

## Effect of Angiopoietin-Like Protein 4 on Severe Acute Pancreatitis-induced Lung Injury in Rats

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### Abstract

Numbers of therapeutic options have been explored for acute pancreatitis (AP), but none has been proven to be effective in clinic. Angiopoietin-like 4 (Angptl4) is a circulating protein, predominantly expressed in the liver and adipose tissue. Overexpression of Angptl4 has been explored in regulating lipid metabolism and angiogenesis. However, *Angptl4* is the target gene of peroxisome proliferation activators-PPARs, the latter has been studied with its anti-inflammatory effects. In this study, we investigated effects of Angptl4 on severe acute pancreatitis-induced lung injury in rats. Acute Pancreatitis was induced by retrograde infusion of 1.5% deoxycholic acid sodium salt (1 mg/kg) into the bile-pancreatic duct. The severity of pancreatitis was verified by serum amylase (AMY) and alanine aminotransferase (ALT) levels. BCL-2 expression was observed in pancreatitis tissue performed by immunohistochemistry staining. Pancreatitis-associated lung injury was verified by oxygen saturation (SpO<sub>2</sub>) levels and pathological changes. Angptl4 and PPAR- $\gamma$  expression were tested using RT-PCR and Western blotting. The levels of Raf-1 and TNF- $\alpha$  in serum, the expression of vascular endothelial growth factor (VEGF) in lung were also analyzed post operation. The results showed that the Angptl4 level was significantly increased after rosiglitazone (the PPAR- $\gamma$  agonist) treatment, but there is no difference in GW9662 (PPAR- $\gamma$  antagonist) group. The rosiglitazone treatment significantly alleviated the level of AMY, ALT and TNF- $\alpha$ , and also decreased the expression of VEGF and BCL-2 in the vascular endothelial cells of lung and acinus of pancreas. Our studies indicated that rosiglitazone could affect Angptl4 expression and function as a role of anti-inflammatory and antiangiogenic effects.

**Keywords:** Angiopoietin-like protein 4; Acute pancreatitis; Pancreatitis-associated lung injury

### Introduction

Acute pancreatitis (AP) is an acute disease of the abdominal syndrome. Its clinical characteristics vary from a mild, transient illness to a fatal disease. About 10%–20% of patients will progress to severe AP, and 25%–30% of the patients with severe acute pancreatitis will die from multiple organ system failure (MOSF) and pulmonary complications [1-4]. It is known that the release of inflammatory mediators and activated leukocytes generated in acute pancreatitis contribute to tissue damage and MOSF [5-7]. The exact mechanisms of AP are not entirely known, and lung injury is considered to be a major factor in mortality [8-11]. Lung injury is also characterized by increased endothelial permeability, activation of the inflammatory cytokines and infiltration of neutrophils into the interstitium in the development of systemic complications. The inflammatory mediators, such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), can recruit neutrophils to the pulmonary microvascular and up-regulate the permeability of endothelial cells. Recent studies demonstrated that secondary infection of the alveoli leads to the leak of lung-derived inflammatory mediators across the injured epithelial barrier into the circulation inducing progressive leukocyte infiltration [12].

Rosiglitazone is widely used as an anti-diabetic reagent functioned the agonists of the nuclear receptor Peroxisome proliferator-activated receptors-gamma (PPAR- $\gamma$ ). PPAR- $\gamma$  was noted as a vital role in adipocyte differentiation, insulin sensitization, and lipid metabolism [13]. Some evidences have demonstrated that PPAR- $\gamma$  is also widely expressed in various types of tumor cells and it has crucial roles in suppressing cell growth and invasion and in promoting differentiation and apoptosis [14-17]. PPAR- $\gamma$  agonists alleviated the inflammatory response by suppressed NF- $\kappa$ B-dependent transcription [18,19].

Notably, angiopoietin-like 4 (Angptl4) is originally identified as one of the target genes of PPAR- $\gamma$  [20,21]. The agonists of PPAR- $\gamma$  can enhance Angptl4 expression and also elevate the circulating levels of this protein in human subjects and rodents [22]. Moreover, Angptl4 mRNA is expressed in various tissues but is highly expressed in liver and adipose tissues [23]. In summary, except regulating insulin sensitivity and lipid partitioning, rosiglitazone is also a modulator of inflammatory response.

Angptl4 is a 50-kDa protein that belongs to the angiopoietin-like family. There is a 75% nucleotide identity and 77% amino acid identity of Angptl4 between human and rats [23], suggesting that Angptl4 may function similar in both species. Till now, seven members of the Angptl family have been identified, all of them had a secondary structural organization similar to angiopoietins, including a NH<sub>2</sub>-terminal coiled domain and a COOH-terminal fibrinogen-like domain. However, Angptl4 does not bind to either the Tie1 or Tie2 angiopoietin receptors [23]. Thus, it is currently considered an orphan ligand of which the mechanism is different from Angptl2, which has receptors for tyrosine

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**Received** January 08, 2013; **Accepted** February 19, 2013; **Published** February 26, 2013

**Citation:** Wang YX, Chen HL, Sun SS, Li HL, Zhang JW, et al. (2013) Effect of Angiopoietin-Like Protein 4 on Severe Acute Pancreatitis-induced Lung Injury in Rats. J Clin Cell Immunol 4: 135. doi:10.4172/2155-9899.1000135

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kinases, such as VEGF receptors. In this study, we aim to elucidate the potential effects of Angptl4 on anti-inflammatory activity and vascular formation.

## Materials and Methods

### Animals

Male Wistar rats, body weight 180 g–220 g, were purchased from the Specific Pathogen Free Animal Laboratory of Dalian Medical University and housed in a controlled environment. Prior to the experiment, rats were deprived of food, but drinking water was available *ad libitum*. The rats were anesthetized by intraperitoneal administration of 10% chloral hydrate (3 ml/kg). The bile-pancreatic duct was exposed, the acute pancreatitis model was induced by a standardized pressure-controlled retrograde infusion of 1.5% deoxycholic acid sodium salt (1 mg/kg) into the bile-pancreatic duct. Then linking part between bile-pancreatic duct and duodenum was compressed using a clip for 5 min. The clip was then removed and the abdomen was closed.

**Experimental designation:** Thirty-two male Wistar rats were randomly divided into the following groups: (1) control (CON) group (n=8): The abdomens of the rats were opened and the pancreas was turned over and then the abdomen was closed; (2) SAP group (n=8): Acute pancreatitis was induced by retrograde infusion of 1.5% deoxycholic acid sodium salts into the bile-pancreatic duct; (3) rosiglitazone (ROSI) group (n=8): Rats were subjected to SAP and received rosiglitazone (dose: 5 mg/kg, Santa Cruz Biotechnology), administered via the venae sublingualis 5 min after the operation; and (4) GW9662 group (n=8): Rats were subjected to SAP and received GW9662 (dose: 5 mg/kg, Santa Cruz Biotechnology) with the same administration method as the ROSI group [1]. All rats were sacrificed 24 h after induction of pancreatitis. All animals received humane care in compliance with the public health service policy on humane care and use of laboratory animals were approved by institutional animal ethics committee of Dalian Medical University.

### Serum assay

Based on preliminary data [1] that a moderate dose of rosiglitazone (5–6 mg/kg) significantly decreased serum amylase (AMY) and alanine aminotransferase (ALT) levels in the AP condition, AMY and ALT were diluted 1:6 and 1:4, respectively, using pure water. AMY, ALT and oxygen saturation (SpO<sub>2</sub>) were measured using standard techniques with an automatic biochemistry analyzer in the first affiliated hospital of Dalian Medical University.

### Semi-quantity Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA from lung tissue was extracted with Trizol reagent. cDNA was synthesized using an iScript cDNA synthesis kit and reverse transcription was then performed on a 0.8 mg total RNA in each sample according to the protocol. The reaction was incubated at 25°C for 5 min, 42°C for 30 min, 99°C for 5 min, and 5°C for 5 min and was then stored at -80°C. PCR was performed with the primers for PPAR- $\gamma$  (F: 5'-ATCTGGCCCACTTCCGG-3'; R: 5'-TGGAAGCCTGATGCTTTATCCCCA-3'); Angptl4 (F: 5'-GCCGCTACTATCCACTAC-3'; R: 5'-CCTGTTGCTCTGACTGTT-3') and  $\beta$ -actin (5'-GGAGTCTGTGGCATCCACG-3'; R: 5'-CTAGAAGCATTTGCGGTGGA-3'). PCR was performed by using a Gene Cyclyer. Amplification steps for PPAR- $\gamma$  and Angptl4 were initial denaturation at 94°C for 2 min then 94°C denaturation for 30 sec, 56°C (55°C) annealing for 30 sec, 72°C extension for 40 sec for 35 cycles and final exten-

sion at 72°C for 5 min.  $\beta$ -actin was chosen as an internal control. Gels were visualized under UV light and then images were photographed. Band intensity was determined by optical density with individual PCR product/ $\beta$ -actin ratios.

### Western blot analysis

Frozen lung tissue was mechanically homogenized in 1 ml of ice-cold extraction buffer (50 mM TrisHis, pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM ethylene diamine tetraacetic acid (EDTA), 1 mM phenylmethylsulfony fluoride, 0.1% sodium dodecylsulfate, and 1  $\mu$ g/ml each of aprotinin and leupeptin). After incubation on ice for 30 min, the homogenate was centrifuged at 12000 $\times$ g for 10 min at 4°C and the supernatant was stored at -80°C until analysis. The protein concentration was determined using the Bradford method with bovine serum albumin as a standard. Equal amount of each samples (10  $\mu$ g) were electrophoresed using 12% SDS-page gels and transferred to a PVDF membrane. Dilutions for primary antibodies were as follows: goat polyclonal anti-rat PPAR- $\gamma$  antibody (200  $\mu$ g/ml; 1:400, Santa Cruz Biotechnology) and rabbit polyclonal anti-rat Angptl4 antibody (200  $\mu$ g/ml; 1:300, Santa Cruz Biotechnology) overnight at 4°C. The membrane was washed with TBS-T (TBS containing 0.05% Tween-20) and then incubated with horseradish peroxidase-conjugated goat anti-rabbit or rabbit anti-goat secondary antibodies (500  $\mu$ g/ml 1:10000) for 1 hour at room temperature. The antibody-antigen complexes were detected with ECL reagent (Immobilon Western HRP Substrate) and exposed to X-OMAT BT films (Kodak). Films were scanned on a UniScan C800 using Photoshop software (Adobe), and the optical density (OD) of each band was determined using one software program (GELPRO4).

### Enzyme-linked immunosorbent assay (ELISA)

The concentration of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and Raf-1 in the culture medium were performed by ELISA kit according to R&D Systems protocols.

### Immunohistochemistry staining

The rat lungs and pancreas were infused with 10% buffered formalin for 24 h and embedded in paraffin. Then 5  $\mu$ m sections were prepared. Continuous sections of the paraffin-embedded tissue were taken for pathological examination with hematoxylin-eosin staining. After deparaffinization, the tissue sections were treated with sodium citrate and boiled for 2 min in a pressure cooker for restoration. After washing 3 times with PBS, they were incubated for 10 min in 3% hydrogen peroxide at room temperature. The lung tissue and pancreas sections were incubated for 1 h with anti-rat VEGF antibody (1:100) and Bcl-2 (1:200) antibody on paraffin sections at room temperature. After washing with PBS, they were incubated for 30 min at room temperature with poly-peroxidase-anti-rat IgG. Diaminobenzidine was used as a substrate for the immunoperoxidase reaction. After rinsing with water, the sections were stained with haematoxylin. Imaging was managed with an Image Pro Plus 6.0.

### Statistical analysis

Data are expressed as mean  $\pm$  s.d. and different groups were compared with a one-way analysis of variance (ANOVA). The Student-Newman-Keuls method was used to evaluate significant differences between the groups. *p* value less than 0.05 was considered to be statistically significant.

## Results

### Rosiglitazone alleviated the pancreatitis-induced lung injury by regulating the expression of AMY, ALT and SpO<sub>2</sub> in serum

To validate the extent of pancreatitis-induced lung injury, we detected the AMY, ALT concentration in serum, and the SpO<sub>2</sub> level which stands for the pulmonary ventilation post lung injury. Baselines of serum AMY, ALT and SpO<sub>2</sub> levels were detected in rats from the CON group (as shown in Table 1). Compared with control group, the AMY and ALT levels in serum post injury were significantly increased, however, SpO<sub>2</sub> was obviously decreased 24 h after SAP administration (p<0.05). We successfully built a model of AP and pancreatitis-associated lung injury. Treatment with rosiglitazone after operation significantly reduced serum AMY and ALT levels compared to the SAP group and GW9662 group (p<0.05). But there was no obvious difference in GW9662 group following the SAP administration. Here the results indicated that rosiglitazone alleviated the severity of SAP.

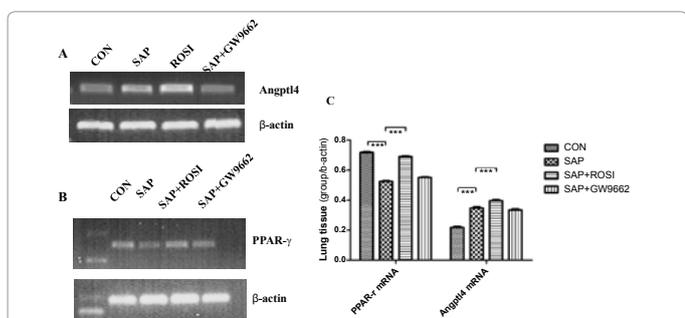
### Rosiglitazone affected the Angptl4 and PPAR-γ expression both in mRNA and protein level

To investigate the function of Angptl4 in severe acute pancreatitis, we observed the variation of Angptl4 and PPAR-γ expression after rosiglitazone and GW9662 administration. In lung tissue, the expression of Angptl4 and PPAR-γ mRNA in CON group 24 h after SAP injury, was used as the baseline. As shown in Figure 1, the changes in mRNA levels of Angptl4 and PPAR-γ in the SAP group were contradict, Angptl4 was a little increased while PPAR-γ was decreased. However, treatment with the PPAR-γ agonist-rosiglitazone significantly increased the expression of Angptl4 and PPAR-γ in the lung post injury compared to its in SAP group and CON group (p<0.05). Furthermore, the magnitude of the mRNA expressed in the GW9662 group was lower than the ROSI group.

As we described above, several upregulated factors in the

Group	SpO <sub>2</sub> (mmHg)	AMY (μl)	ALT (μl)
CON	97.53 ± 6.5	1715.00 ± 243.2	82.54 ± 5.5
SAP	72.34 ± 5.8*	7568.13 ± 571.7*	310.04 ± 16.1*
ROSI	85.84 ± 5.2*	5353.75 ± 900.4*	138.83 ± 14.9*
GW9662	73.08 ± 8.1#	7567.63 ± 651.6#	390.00 ± 16.7*

**Table 1:** Rats from different groups were killed 24h after sodium taurocholate infusion. Date are presented as mean ± sd. <sup>SAP,ROSI,GW9662</sup>P\* < 0.05 vs. the CON group. <sup>ROSI</sup>P\* < 0.05 vs. the SAP group. <sup>GW9662</sup># p > 0.05 vs. the SAP group.



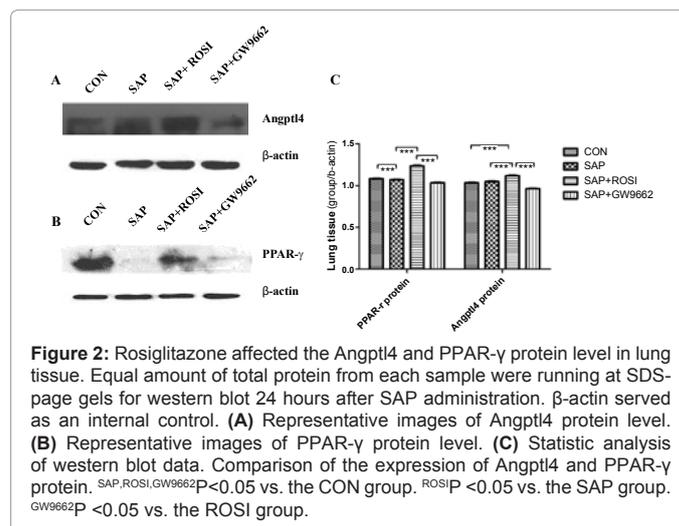
**Figure 1:** Rosiglitazone affected the Angptl4 and PPAR-γ mRNA expression in lung tissue. Total RNA was isolated 24 hours after SAP administration following RT-PCR analysis. β-actin served as an internal control. (A) Representative images of Angptl4 mRNA expression. (B) Representative images of PPAR-γ mRNA expression. (C) Statistic analysis of RT-PCR data. Comparison of the expression of Angptl4 mRNA and PPAR-γ mRNA. <sup>SAP,ROSI</sup>P\* < 0.05 vs. the CON group. <sup>ROSI</sup>P\* < 0.05 vs. the SAP group. <sup>GW9662</sup>P\* < 0.05 vs. the ROSI group.

inflammatory response were involved in the models of SAP, Angptl4 treatment resulted in a significant increase of their expression levels. Angptl4 and PPAR-γ proteins were not highly expressed in the lung tissue in CON group (Figure 2). However, after AP administration, the protein level of Angptl4 in the lung were slightly increased. But after treated with rosiglitazone (the PPAR-γ agonist), the expression of PPAR-γ proteins was significantly elevated (p<0.05). Angptl4 expression was also significantly increased. Accordingly, treatment with the PPAR-γ antagonist GW9662 inhibited the expression of PPAR-γ and Angptl4 proteins (p<0.05). Taken together, these results suggest that Angptl4 might play an important role in regulating the inflammatory response.

### Overexpression of Angptl4 affected the levels of TNF-α and Raf-1 in serum

To determine whether overexpression of Angptl4 affects angiogenic and inflammatory molecules, related to protein levels of TNF-α was analyzed by ELISA. The serum TNF-α concentration of the SAP group was obviously increased compared to the CON group (Table 2, p<0.05). However, the decrease in the TNF-α concentration in the GW9662 group was lower than in the ROSI group (p<0.05).

As expected, there was an increase in Raf-1 concentrations in the SAP group compared to the CON group. Whereas the concentration of Raf-1 in the ROSI group was greatly inhibited compared to the SAP group (p<0.05). These results suggest that the improved anti-inflammatory functions might partly contribute to the beneficial metabolic effects of Angptl4. Above all, we demonstrated that the secreted protein of Angptl4 in metabolic of serum might influence the TNF-α and Raf-1 concentrations, but the signal mechanism needs further studies.



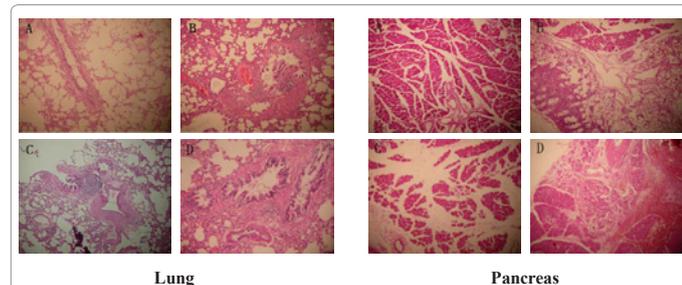
**Figure 2:** Rosiglitazone affected the Angptl4 and PPAR-γ protein level in lung tissue. Equal amount of total protein from each sample were running at SDS-page gels for western blot 24 hours after SAP administration. β-actin served as an internal control. (A) Representative images of Angptl4 protein level. (B) Representative images of PPAR-γ protein level. (C) Statistic analysis of western blot data. Comparison of the expression of Angptl4 and PPAR-γ protein. <sup>SAP,ROSI,GW9662</sup>P\* < 0.05 vs. the CON group. <sup>ROSI</sup>P\* < 0.05 vs. the SAP group. <sup>GW9662</sup>P\* < 0.05 vs. the ROSI group.

Group	TNF-α (ng/L)	Raf-1 (ng/L)
CON	79.67 ± 14.3	262.98 ± 19.6
SAP	188.53 ± 20.1*	391.89 ± 48.2*
ROSI	136.99 ± 23.4*	262.28 ± 65.9#
GW9662	216.45 ± 25.2*	444.05 ± 35.5*

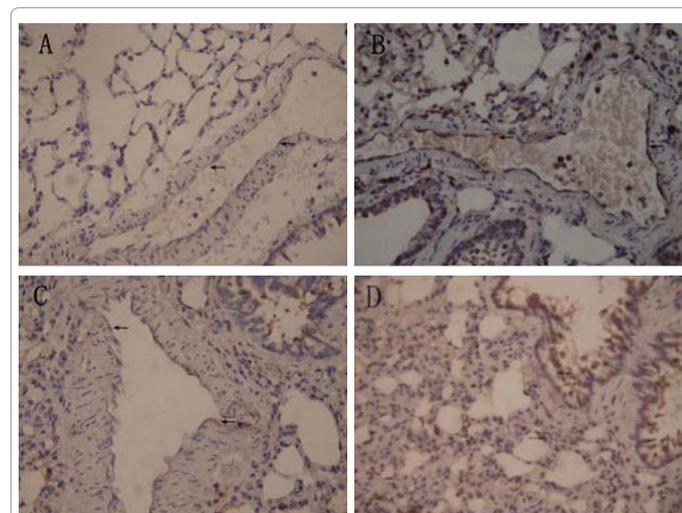
**Table 2:** Serum TNF-α and Raf-1 concentrations were examined by ELISA method. Date are presented as mean ± sd. <sup>SAP,ROSI,GW9662</sup>P\* < 0.05 vs. the CON group. <sup>ROSI</sup>P\* > 0.05 vs. the CON group. <sup>ROSI</sup>P\* < 0.01 vs. the SAP group. <sup>GW9662</sup>P\* < 0.05 vs. the ROSI group.

## Overexpression of Angptl4 alleviated the inflammatory cell infiltration and VEGF expression during SAP-associated lung injury

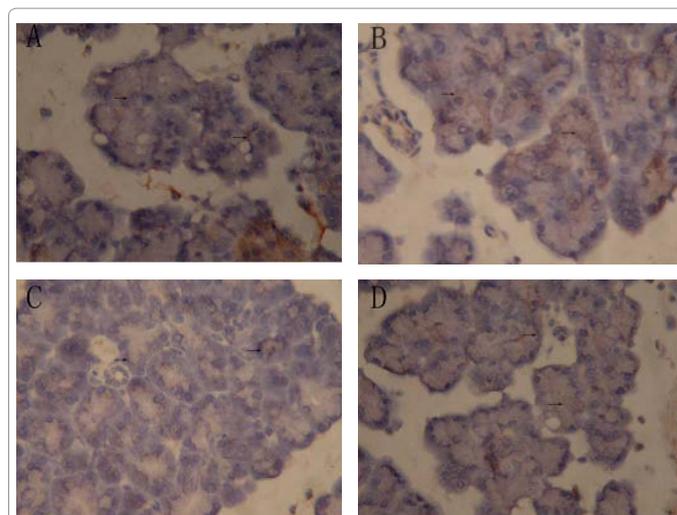
In order to observe the lung tissue and pancreas morphology, representative histological results are shown in Figures 3a-3d. Sodium taurocholate-induced pancreatitis associated lung injury was evident by interstitial and intra-alveolar edema and inflammatory cell infiltration. Prophylactic administration of rosiglitazone reduced the infiltration of inflammatory cells, particularly neutrophils, into the pulmonary interstitial and alveolar edematous areas. Also, there is no hemorrhagic edema and inflammatory cells infiltration in pancreatic lobular structure of control group, while the lobular structure of SAP group turned fuzzy, widely separated and more inflammatory cells invasion. Pathological damage of the ROSI group were significantly reduced compared to the SAP group. In the control animals, immunohistochemistry (Figures 4 and 5) results showed vascular endothelial cells of the lung were positive for VEGF. VEGF<sup>+</sup> cell populations increased 24 h after pancreatitis administration. However, after rosiglitazone treatment, VEGF concentrations in vascular endothelial cells changed in the opposite direction ( $p < 0.05$ ). At the same time, increased acinus of the pancreas with BCL-2 positive signals demonstrated that the ROSI group can obviously inhibit apoptosis of the acinus of the pancreas. Above all, one of the major findings of this



**Figure 3:** Representative hematoxylin-eosin staining sections of lung and pancreas histopathology. (A) CON group at 24h. (B) SAP group at 24h. (C) ROSI group at 24h. (D) GW9662 group at 24h (magnification of  $\times 10$ ).



**Figure 4:** Representative VEGF<sup>+</sup> immunohistochemistry of lung vascular endothelial histopathology. (A) CON group at 24h. (B) SAP group at 24h. (C) ROSI group at 24h. (D) GW9662 group at 24h. Comparison of the gray value of VEGF<sup>+</sup> of lung. <sup>B,C,D</sup> $P < 0.05$  vs. the CON group. <sup>C</sup> $P < 0.05$  vs. the SAP group. <sup>D</sup> $P < 0.05$  vs. the ROSI group (magnification of  $\times 40$ ).



**Figure 5:** Representative BCL-2 immunohistochemistry of pancreas histopathology. (A) CON group at 24h. (B) SAP group at 24h. (C) ROSI group at 24h. (D) GW9662 group at 24h. Comparison of the gray value of acinus of pancreas. <sup>B,C,D</sup> $P < 0.05$  vs. the CON group. <sup>C</sup> $P < 0.05$  vs. the SAP group. <sup>D</sup> $P < 0.05$  vs. the ROSI group (magnification of  $\times 40$ ).

study was the much lower levels of VEGF in vascular endothelial cells from a ROSI group than the SAP group. On the other hand, we also found that the increase expression of BCL-2 in pancreas indicated the protective of ROSI during the lung injury.

## Discussion

In this study, we further demonstrated that rosiglitazone treatment enhanced PPAR- $\gamma$  expression following increased expression of Angptl4. It might play an important role as a negative regulator in inflammatory and vascular endothelial cell damage in severe acute pancreatitis-induced lung injury. Nevertheless, the mechanism for the role of Angptl has not been fully elucidated [24-26]. It has been reported that Angptl4 is a downstream target gene of the ligand activated transcription factor PPAR- $\gamma$ . Recent studies have demonstrated that the PPAR- $\gamma$  ligand exerts potent anti-inflammatory properties [27]. However, the PPAR- $\gamma$  antagonist GW9662 attenuates the observed protective effects of PPAR- $\gamma$  ligands [28]. Although the function of Angptl4 is known to be involved in the regulation of lipid metabolism, the definite mechanisms of how Angptl4 affects SAP-associated lung injury have not been completely elucidated. Alterations in the metabolic and inflammatory milieu induced by PPAR- $\gamma$  activator rosiglitazone effectively dissociate obesity from sever AP, and there is a fine balance between the anti-inflammatory and adiposity-inducing effects of rosiglitazone [29]. Increased mRNA expression of Angptl4 has been observed in acute injury, and Angptl4 expression was significantly increased than PPAR- $\gamma$  level after acute pancreatitis. We demonstrated that Angptl4 is positive protected protein, which probably influenced by other cytokines except PPAR- $\gamma$ .

Although increase of Angptl4 expression has been demonstrated in certain tumors [30], and earlier studies suggested potential pro-angiogenic activity of Angptl4 [31]. More recent studies from several independent laboratories have demonstrated Angptl4 is a potent anti-angiogenic factor [32-34]. Kim et al. [23] showed that Angptl4 protects endothelial cells from apoptosis through an endocrine action, whereas Ito et al. showed that Angptl4 inhibits VEGF-induced vascular leakiness and neo-angiogenesis. In other studies, VEGF had the ability

to induce vascular permeability and led to neutrophil infiltration and even tissue edema. However, Angptl4 also inhibited VEGF-induced phosphorylation of ERK1/2, attributed to its specific suppression of the ERK1/2 MAP kinase pathway [35]. Our results showed that increases of Angptl4 in rosiglitazone group reduced the concentration of Raf-1, which is an important relay point of the ERK1/2 MPAK pathway, integrating positive and negative inputs from upstream stimuli [36]. Considering immunohistochemistry results from our studies, the rosiglitazone stimulates Angptl4 secretion and influences VEGF expression in endothelial cells in lung and function as protector for vascular formulation.

While VEGF-induced angiogenesis and vascular leakiness were significantly inhibited by recombinant Angptl4, the infiltration of neutrophils was impaired. Neutrophil sequestration is a hallmark of acute lung injury and occurs within the lung due to impaired neutrophils transiting out of the pulmonary microvascular into the lung, where they release cytotoxic agents, such as TNF- $\alpha$ . TNF- $\alpha$  is a pro-inflammatory mediator that promotes adhesion of leukocytes to the endothelium and plays a vital role in the pathogenesis of SAP [37]. The increase of TNF- $\alpha$  concentrations in tissue and serum directly correlates with the severity of pancreatic damage and inflammation in ALI [38]. It can overturn damage the vascular barrier to promote the leakiness. Also as we know, TNF- $\alpha$  is mediated by many signaling pathways, such as the ERK1/2-MPAK pathway and TPK-Ras-MPAK pathways. The down-regulation of TNF- $\alpha$  concentration might also relate to the impeded the Raf/MEK/ERK cascades. Also, anti-TNF $\alpha$  therapy in rats is able to reduce the severity and mortality resulting from ALI [39,40]. In this study, we demonstrated that, TNF- $\alpha$  concentration was obviously down regulated after treated by rosiglitazone. The mechanisms of it may inhibit neutrophil sequestration and influence TNF- $\alpha$  concentration, possibly amplified by PPAR- $\gamma$  expression. In addition, Rollins et al. demonstrated pretreatment with another PPAR- $\gamma$  ligand and rosiglitazone completely abolished the increase in the pro-inflammatory cytokine IL-6 and TNF- $\alpha$  mRNA expression in cerulein-induced mice [41].

Many studies had been carried out to investigate the various aspects of regeneration after acute pancreatitis. Sidhu et al. showed that rosiglitazone explore the activation of pancreatic stellate cells, which further synthesis ECM components that creates a platform for pancreatic regeneration [42]. In this study, we demonstrated that increase of Angptl4 expression in the pancreas of rats by immunohistochemistry and induced apoptosis of the acinus of the pancreas by influencing BCL-2 level. Since higher level of Angptl4 could suppress the ERK1/2-MPAK pathway and TPK-Ras-MPAK pathway, BCL-2 protein expression was decreased which would induce pancreas acinus apoptosis. The latter leads to acute pancreatitis and negative effects in this disease [43].

In conclusion, the molecular mechanisms that underlie the observed functional response heterogeneity to Angptl4 in SAP are unknown but presumably involve the differential activation of signals downstream of Angptl4. More experiments need to be explored on pulmonary microvascular cells of Angptl4 expression. Based on previous reports, we found that rosiglitazone induced the increased expression of Angptl4 which lead to pancreas acinus apoptosis earlier inflammatory station and inhibits inflammatory proliferation, the migration of endothelial cells of the lung, VEGF-induced angiogenesis and vascular leakage. All together, our results indicated that Angptl4 might play an important role in SAP and pancreatitis-associated lung injury.

## Authors' Contributions

YuXi Wang carried out the entire experiment and drafted the manuscript. JingWen Zhang performed the Western-blot procedure and carried out animal experiments. ShangShao Sun, HaiLong Li and XiaoYu Sun, Liang Cao helped to draft the manuscript. HaiLong Chen conceived the study and participated in the design of the study. All authors read and approved the final manuscript.

## Acknowledgment

This research was supported by The National Natural Science Foundation of China (FNSFC\_81173452). We complete this study at the First Affiliated Hospital of DaLian Medical University and DaLian Center Hospital. The authors thank HaiLong Li, Jun Ji, DongShang and Yuefeng Ma for helpful discussions. This manuscript was edited by International Science Editing. Thank you to David Cushley for editing the manuscript. The authors declare no conflicts of interest.

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