

## Basic Study

# Effects of $\omega$ -3 fatty acids on toll-like receptor 4 and nuclear factor- $\kappa$ B p56 in lungs of rats with severe acute pancreatitis

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**Author contributions:** Wang B designed and performed the research and wrote the paper; Wu XW, Xu XB and Jin XX performed the research; Guo MX and Li ML provided analytic tools; Zhang XH designed the paper.

**Supported by** Jinling Hospital Research Fund, No. 2013064.

**Institutional review board statement:** This study has been approved by the Institutional Review Board of Jinling Hospital, Nanjing, China.

**Institutional animal care and use committee statement:** The study was approved by the Institutional Animal Care and Use Committee of Jinling Hospital. All operations were performed according to international guidelines concerning the care and treatment of experimental animals.

**Conflict-of-interest statement:** The authors declare that there is no conflict of interest related to this study.

**Data sharing statement:** No additional unpublished data are available.

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**Manuscript source:** Unsolicited manuscript

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Received: July 1, 2016  
Peer-review started: July 4, 2016  
First decision: August 29, 2016  
Revised: September 13, 2016  
Accepted: October 10, 2016  
Article in press: October 10, 2016  
Published online: November 28, 2016

## Abstract

### AIM

To determine the effects of  $\omega$ -3 fatty acids ( $\omega$ -3FA) on the toll-like receptor 4 (TLR4)/nuclear factor  $\kappa$ B p56 (NF- $\kappa$ Bp56) signal pathway in the lungs of rats with severe acute pancreatitis (SAP).

### METHODS

A total of 56 Sprague-Dawley rats were randomly divided into 4 groups: control group, SAP-saline group, SAP-soybean oil group and SAP- $\omega$ -3FA group. SAP was induced by the retrograde infusion of sodium taurocholate into the pancreatic duct. The expression of TLR4 and NF- $\kappa$ Bp56 in the lungs was evaluated by immunohistochemistry and Western blot analysis. The levels of inflammatory cytokines interleukin-6 and tumor necrosis factor-alpha in the lungs were measured by enzyme-linked immunosorbent assay.

### RESULTS

The expression of TLR4 and NF- $\kappa$ Bp56 in lungs and of inflammatory cytokines in serum significantly increased in the SAP group compared with the control group ( $P < 0.05$ ), but was significantly decreased in the  $\omega$ -3FA group compared with the soybean oil group at 12 and 24 h ( $P < 0.05$ ).

## CONCLUSION

During the initial stage of SAP,  $\omega$ -3FA can efficiently lower the inflammatory response and reduce lung injury by triggering the TLR4/NF- $\kappa$ Bp56 signal pathway.

**Key words:** Severe acute pancreatitis;  $\omega$ -3 fatty acids; Lung injury; Toll-like receptor 4; Nuclear factor- $\kappa$ B p56; Cytokine

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**Core tip:** There is no report about the correlation between  $\omega$ -3 fatty acids and toll-like receptor 4 (TLR4) expression in lungs of animals with severe acute pancreatitis (SAP). In this study, we investigated the effects of  $\omega$ -3 fatty acids ( $\omega$ -3FA) on TLR4 and nuclear factor  $\kappa$ B p56 (NF- $\kappa$ Bp56) in lungs of rats with SAP and the levels of cytokines in serum to examine the effects of  $\omega$ -3FA on TLR4 and NF- $\kappa$ Bp56 of lungs in rats with SAP.

Wang B, Wu XW, Guo MX, Li ML, Xu XB, Jin XX, Zhang XH. Effects of  $\omega$ -3 fatty acids on toll-like receptor 4 and nuclear factor- $\kappa$ B p56 in lungs of rats with severe acute pancreatitis. *World J Gastroenterol* 2016; 22(44): 9784-9793 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v22/i44/9784.htm> DOI: <http://dx.doi.org/10.3748/wjg.v22.i44.9784>

## INTRODUCTION

Severe acute pancreatitis (SAP) is a critical illness associated with long-term treatment and high mortality. Mortality can approach 50% due to induction of systemic inflammatory response syndrome (SIRS) during the early stages of the disease, subsequently leading to multiple organ dysfunction syndrome (MODS)<sup>[1]</sup>. Acute lung injury is common, with approximately 20% of patients developing acute respiratory distress syndrome (ARDS). ARDS is a primary cause of death during the early stages of SAP<sup>[2,3]</sup>.

Recent reports have highlighted the role of activation of inflammatory cytokines and signal pathways in pancreatic tissues during the process of SAP<sup>[4,5]</sup>. Activation of interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and infiltration of inflammatory cells in early SAP can lead to pathological injury not only in pancreatic tissue but also in extra-pancreatic organs, through activation of downstream inflammatory mediators by the amplification of a series of cascade reactions<sup>[6]</sup>. These injuries can then induce SIRS or even MODS<sup>[7,8]</sup>. It has been shown that toll-like receptor 4 (TLR4) plays a critical role in the initiation of SAP. TLR4 can regulate the transcription of inflammatory cytokines, which leads to local inflammation in multiple systems and/or organs<sup>[9-12]</sup>.

Toll-like receptors (TLRs) are key modulators of

the innate immune response. Members of the TLR family recognize and bind to their corresponding ligand to activate signal transduction pathways and thus produce different biological functions in response to various stimuli<sup>[13]</sup>. TLR4 is the first reported TLR by which the mediated signal pathway can non-specifically bind in pathogen-associated molecular patterns<sup>[14]</sup>. Nuclear factor- $\kappa$ B p56 (NF- $\kappa$ Bp56), a nuclear transcription factor present in a wide variety of cells, shares an upstream/downstream relationship with TLR4<sup>[15]</sup>. NF- $\kappa$ Bp56 mainly functions at the level of regulating inflammation, cell survival, and apoptosis<sup>[16]</sup>.

Under normal circumstances, members of NF- $\kappa$ Bp56 form homomeric or heteromeric dimers in cytoplasm, which exert their function on the activation/inhibition of transcription<sup>[17]</sup>. Following activation of the TLR4-mediated signal transduction pathway, NF- $\kappa$ Bp56 activation and transcription of related inflammatory cytokines are then stimulated<sup>[18]</sup>. Several studies have confirmed that the expression and activation of TLR4 and NF- $\kappa$ Bp56 were up-regulated, and a large number of inflammatory cytokines were detected in the SAP rat model induced in a variety of ways<sup>[19-21]</sup>.

Thus, it is highly likely that the TLR4/NF- $\kappa$ Bp56 signal pathway is closely related to the occurrence and development of SAP. The global inflammation in SAP is an important step in the initiation of MODS, in which TLR4 functions as a "messenger"<sup>[22]</sup>. During the onset stage of SAP, through TLR4, local inflammation mediates the activation of various inflammatory cytokines, which in turn spread to other organs and lead to inflammation of multiple organs<sup>[23]</sup>.

Parenteral nutrition support with  $\omega$ -3 fatty acids ( $\omega$ -3FA) alters cytokine production and reduces the rate of complications, for example, the duration of mechanical ventilation and the prevalence of nosocomial infections<sup>[24-26]</sup>. Thus,  $\omega$ -3FA, which are major components of fish oil-supplemented parenteral nutrition, offers a potential positive effect on the anti-inflammatory response. Recent animal studies have also shown that  $\omega$ -3FA can have an anti-inflammatory role in pancreatitis<sup>[27-29]</sup>. However, there are no data on the correlation between  $\omega$ -3FA and the TLR4/NF- $\kappa$ Bp56 signal pathway in the lungs of subjects with SAP. In this study, we investigated the effects of  $\omega$ -3FA on the expression of TLR4 and NF- $\kappa$ Bp56 in the lungs of rats with SAP, as well as the levels of cytokines in lungs.

## MATERIALS AND METHODS

### Experimental animal model and grouping

All experiments were approved by the Ethics Committee for Animal Research at Jinling Hospital, Nanjing, China. Healthy adult male Sprague-Dawley rats, weighing 230-250 g, were provided by the Experimental Animal Center of Jinling Hospital. All 56 animals were randomly divided into 4 groups: healthy controls ( $n = 8$ ); SAP-

saline group ( $n = 16$ ); SAP-soybean oil group ( $n = 16$ ); and SAP- $\omega$ -3FA group ( $n = 16$ ). Under pentobarbital anesthesia (50 mg/kg body weight), a laparotomy was performed and 5% sodium taurocholate in distilled water (1 mL/kg body weight) was injected into the bilio-pancreatic duct at the rate of 0.2 mL/min using a micro-infusion pump. Controls received an intraductal infusion of saline (0.2 mL/min)<sup>[30]</sup>. After SAP induction, the SAP- $\omega$ -3FA group received an intravenous injection of a combination of the soybean-based compound and 0.2 g/kg fish oil (Omegaven; Fresenius, Bad Homburg, Germany). The SAP-soybean oil group received a soybean-based fat solution without additional fish oil [Intralipid medium-chain triglyceride/long-chain triglyceride 20%; Braun, Melsungen, Germany], and the SAP-saline group received the same volume of saline.

Eight animals from each group were sequentially killed after 12 and 24 h (SAP-saline group, SAP-soybean oil group and SAP- $\omega$ -3FA group) by a lethal dose of pentobarbital (200 mg/kg intravenous). The entire lung was removed, and a sample was immediately frozen at  $-80^{\circ}\text{C}$  for biochemical analysis. The lung was then fixed in 10% formalin in anatomic orientation for histological analysis. The lung tissues and whole blood were obtained for subsequent analysis.

#### **Measurement of amylase levels in serum**

Serum measurement of measurement of amylase (AMY) concentration was conducted by an automated HITACHI-7150 analyzer to ensure successful SAP models.

#### **Measurement of TNF- $\alpha$ and IL-6 levels in lungs**

TNF- $\alpha$  and IL-6 levels in lungs were measured using a commercial ELISA according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, United States). The levels of TNF- $\alpha$  and IL-6 were found to be a minimum of 7 pg/mL and 60 pg/mL, respectively.

#### **Pathological examination of lungs**

The lung tissue was fixed by 40 g/L formaldehyde, embedded in paraffin, and stained with hematoxylin-eosin. All microscopic sections were analyzed blindly. One slide for each rat per group was studied, and 10 random fields per slide were evaluated. Lung histopathological changes were graded on a scale of 0 to 3 (normal 0, mild 1, moderate 2, severe 3) for alveolar and interstitial edema, inflammatory cell infiltration, and alveolar and interstitial hemorrhage<sup>[31]</sup>.

#### **Immunohistochemistry of TLR4 and NF- $\kappa$ Bp56 in lung tissues**

The S-P method was used to detect the expression of TLR4 and NF- $\kappa$ Bp56 proteins in lung tissues. Fixed sections were incubated with the appropriate primary and secondary antibodies. Color reactions were developed using diaminobenzidine solution according to

the manufacturer's instructions. Phosphate buffered saline was used as a negative control, in lieu of antibody. For semi-quantitative analyses, areas of positive staining were defined by two independent investigators using Image-Pro 6.0 Plus (MediaCybernetics). Five fields of view for each section were randomly selected, images acquired, and integrated optical density (IOD) determined [density (mean) = IOD/area].

#### **Western blot of TLR4 and NF- $\kappa$ Bp56 in lung tissues**

Lung tissue samples were homogenized with lysate buffer (10 mmol/L Tris at pH 7.5, 10 mmol/L NaCl, 0.1 mmol/L EDTA, 0.5% Triton-X 100, 0.02 mmol/L Na<sub>3</sub>N, and 0.2 mmol/L phenylmethanesulphonylfluoride), treated with a sodium dodecyl sulfate-polyacrylamide loading buffer at  $95^{\circ}\text{C}$  for 5 min, and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The separated protein was transblotted from the gel to the polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, United States) at 300 mA for 1.5 h at  $4^{\circ}\text{C}$ . The membrane was blocked with 5% non-fat dried milk in Tris-buffered saline with 0.05% Tween20 (TBST) for 1 h at room temperature, washed 3 times for 10 min each time in TBST, and incubated with a primary antibody in a 1:500 dilution of goat anti-rat TLR4 and anti-rat NF- $\kappa$ Bp56 monoclonal antibody (Upstate, Charlottesville, VA, United States) in TBST containing 5% non-fat dried milk for 2 h at room temperature. After washing 3 times with TBST for 10 min each time, the membranes were incubated with a 1:5000 dilution of peroxidase-conjugated goat anti-rat immunoglobulin G (Sigma-Aldrich) for 1 h at room temperature. After washing, the membranes were analyzed by the enhanced fluorescence system (Pierce Biotechnology, Rockford, IL, United States).

#### **Statistical analysis**

The results for AMY, cytokine and histopathology score of the lungs represent the mean of 8 experiments and are expressed as mean  $\pm$  SE. Experimental data from different groups were compared using the non-paired Student's *t*-test and one-way analysis of variance. The western blot data were from 8 experiments and are expressed as mean optical density, which was analyzed using the Mann-Whitney nonparametric *U* test. A *P* value less than 0.05 (2-tailed) was considered statistically significant. All tests were performed using the statistical package SPSS software 11.0 (SPSS, Chicago, IL, United States) in Windows 7.

## **RESULTS**

#### **Serum levels of AMY in rats**

Serum AMY was determined and showed a consistent change. Hyperamylasemia was found in all SAP groups, and AMY levels were significantly higher than those in the control group (Table 1).

**Table 1 Serum amylase levels in the different groups**

Group	Time	Cases	Mean $\pm$ SE (IU/L)
Control		8	1316.34 $\pm$ 115.21
SAP-saline	12 h	8	4637.11 $\pm$ 621.57 <sup>a</sup>
	24 h	8	4352.54 $\pm$ 436.41 <sup>d</sup>
SAP-soybean oil	12 h	8	4978.25 $\pm$ 918.48 <sup>b</sup>
	24 h	8	4245.89 $\pm$ 646.27 <sup>e</sup>
SAP- $\omega$ -3FA	12 h	8	4638.63 $\pm$ 586.14 <sup>c</sup>
	24 h	8	4163.47 $\pm$ 653.64 <sup>f</sup>

<sup>a</sup> $P < 0.05$ ; <sup>d</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.05$ ; <sup>e</sup> $P < 0.05$ ; <sup>f</sup> $P < 0.05$  vs control group.  
SAP: Severe acute pancreatitis;  $\omega$ -3FA:  $\omega$ -3 fatty acids.

### Measurement of TNF- $\alpha$ and IL-6 levels in lungs

Compared with the control group, TNF- $\alpha$  and IL-6 levels in the lungs of the SAP-saline group, SAP-soybean oil group and SAP- $\omega$ -3FA group were significantly higher at each time point ( $P < 0.05$ ). Moreover, lung TNF- $\alpha$  and IL-6 levels in the SAP- $\omega$ -3FA group were lower than those in the SAP-soybean oil group at each time point ( $P < 0.05$ ) (Table 2).

### Histopathology of lung tissues

Significantly greater pulmonary interstitial alveolar edema, and a larger number of alveolar bleeding inflammatory cells were detected in the lungs of the SAP-saline, SAP-soybean oil and SAP- $\omega$ -3FA groups, but the progression of inflammation was different among the groups: lung inflammation was significantly decreased in the SAP- $\omega$ -3FA group compared with the SAP-soybean oil group at the same time point ( $P < 0.05$ ); no significant differences in histopathological changes were detected between the SAP-saline group and the SAP-soybean oil group at each time point; and no histopathological changes were detected in the control group (Figure 1, Table 3).

### Immunohistochemistry of TLR4 and NF- $\kappa$ Bp56 in lung tissues

The immunohistochemistry results indicated that TLR4 and NF- $\kappa$ Bp56 expression in the lungs was mainly in neutrophils, macrophages, bronchial epithelial cells, alveolar epithelial cells and microvascular endothelium cells. The TLR4 and NF- $\kappa$ Bp56 positive rate was significantly higher in the SAP-soybean oil group than in the SAP- $\omega$ -3FA group ( $P < 0.05$ ) (Figures 2 and 3).

### Western blot of TLR4 and NF- $\kappa$ Bp56 in lung tissues

The Western blotting results showed the expression level of TLR4 and NF- $\kappa$ Bp56 in the lungs of the SAP-saline, SAP-soybean oil and SAP- $\omega$ -3FA groups compared with the control group at 12 and 24 h. The expression levels of both proteins in the groups are shown in Figure 4 and Table 4. Compared with the control group, the SAP-saline group and SAP-soybean oil group showed a significantly higher expression level of TLR4 in the lungs at each time point ( $P <$

0.05). TLR4 expression was much lower in the SAP- $\omega$ -3FA group. Furthermore, NF- $\kappa$ Bp56 expression level was also significantly increased in the SAP groups compared with the control group, while NF- $\kappa$ Bp56 expression in the SAP- $\omega$ -3FA group was a little lower than that in the SAP-soybean oil group ( $P < 0.05$ ). These results suggest that while the expression of TLR4 and NF- $\kappa$ Bp56 significantly increased in the lungs of rats with SAP,  $\omega$ -3FA down-regulated TLR4 expression and inhibited NF- $\kappa$ Bp56 expression in the lungs of rats with SAP.

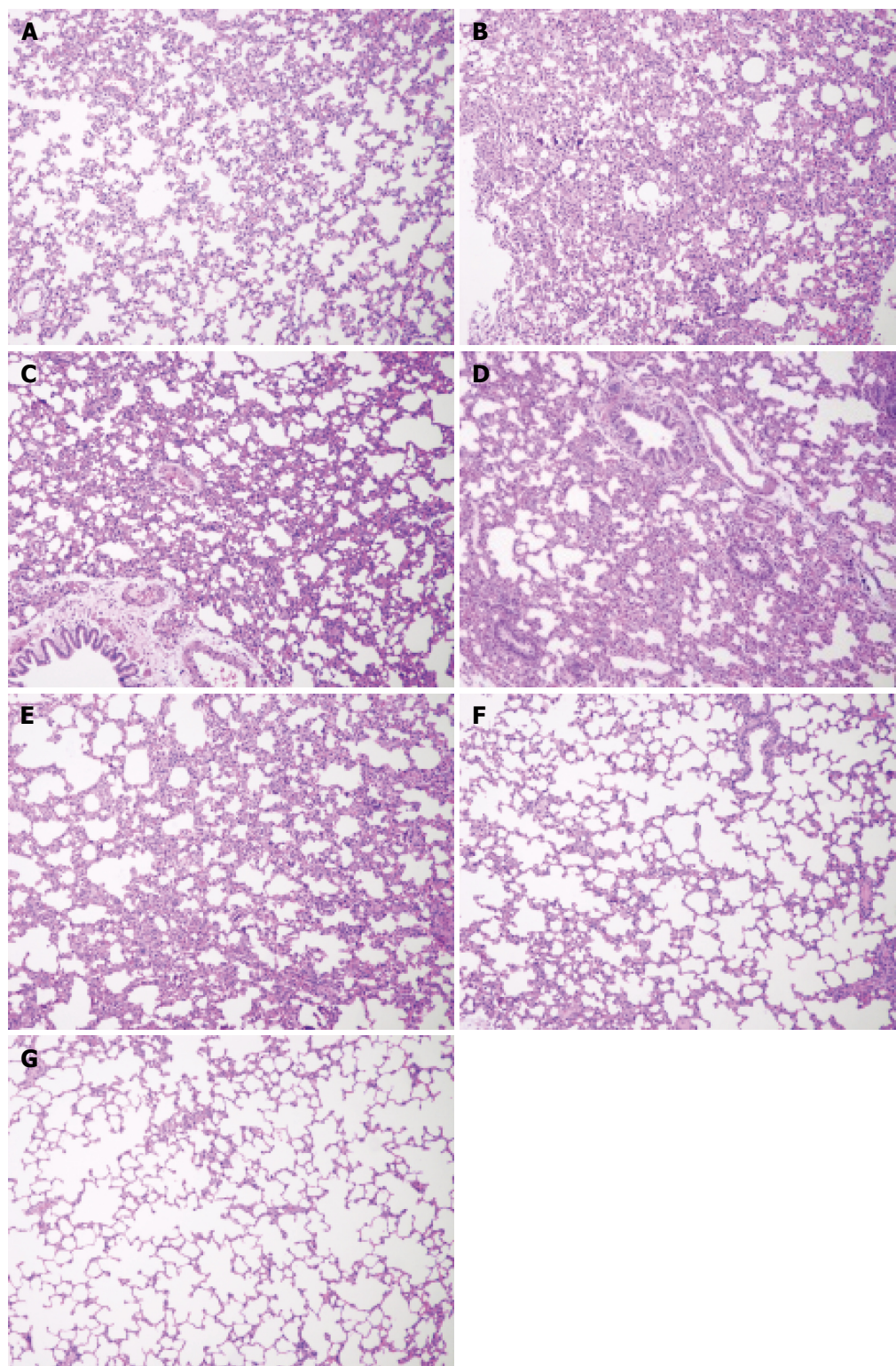
## DISCUSSION

The first sign of multiple organ failure in SAP is often impaired lung function due to ARDS<sup>[32]</sup>. As a consequence of overactive SIRS, TNF- $\alpha$  and IL-6 are activated within the circulation and attack the pulmonary vascular endothelium<sup>[33,34]</sup>. Inflammatory cytokines play an important role in lung injury in SAP and in the translation of local pancreatic damage to a systemic inflammatory response<sup>[35,36]</sup>. The increase in lung pathological damage and lung edema is evidence of SAP-associated lung injury. TLRs are proteins that can trigger the inflammatory cascade reaction. Therefore, an investigation into the tissue-specific expression of TLRs (mainly TLR4) in lungs and determining their roles is of great significance in understanding the pathogenesis of SAP.

It is currently thought that TLRs may play a central role in the recognition of endogenous or exogenous antigens in the immune system and in the initiation of signal transduction in the inflammatory reaction during SAP. Some researchers believe that TLR4 may play an important role in the synthesis and release of inflammatory cytokines, and up-regulation of the TLR4 gene may be related to the development and progression of organ injury during SAP<sup>[37,38]</sup>. Some studies have indicated that when SAP is stimulated, the expression of cytokines and cell adhesion molecules is significantly up-regulated in the pancreas, thereby promoting the accumulation of excessive neutrophils in the area of inflammation leading to injury of the pancreas and other organs<sup>[39,40]</sup>. Our study showed that, during the early stages of SAP, there was obvious expression of TLR4 and NF- $\kappa$ Bp56 in lung tissues. This expression increased as SAP developed; peak expression was seen at 24 h. Changes in the concentration of TNF- $\alpha$  and IL-6 in lungs were consistent with pathological changes in lung tissues.

In recent years, a new  $\omega$ -3 fish oil emulsion was found to not only supply energy but also to exert some important biological effects, such as immune regulation and organ protection<sup>[41-43]</sup>. The main mechanisms of action are as follows. On the one hand,  $\omega$ -3FA can enter the phospholipid pool of the cell membrane to replace  $\omega$ -6FA, and reach an appropriate  $\omega$ -3/ $\omega$ -6 ratio



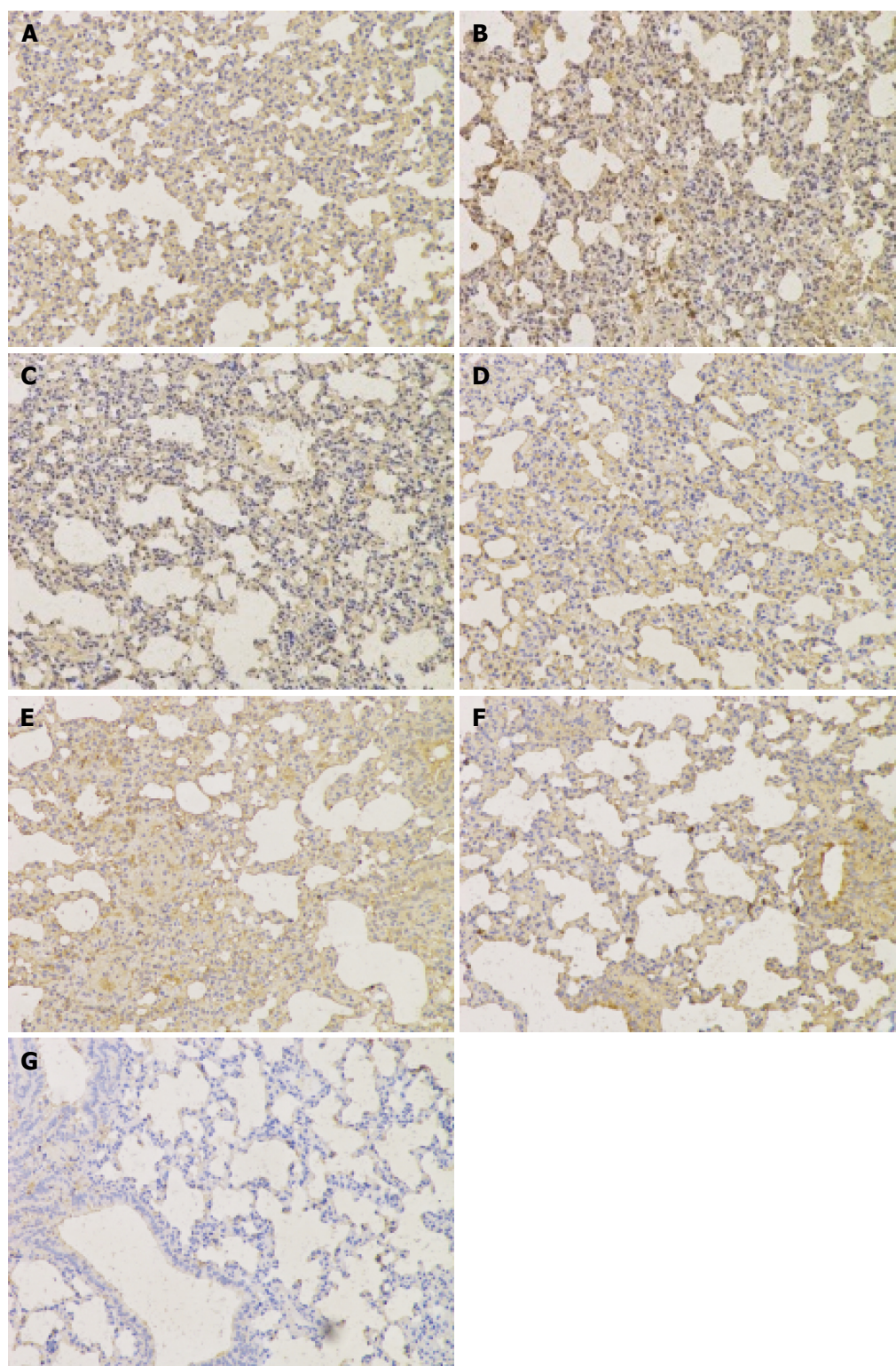


**Figure 1** Representative photographs of lung histology in rats from each group (magnification  $\times 200$ ). A: HC group; B: SAP-saline 12 h group; C: SAP-saline 24 h group; D: SAP-soybean oil 12 h group; E: SAP-soybean oil 24 h group; F: SAP- $\omega$ -3FA 12 h group; G: SAP- $\omega$ -3FA 24 h group. HC: Healthy control; SAP: Severe acute pancreatitis;  $\omega$ -3FA:  $\omega$ -3 fatty acids.

(1:2 to 1:4). They can competitively combine with cyclooxygenase and lipoxygenase to inhibit production of inflammatory mediators from  $\omega$ -6FA which have a strong pro-inflammatory action and immune regulation. On the other hand, by reducing the expression of

surface receptors and molecules in immune cell membranes,  $\omega$ -3FA can participate in the immune response, reduce the activity of immune cells and the production of cytokines, and inhibit the inflammatory response<sup>[44-46]</sup>. In our study,  $\omega$ -3FA reduced the release





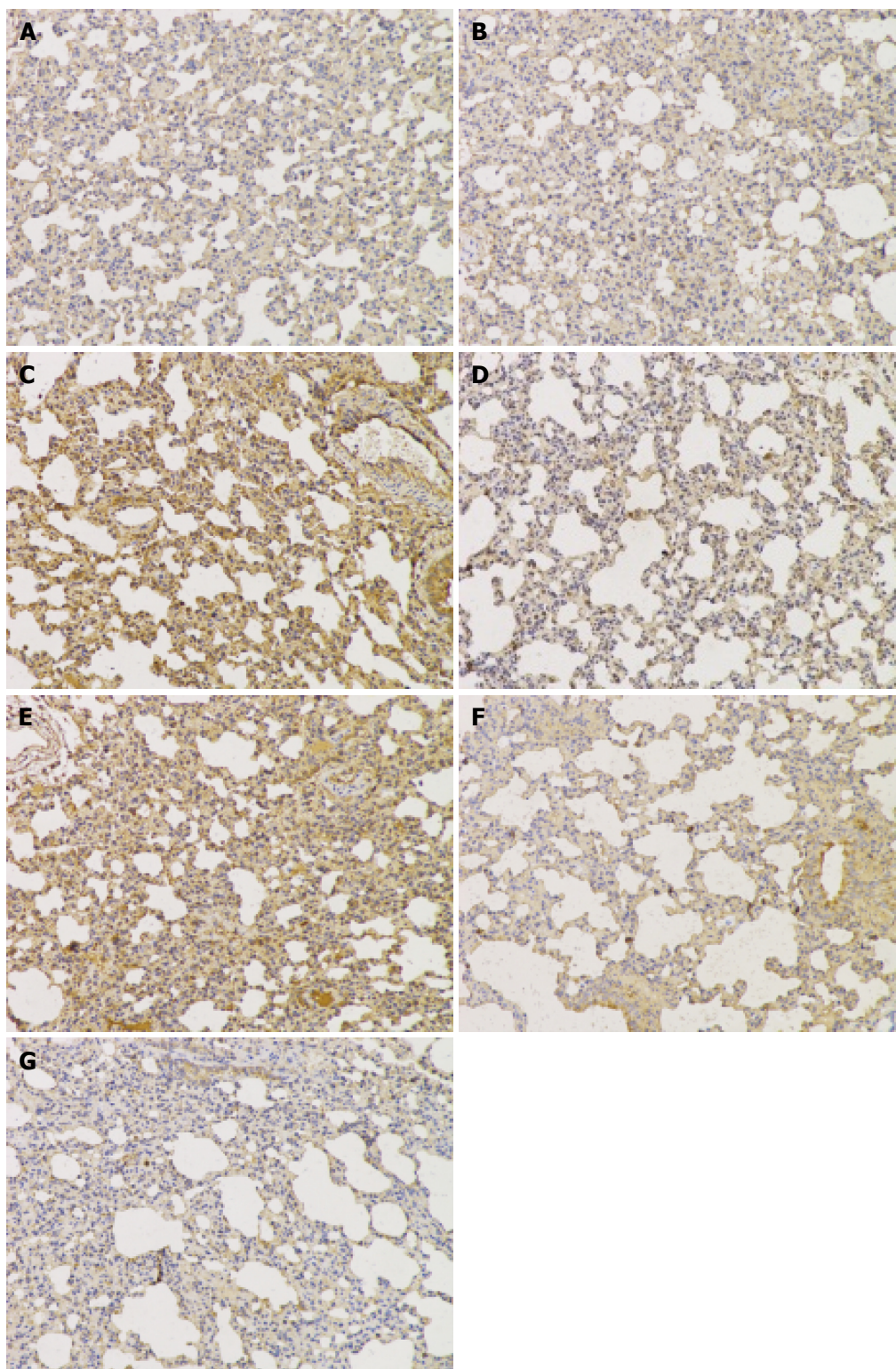
**Figure 2** Immunohistochemical analysis of the toll-like receptor 4 protein in lungs from each group (magnification  $\times 200$ ). A: HC group; B: SAP-saline 12 h group; C: SAP-saline 24 h group; D: SAP-soybean oil 12 h group; E: SAP-soybean oil 24 h group; F: SAP- $\omega$ -3FA 12 h group; G: SAP- $\omega$ -3FA 24 h group. HC: Healthy control; SAP: Severe acute pancreatitis;  $\omega$ -3FA:  $\omega$ -3 fatty acids.

of the inflammatory cytokines TNF- $\alpha$  and IL-6, and reduced lung injury.

However, it is unclear whether  $\omega$ -3FA inhibits the inflammatory response by reducing the expression of TLR4 in lungs. In our study, we found that the ex-

pression of TLR4 and NF- $\kappa$ Bp56 was decreased in the lungs of rats treated with  $\omega$ -3FA. The inflammatory response and pathological changes in the lungs improved after the application of  $\omega$ -3FA. This suggested that  $\omega$ -3FA indirectly inhibits the expression of TLR4 by





**Figure 3** Immunohistochemical analysis of the nuclear factor- $\kappa$ B p56 protein in lungs from each group (magnification  $\times 200$ ). A: HC group; B: SAP-saline 12 h group; C: SAP-saline 24 h group; D: SAP-soybean oil 12 h group; E: SAP-soybean oil 24 h group; F: SAP- $\omega$ -3FA 12 h group; G: SAP- $\omega$ -3FA 24 h group. HC: Healthy control; SAP: Severe acute pancreatitis;  $\omega$ -3FA:  $\omega$ -3 fatty acids.

inhibiting activation of NF- $\kappa$ Bp56.

In conclusion, TLR4 may play an important role during SAP-induced lung injury. TLR4 activation results in a variety of inflammatory reactions, and  $\omega$ -3FA can

inhibit the inflammatory response and reduce lung injury by inhibiting the TLR4/NF- $\kappa$ Bp56 signal pathway. Development of these therapeutic strategies should help reduce the complications and mortality associated

**Table 2** Changes in tumor necrosis factor-alpha and interleukin-6 in the lungs of the groups at each time point (pg/mL, mean  $\pm$  SE,  $n = 8$ )

	Control	SAP-saline	SAP-soybean oil	SAP- $\omega$ -3FA
TNF- $\alpha$ (12 h)	24.18 $\pm$ 1.05	43.24 $\pm$ 4.26 <sup>a</sup>	46.17 $\pm$ 13.63 <sup>ad</sup>	33.42 $\pm$ 6.37 <sup>abc</sup>
IL-6 (12 h)	625.11 $\pm$ 124.1	826.31 $\pm$ 76.34 <sup>a</sup>	827 $\pm$ 44.36 <sup>ad</sup>	739.67 $\pm$ 65.23 <sup>abc</sup>
TNF- $\alpha$ (24 h)	24.22 $\pm$ 1.03	38.67 $\pm$ 4.87 <sup>a</sup>	42.45 $\pm$ 11.14 <sup>ad</sup>	30.37 $\pm$ 10.23 <sup>abc</sup>
IL-6 (24 h)	623.21 $\pm$ 124.3	909.54 $\pm$ 26.23 <sup>a</sup>	925.18 $\pm$ 34.52 <sup>ad</sup>	801.27 $\pm$ 51.29 <sup>abc</sup>

<sup>a</sup> $P < 0.05$  vs control group at the same time point; <sup>d</sup> $P > 0.05$ , <sup>b</sup> $P < 0.05$  vs SAP-saline group; <sup>c</sup> $P < 0.05$  vs SAP-soybean oil group. SAP: Severe acute pancreatitis;  $\omega$ -3FA:  $\omega$ -3 fatty acids.

**Table 3** Lung histopathology score in each group of rats (mean  $\pm$  SE,  $n = 8$ )

Group	Pulmonary interstitial edema	Exudation of inflammatory cells	Alveolar bleeding	Alveolar edema
Control	0.32 $\pm$ 0.06	0.21 $\pm$ 0.01	1.14 $\pm$ 0.02	0.14 $\pm$ 0.02
SAP-saline				
12 h	1.24 $\pm$ 0.11 <sup>a</sup>	1.37 $\pm$ 0.14 <sup>a</sup>	0.87 $\pm$ 0.09 <sup>a</sup>	1.13 $\pm$ 0.08 <sup>a</sup>
24 h	2.53 $\pm$ 0.21 <sup>a</sup>	2.67 $\pm$ 0.26 <sup>a</sup>	1.12 $\pm$ 0.12 <sup>a</sup>	2.14 $\pm$ 0.11 <sup>a</sup>
SAP-soybean oil				
12 h	1.24 $\pm$ 0.21 <sup>ad</sup>	1.35 $\pm$ 0.24 <sup>ad</sup>	0.90 $\pm$ 0.25 <sup>ad</sup>	1.14 $\pm$ 0.12 <sup>ad</sup>
24 h	2.15 $\pm$ 0.28 <sup>abc</sup>	2.38 $\pm$ 0.30 <sup>abc</sup>	0.85 $\pm$ 0.15 <sup>abc</sup>	1.95 $\pm$ 0.21 <sup>abc</sup>
SAP- $\omega$ -3FA				
12 h	0.94 $\pm$ 0.16 <sup>abc</sup>	1.12 $\pm$ 0.15 <sup>abc</sup>	0.72 $\pm$ 0.38 <sup>abc</sup>	0.94 $\pm$ 0.36 <sup>abc</sup>
24 h	2.15 $\pm$ 0.28 <sup>abc</sup>	2.38 $\pm$ 0.30 <sup>abc</sup>	0.85 $\pm$ 0.15 <sup>abc</sup>	1.95 $\pm$ 0.21 <sup>abc</sup>

<sup>a</sup> $P < 0.05$  vs the control group; <sup>d</sup> $P > 0.05$ , <sup>b</sup> $P < 0.05$  vs SAP-saline group at the same time point; <sup>c</sup> $P < 0.05$  vs SAP-soybean oil group at the same time point. SAP: Severe acute pancreatitis;  $\omega$ -3FA:  $\omega$ -3 fatty acids.

**Table 4** Densitometrically quantified toll-like receptor 4 and nuclear factor- $\kappa$ B p56 bands in the lungs of each group of rats, optical density (mean  $\pm$  SE)

Group	Time	Cases	TLR4	NF- $\kappa$ Bp56
Control		8	2.3 $\pm$ 0.3	2.5 $\pm$ 0.2
SAP-saline	12 h	8	3.9 $\pm$ 0.4 <sup>a</sup>	4.0 $\pm$ 0.3 <sup>a</sup>
	24 h	8	4.0 $\pm$ 0.2 <sup>a</sup>	4.1 $\pm$ 0.4 <sup>a</sup>
SAP-soybean oil	12 h	8	3.8 $\pm$ 0.3 <sup>ad</sup>	3.9 $\pm$ 0.3 <sup>ad</sup>
	24 h	8	3.9 $\pm$ 0.4 <sup>ad</sup>	3.8 $\pm$ 0.2 <sup>ad</sup>
SAP- $\omega$ -3FA	12 h	8	2.7 $\pm$ 0.3 <sup>abc</sup>	3.0 $\pm$ 0.2 <sup>abc</sup>
	24 h	8	3.1 $\pm$ 0.4 <sup>abc</sup>	2.9 $\pm$ 0.4 <sup>abc</sup>

<sup>a</sup> $P < 0.05$  vs the control group; <sup>d</sup> $P > 0.05$ , <sup>b</sup> $P < 0.05$  vs SAP-saline group at the same time point; <sup>c</sup> $P < 0.05$  vs SAP-soybean oil group at the same time point. NF- $\kappa$ Bp56: Nuclear factor- $\kappa$ B p56; SAP: Severe acute pancreatitis; TLR4: Toll-like receptor 4;  $\omega$ -3FA:  $\omega$ -3 fatty acids.

with SAP.

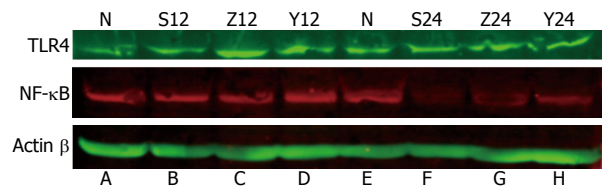
## COMMENTS

### Background

Acute lung injury (ALI) is one of the most common complications of severe acute pancreatitis (SAP) and often occurs in the early stage of the disease, and may progress to adult respiratory distress syndrome. Toll-like receptor 4 (TLR4) can regulate the transcription of inflammatory cytokines, which leads to ALI.

### Research frontiers

It has been found that  $\omega$ -3 fatty acids ( $\omega$ -3FA) reduces the release of the inflammatory cytokines tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6), and reduces lung injury. It is unclear whether  $\omega$ -3FA can inhibit the inflammatory response by reducing expression of TLR4 in the lungs.



**Figure 4** Western blot analysis of the toll-like receptor 4 and nuclear factor- $\kappa$ B protein levels in lungs from each group. A: HC group; B: SAP-saline 12 h group; C: SAP-soybean oil 12 h group; D: SAP- $\omega$ -3FA 12 h group; E: HC group; F: SAP-saline 24 h group; G: SAP-soybean oil 24 h group; H: SAP- $\omega$ -3FA 24 h group. HC: Healthy control; SAP: Severe acute pancreatitis;  $\omega$ -3FA:  $\omega$ -3 fatty acids.

### Innovations and breakthroughs

In this study, the authors found that  $\omega$ -3FA inhibited the inflammatory response and reduced lung injury by inhibiting the TLR4/nuclear factor- $\kappa$ B p56 (NF- $\kappa$ Bp56) signal pathway.

### Applications

The findings of this study may help to further understand the role of TLR4 during the course of SAP-induced lung injury.

### Peer-review

This is a well-intentioned basic study that has evaluated the effects of  $\omega$ -3FA on expression of TLR4 and NF- $\kappa$ Bp56 of lungs with SAP and the levels of cytokines in rat.

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ISSN 1007-9327

