **29**: 157-161
8 **Zhang Y**, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**: 425-432
9 **Ahima RS**, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS. Role of leptin in the neuroendocrine response to fasting. *Nature* 1996; **382**: 250-252
10 **Fantuzzi G**, Faggioni R. Leptin in the regulation of immunity, inflammation, and hematopoiesis. *J Leukoc Biol* 2000; **68**: 437-446
11 **Friedman JM**. The function of leptin in nutrition, weight, and physiology. *Nutr Rev* 2002; **60**: S1-S14; discussion S68-S84, S85-S87
12 **White DW**, Tartaglia LA. Leptin and OB-R: body weight regulation by a cytokine receptor. *Cytokine Growth Factor Rev* 1996; **7**: 303-309
13 **Tartaglia LA**. The leptin receptor. *J Biol Chem* 1997; **272**: 6093-6096
14 **Yavuz N**, Unal E, Memisoglu K, Krand O, Kiziler AR, Aydemir B, Kusaslan R, Dogan M, Gunes P, Titiz I. Plasma leptin levels in rats with pancreatitis. *Tohoku J Exp Med* 2004; **204**: 243-248
15 **Jaworek J**, Bonior J, Pierzchalski P, Tomaszewska R, Stachura J, Sendur R, Leja A, Jachimczak B, Konturek PC, Bielański W, Pawlik W, Konturek SJ. Leptin protects the pancreas from damage induced by caerulein overstimulation by modulating cytokine production. *Pancreatology* 2002; **2**: 89-99
16 **Konturek PC**, Jaworek J, Maniatoglou A, Bonior J, Meixner H, Konturek SJ, Hahn EG. Leptin modulates the inflammatory response in acute pancreatitis. *Digestion* 2002; **65**: 149-160
17 **Warzecha Z**, Dembiński A, Ceranowicz P, Jaworek J, Konturek PC, Dembiński M, Bilskl J, Konturek SJ. Influence of leptin administration on the course of acute ischemic pancreatitis. *J Physiol Pharmacol* 2002; **53**: 775-790
18 **Tani S**, Otsuki M, Itoh H, Fujii M, Nakamura T, Oka T, Baba S. Histologic and biochemical alterations in experimental acute pancreatitis induced by supramaximal caerulein stimulation. *Int J Pancreatol* 1987; **2**: 337-348
19 **Archer S**. Measurement of nitric oxide in biological models. *FASEB J* 1993; **7**: 349-360
20 **Schmidt J**, Lewandrowsi K, Warshaw AL, Compton CC, Rattner DW. Morphometric characteristics and homogeneity of a new model of acute pancreatitis in the rat. *Int J Pancreatol* 1992; **12**: 41-51
21 **Bhatia M**, Saluja AK, Hofbauer B, Lee HS, Frossard JL, Steer ML. The effects of neutrophil depletion on a completely noninvasive model of acute pancreatitis-associated lung injury. *Int J Pancreatol* 1998; **24**: 77-83
22 **Leifeld L**, Trautwein C, Dumoulin FL, Manns MP, Sauerbruch T, Spengler U. Enhanced expression of CD80 (B7-1), CD86 (B7-2), and CD40 and their ligands CD28 and CD154 in fulminant hepatic failure. *Am J Pathol* 1999; **154**: 1711-1720
23 **Bhatia M**, Proudfoot AE, Wells TN, Christmas S, Neoptolemos JP, Slavin J. Treatment with Met-RANTES reduces lung injury in caerulein-induced pancreatitis. *Br J Surg* 2003; **90**: 698-704
24 **Makhija R**, Kingsnorth AN. Cytokine storm in acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2002; **9**: 401-410
25 **Pastor CM**, Rubbia-Brandt L, Hadengue A, Jordan M, Morel P, Frossard JL. Role of macrophage inflammatory peptide-2 in cerulein-induced acute pancreatitis and pancreatitis-associated lung injury. *Lab Invest* 2003; **83**: 471-478
26 **Murphy PM**, Baggiolini M, Charo IF, Hébert CA, Horuk R, Matsushima K, Miller LH, Oppenheim JJ, Power CA. International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev* 2000; **52**: 145-176
27 **Zarkesh-Esfahani H**, Pockley G, Metcalfe RA, Bidlingmaier M, Wu Z, Ajami A, Weetman AP, Strasburger CJ, Ross RJ. High-dose leptin activates human leukocytes via receptor expression on monocytes. *J Immunol* 2001; **167**: 4593-4599
28 **Zarkesh-Esfahani H**, Pockley AG, Wu Z, Hellewell PG, Weetman AP, Ross RJ. Leptin indirectly activates human neutrophils via induction of TNF-alpha. *J Immunol* 2004; **172**: 1809-1814
29 **Takahashi N**, Waelput W, Guisez Y. Leptin is an endogenous protective protein against the toxicity exerted by tumor necrosis factor. *J Exp Med* 1999; **189**: 207-212
30 **Mach F**, Schönbeck U, Libby P. CD40 signaling in vascular cells: a key role in atherosclerosis? *Atherosclerosis* 1998; **137** Suppl: S89-S95
31 **Frossard JL**, Morel P, Kwak B, Pastor C, Berney T, Buhler L, Von Laufen A, Demulder S, Mach F. Soluble CD40 ligand in prediction of acute severe pancreatitis. *World J Gastroenterol* 2006; **12**: 1613-1616
32 **Daoussis D**, Andonopoulos AP, Liossis SN. Targeting CD40L: a promising therapeutic approach. *Clin Diagn Lab Immunol* 2004; **11**: 635-641

S- Editor Liu Y **L- Editor** Anand BS **E- Editor** Chen GJ