Protective effects of dexmedetomidine on blunt chest trauma–induced pulmonary contusion in rats

Xiaojing Wu, MD, Xuemin Song, PhD, Ningtao Li, MD, Lijing Zhan, MD, Qingtao Meng, MD, and Zhongyuan Xia, PhD, Wuhan, China

BACKGROUND: Dexmedetomidine is a new and highly selective a2-adrenoreceptor agonist with potent anti-inflammatory capacity. This study explored the effects of dexmedetomidine on regulating hemodynamics, the plasma tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) levels, immunohistochemical localization of nuclear factor κB (NF-κB) from blunt chest trauma–induced pulmonary contusion in rats.

METHODS: Fifty Sprague-Dawley rats were randomly assigned into five equal groups (n = 10) as follows: uninjured control group, uninjured plus dexmedetomidine group, injured group, injured plus dexmedetomidine group, injured plus dexmedetomidine plus yohimbine (IDY) group. Dexmedetomidine was infused continuously through the left femoral vein cannula at the rate of 5.0 μg/kg per hour after blunt chest trauma 30 minutes in uninjured plus dexmedetomidine group, injured plus dexmedetomidine group, and IDY group. Animals in the IDY group received 0.2-mg/kg yohimbine immediately after the administration of dexmedetomidine. The right femoral artery was cannulated to monitor mean arterial pressure and heart rate and to draw blood samples. The plasma TNF-α and IL-1β levels were measured using enzyme-linked immunosorbent assays. The lung tissue NF-κB expression was determined by immunohistochemistry.

RESULTS: Bilateral blunt chest trauma produced progressive hypotension and a prolonged descent in heart rate. The plasma TNF-α and IL-1β levels were significantly increased after blunt chest trauma challenge alone. Dexmedetomidine not only significantly modified hemodynamics and relieved the infiltration of inflammatory cells into alveolar spaces but also inhibited the plasma TNF-α and IL-1β production as well as the lung NF-κB activation (p < 0.05, respectively). Yohimbine treatment significantly reversed the effects of dexmedetomidine (p < 0.05).

CONCLUSION: The administration of dexmedetomidine has beneficial effects on pulmonary contusion from blunt chest trauma in rats. The mechanisms were likely to inhibit the NF-κB activation via α2-adrenergic receptors and attenuate the proinflammatory cytokine responses. (J Trauma Acute Care Surg. 2013;74: 524–530. Copyright © 2013 by Lippincott Williams & Wilkins)

KEY WORDS: Dexmedetomidine; blunt chest trauma; inflammatory response; nuclear factor κB; rat.

Blunt chest trauma is commonly sustained in modern life. Blunt chest trauma resulting in pulmonary contusion (PC) often carries a high risk of morbidity and mortality.1–2 Although blunt chest trauma has a mechanical element leading to hypoxemia and lactic acid accumulation, pulmonary and systemic inflammatory responses play an important role in mediating the development of PC. In 2008, Seitz et al.3 reported that blunt chest trauma had activated alveolar macrophages, resulting in proinflammatory cytokines release, and caused alveolar type 2 epithelial cell apoptosis. In 2009, Hoth et al.4 demonstrated that blunt chest trauma resulting in PC primed systemic innate immunity responses and promoted proinflammatory cytokines production. In 2010, Fang et al.5 revealed that nuclear factor κB (NF-κB) plays a crucial role during severe lung contusion in rabbits. NF-κB is known as a nuclear protein critical for controlling the expression of inflammation–associated factors, including that encode the proinflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), adhesion molecules, and additional inflammatory mediators involved in acute lung injury and acute respiratory distress syndrome.6–7

Dexmedetomidine is a highly selective α2-adrenoreceptor agonist approved by the US Food and Drug Administration for short-term postoperative sedation, which is a potent sedative and analgesic agent for critically ill patients in intensive care units.8 Recently, increasing evidences have indicated that dexmedetomidine could suppress proinflammatory cytokines production.9 In 2008, Taniguchi et al.10 discovered that dexmedetomidine had inhibited the inflammatory responses and drastically reduced the high mortality rate in endotoxin-induced shock rats. In 2009, Can et al.11 reported that dexmedetomidine had decreased inflammatory cytokines production in spinal cord injury. In addition, Memis et al.12 elaborated that dexmedetomidine had significant effects on inhibiting the up-regulation of inflammatory molecules in critically ill septic patients. However, so far, there are few reports about the effects of dexmedetomidine on mechanical PC and NF-κB activation.

Therefore, we thus hypothesized that dexmedetomidine would inhibit proinflammatory cytokines production by limiting NF-κB activation during PC from blunt chest trauma. To elucidate further, we replicate the model of PC by bilateral blunt chest trauma and investigate the effects of dexmedetomidine on regulating hemodynamics, arterial blood gas, lactic acid, the histopathology of lung tissue, the plasma TNF-α and IL-1β levels,

From the Department of Anesthesiology (X.W., N.L., L.Z., Q.M., Z.X.), Renmin Hospital of Wuhan University; and Department of Anesthesiology and Critical Care Medicine (X.S.), Zhongnan Hospital of Wuhan University, Wuhan, Hubei Province, China.
Address for reprints: Zhongyuan Xia, PhD, Department of Anesthesiology, Renmin Hospital of Wuhan University, Wuhuan, 430060, Wuchang, 238 of Liberation Rd, Hubei Province, China; email: xiazhongyuan2005@yahoo.com.cn.
DOI: 10.1097/TA.0b013e31827d6de3
immunohistochemical localization, and activation of NF-κB, elaborating the possible mechanism on PC in rats.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats weighing 250 g to 300 g were maintained on sterile, standard laboratory chow and water ad libitum in individual ventilated cages under specific pathogen-free conditions in the animal facility of the Experimental Research Centre of Wuhan University. All animal experiments were approved by the institutional animal care committee and were in accordance with the guidelines of the National Institutes of Health on the care and use of animals.

**Animal PC Model Procedure**

After an intraperitoneal injection of 2% pentobarbital (30 mg/kg), the 14- or 16-gauge catheter was inserted into the tracheal tube using wire under direct vision. The catheter was connected to a pressure-controlled ventilator (DW-2000, Shanghai, China) delivering 30% (inspired oxygen fraction, FIO₂) oxygen at a frequency of 60 bpm is expanded as breaths per minute. The tidal volume of 8 mL/kg with an inspiratory-expiratory ratio of 1:1. Dexmedetomidine was infused continuously through the left femoral vein cannula at the rate of 5.0 μg/kg per hour 30 minutes after blunt chest trauma. A stretched polyethylene catheter filled with heparinized saline was inserted into the right femoral artery. An arterial cannula was tied around the artery, and the catheter was tunneled subcutaneously to exit at the back of the leg. The arterial cannula was connected to a pressure transducer. Mean arterial pressure (MAP) and heart rate (HR) were recorded on monitor (IntelliVueMP20, Philips, Netherlands). Blunt chest trauma resulting in PC was induced in anesthetized rats at fixed chest impact energy of 2.45 J described by Raghavendran et al. The precordial shield directed the impact force bilaterally to the lungs to prevent cardiac trauma. Blood samples were drawn via the femoral artery at 6 hours after blunt chest trauma challenge for measuring arterial blood gas (ABG), lactic acid, and the blood samples using cytokines measurements were immediately separated by centrifugation at 3,000 rpm for 15 minutes at 4°C. The plasma was divided into aliquots and stored at −70°C until assayed. All animals were killed through bleeding from the right femoral artery 6 hours after blunt chest trauma challenge. For histological evaluation of pulmonary tissue, the lungs were gradually inflated with 10% formalin, and tissue sections were stained with hematoxylin and eosin (H & E).

**Experimental Protocols**

Fifty rats were randomly assigned into five equal groups (n = 10) as follows: uninjured control (UC) group, uninjured plus dexmedetomidine (UD) group, injured (I) group, injured plus dexmedetomidine (ID) group, injured plus dexmedetomidine plus yohimbine (IDY), an α2-adrenergic receptor antagonist, group. Except for the UC and UD groups, all animals from I, ID, and IDY group had a severe bilateral blunt chest trauma. Rats in UD, ID, and IDY groups received dexmedetomidine infusion continuously through the left femoral vein cannula at the rate of 5.0 μg/kg per hour 30 minutes after blunt chest trauma until the end of the experiment. Rats in the IDY group also received yohimbine (0.2 mg/kg, intravenous infusion over 10 minutes) immediately after the administration of dexmedetomidine. Rats in the UC and I groups were intravenously administered with isotonic sodium chloride solution at the rate of 5.0 mL/kg per hour.

**Polymorphonuclear Neutrophils Count in Bronchoalveolar Lavage Fluid**

At 6 hours after blunt chest trauma challenge, bronchoalveolar lavage fluid (BALF) was prepared by washing the lungs for three times with 4.0-mL phosphate-buffered saline (PBS). All three BALs was pooled and then centrifuged at 1,000 G for 10 minutes at 4°C. The supernatant of the BALs was used for polymorphonuclear neutrophils (PMNs) analysis. BALF cells, stained with Thomas, were evaluated in a Neubauer chamber, and cell differentiation was performed on cytocentrifuge slides with Giemsa.

**Lung Histopatology Evaluation**

**H & E Stain**

The lung specimens were fixed in 10% formalin, sectioned, and stained with H & E. Slides (100× magnification) were evaluated and graded for the presence of interstitial neutrophilic infiltrate, intra-alveolar hemorrhage, and pulmonary edema with a microscope (BX51, Olympus, Tokyo, Japan). Histopathological evaluations were scored by a blinded and experienced laboratory pathologist using a five-point scale according to combined assessments of alveolar congestion, hemorrhage, infiltration or aggregation of neutrophils in the airspace or vessel wall, and thickness of alveolar wall/hyaline membrane formation: 0, minimum damage; 1+, mild damage; 2+, moderate damage; 3+, severe damage; and 4+, maximum damage.

**Transmission Electron Microscope**

The fragments of the lung tissue were cut into 1-mm-thick slices, immersion-fixed in 2.5% buffered glutaraldehyde at 0°C to 4°C for 2 hours, buffered in PBS for three times, fixed with 1% osmic acid for 1 hour, washed with distilled water, and dehydrated by dimethylketone. After being embedded in Epon-812, they were cut into ultrathin sections (60 nm) by an LKB-V ultramicromtome (Bromma, Sweden) and stained with uranyl acetate and plumbum citrate. Sections were examined with a Hitachi H-600 transmission electron microscope (Hitachi, Tokyo, Japan).

**Lung Wet-to-Dry Weight Ratio**

After the lungs were drawn and the blood on the surface of the lung tissue was wiped with gauze, the lungs were weighed and then dried in an oven at 60°C for 72 hours. Determined wet/dry weight (W/D) ratio was reported as a measure of pulmonary edema.

**ABG, Lactic Acid, and Cytokine Measurements**

ABG and blood lactic acid values were determined using a portable blood gas analyzer (i-STAT, Princeton, NJ). The plasma cytokines (TNF-α and IL-1β) concentration were measured using enzyme-linked immunosorbent assay kits (American R & D, Washington, DC). The absorbance of each well was read at 450 nm with an enzyme-linked immunosorbent assay plate reader.
Nuclear Factor κB
The lung tissues were immersed in 4% paraformaldehyde and dehydrated, embedded in paraffin, and made into 5-μm slices using standard histological techniques. The sections were warmed at 60°C for 60 minutes in the oven and were deparaffinized in xylene. After the endogenous peroxidase reaction was blocked with 3% H2O2 for 30 minutes, the sections were rinsed in PBS three times then incubated overnight with 1:100 dilution of primary rabbit anti-rat NF-κB polyclonal antibody (Santa Cruz, CA) at room temperature. Bound antibody was decorated with 1:1,000 goats anti-rabbit IgG (Sigma, St. Louis, MO) diluted in the blocking solution for 30 minutes at room temperature, after washing three times with PBS for 10 minutes each. The biotin-peroxide and diaminobenzidine were used as substrates to develop signals in brown-yellow colors. The mean optical densities of NF-κB–positive cells from each section were analyzed by image cytometry with HIPAS-2000 image analysis software. The number of positive microvessels in each section was counted in 10 microscopic fields (at 400× magnification) and averaged for the positively immunostained vessel number per visual field.

Statistical Analysis
Data were presented as mean (SD). Data analysis was performed using SPSS 15.5 software (SPSS Inc., Chicago, IL). Differences associated with main sources of variation were tested with one-way analysis of variance. When the F statistic was significant for analysis-of-variance comparisons, differences between individual means were tested for significance using Bonferroni test. The Bonferroni is a post hoc test that adjusts α for multiple comparisons. Statistical significance was defined as p < 0.05.

RESULTS

Hemodynamic Parameter
Mean Arterial Pressure
No significant differences were noted in baseline in MAP among all groups. Blunt chest trauma challenge decreased MAP in I, ID, and IDY group. At the time point 6 hours after PC, MAP decreased by 30.9%, 10.6%, and 23.6% in I, ID, and IDY groups, respectively. Significant differences in MAP were found between I group and ID group at 2-, 4-, and 6-hour points following PC (p < 0.05, respectively). There were significant differences between ID and IDY group at 4- and 6-hour time points (p < 0.05, respectively) (Fig. 1A).

Heart Rate
There were no significant differences in baseline HR among all groups. The HR level was firstly ascending and followed by descending in I group animals. No significant differences were between the normal control (NC) and UD groups. There were significant differences between ID and IDY group at 1-, 2-, and 6-hour time points following the infusion of dexmedetomidine 5.0 μg/kg per hour (p < 0.05) (Fig. 1B).

Pulmonary Pathophysiologic Changes
As shown in Figure 2, the lung H & E staining revealed normal lung parenchyma in the NC and UD group. In contrast, rats in I and IDY groups had disruption of normal alveolar structure with severe congestion and hemorrhage associated with infiltrating leukocytes. Rats in ID group had significantly less hemorrhage and leukocyte infiltration than those in I group.

As shown in Figure 3, electron microscopy observation showed mitochondrial vacuolization and ridge dissolution in some alveolar epithelial cells and vascular endothelial cells as well as a number of emptied lamellar bodies in injured group rats. In comparison, dexmedetomidine treatment resulted in a marked attenuation of those pathologic alterations.

PMN Count in BALF
PMNs reflecting the inflammation status of the lungs were identified by using coulter counter. The leukocytes in the UC group were mostly macrophages and a small quantity of neutrophils. All injury groups had significantly larger numbers of PMNs in BALF compared with UC group (p < 0.05). The PMNs (PMN%) in the BALF was as high as 69.4%. However, after the administration of dexmedetomidine, PMNs in BALF was remarkably lower than in injured rats (p < 0.05) (Table 1).

Pulmonary Edema and Analysis of ABG and Lactic Acid Values
Results of lung W/D ratio are presented in Table 1. As a further indicator of pulmonary edema, the lung W/D ratio was significantly elevated after blunt chest trauma challenge. The lung W/D ratio was decreased after the infusion

Figure 1. A and B, Changes of MAP and HR (n = 10, mean [SD]). Closed squares, UC group; open squares, UD group; closed trilaterals, I group; closed circles, ID group; open circles, IDY group. *p < 0.05 versus I group; #p < 0.05, ID group versus IDY group.
of dexmedetomidine 5.0 μg/kg per hour of \( p < 0.05 \) compared with the I group. However, yohimbine treatment significantly reversed the inhibitory effect of dexmedetomidine \( p < 0.05 \).

ABG analysis was assessed at 6 hours after blunt chest trauma challenge. Results are shown in Table 1. The carbon dioxide tension increased, and the oxygen tension and the arterial blood pH values decreased after blunt chest trauma challenge. The administration of dexmedetomidine resulted in a significant decrease in arterial blood PaCO\(_2\) and an increased PaO\(_2\) and pH values compared with the I group \( p < 0.05 \). However, yohimbine treatment significantly reversed the effects of dexmedetomidine \( p < 0.05 \).

The blood lactic acid concentration was significantly increased after blunt chest trauma challenge. The administration of dexmedetomidine significantly reduced lactic acid level...
compared with the I group ($p < 0.05$). However, yohimbine treatment significantly reversed the inhibitory effect of dexmedetomidine ($p < 0.05$) (Table 1).

**The Plasma Cytokine Levels (TNF-α and IL-1β)**

The plasma TNF-α and IL-1β levels were significantly increased after blunt chest trauma challenge (Fig. 4A and B). The administration of dexmedetomidine significantly reduced the plasma TNF-α and IL-1β levels owing to bilateral blunt chest trauma. However, yohimbine treatment significantly reversed the inhibitory effects of dexmedetomidine ($p < 0.05$).

**NF-κB Activation**

The significantly increased expression of NF-κB protein in lung tissue were observed in the I group. The administration of dexmedetomidine had significantly lower activation of NF-κB compared with the I group. However, yohimbine treatment significantly reversed the inhibitory effects of dexmedetomidine ($p < 0.05$) (Fig. 5).

### DISCUSSION

PC is a relatively common injury seen after blunt chest trauma and has been demonstrated to be associated with increased morbidity and mortality. Our experiment has used a recently developed model to examine lung injury severity and innate pulmonary inflammation in rats. This model simulates the clinically relevant lung injury that follows blunt chest trauma. At 6 hours after bilateral blunt chest trauma challenge, rats had significant hypotension, arterial hypoxemia, alveolar edema, leucocytosis in the interstitial capillaries, and alveolar hemorrhage in histological assessments, which was consistent with the study of Raghavendran et al. In addition, we found that PMNs population was significantly increased in during PC. It indicated that we succeeded in replicating PC in rats.

In the present study, the administration of dexmedetomidine markedly elevated MAP, made HR stable, improved the extent of the damage of lung, maintained normal structure of the lung tissue, inhibited pulmonary edema formation, and prevented infiltration of activated PMNs into the lung tissue, and that the result of dexmedetomidine treatment significantly reduced the lung W/D ratio demonstrated that dexmedetomidine effectivly decreased the lung vascular permeability and promoted the resolution of lung edema. In addition, the ultrastructure phenomenon of mitochondrial vacuolization and emptied lamellar bodies in epithelial cells and endothelial cells revealed that using dexmedetomidine might inhibit apoptosis of alveolar surfactant Type II epithelial cells after blunt chest trauma challenge. These are the most important findings of this study.

Blunt chest trauma alone had activated local inflammatory response, such as increase in PMNs in BALFs. Furthermore, blunt chest trauma resulted in a relevant systemic inflammatory response. Therefore, we detected the proinflammatory cytokine (TNF-α and IL-1β) levels in peripheral plasma and examined the correlation with clinical outcomes.

#### TABLE 1. Changes of PMN in BALF, Lung W/D, ABG and Lactic Acid (n = 8, mean (SD))

<table>
<thead>
<tr>
<th>Group</th>
<th>UC</th>
<th>UD</th>
<th>I</th>
<th>ID</th>
<th>IDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMN, %</td>
<td>12.2 (3.2)*</td>
<td>11.1 (3.1)*</td>
<td>69.4 (4.6)</td>
<td>43.2 (4.0)*</td>
<td>60.8 (4.9)*†</td>
</tr>
<tr>
<td>W/D ratio</td>
<td>3.77 (0.14)*</td>
<td>3.79 (0.14)*</td>
<td>5.22 (0.17)</td>
<td>4.63 (0.16)*</td>
<td>5.13 (0.12)*†</td>
</tr>
<tr>
<td>$\text{PaO}_2$, mm Hg</td>
<td>38.3 (3.7)*</td>
<td>37.6 (3.0)*</td>
<td>54.4 (6.0)</td>
<td>44.0 (5.0)*</td>
<td>53.9 (5.4)*†</td>
</tr>
<tr>
<td>$\text{PaCO}_2$, mm Hg</td>
<td>104.6 (4.4)*</td>
<td>106.6 (7.5)*</td>
<td>71.6 (5.6)</td>
<td>87.8 (5.0)*</td>
<td>76.9 (5.3)*†</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 (0.05)*</td>
<td>7.35 (0.03)*</td>
<td>7.23 (0.04)</td>
<td>7.33 (0.04)*</td>
<td>7.25 (0.05)*†</td>
</tr>
<tr>
<td>Lactic acid, mmol/L</td>
<td>1.13 (0.19)*</td>
<td>1.06 (0.22)*</td>
<td>2.34 (0.38)</td>
<td>1.67 (0.32)*</td>
<td>2.16 (0.36)*†</td>
</tr>
</tbody>
</table>

* $p < 0.05$ versus I group.
† $p < 0.05$, IDY group versus ID group.

To convert millimeters of mercury to kilopascal, multiply value by 0.1333.

**Figure 4.** A and B, Changes of the plasma TNF-α and IL-6 (mean [SD]). * $p < 0.05$ versus I group; # $p < 0.05$, IDY group versus ID group.
血。最近，越来越多的证据表明， dexmedetomidine 在动物损伤模型中显示抗炎效果。10,11,14,18 2008年，Yang et al. 14 发现，剂量约为临床剂量10倍的 dexmedetomidine 显著降低了通气诱导的肺损伤大鼠肺部炎症分子的上调。2009年，Qiao et al. 18 发现，用 sedation with midazolam and dexmedetomidine 都能改善多菌群严重感染的动物的预后。同年，Lai et al. 19 发现 dexmedetomidine 显著影响了巨噬细胞中炎性细胞因子的表达。2011年，Gu et al. 20 发现， dexmedetomidine 可以减轻由肾缺血再灌注引起的远端肺损伤。从我们的数据来看， blunt chest trauma 所引起的 TNF-α 和 IL-1β 的表达显著降低，而 dexmedetomidine 可以显著降低 TNF-α 和 IL-1β 的表达，这表明 dexmedetomidine 具有潜在的抗炎能力。

此外， dexmedetomidine 在本研究中的剂量是 5.0 μg/kg/小时。在动物模型中，临床剂量与人类剂量的比值约为 10:1。因此，在当前研究中， dexmedetomidine 的剂量并不是出乎意料的。

一些研究已被证明可以改善对 blunt chest trauma 所引起的 PC 的理解。21 许多研究者已经发现了 NF-κB 的抑制可以减少炎症激活，NF-κB 是核蛋白，对于控制炎性相关因素的表达至关重要，从而激活了可以最终激活释放的 proinflammatory cytokines, such as TNF-α, IL-1β, and additional proinflammatory mediators involved in PC. NF-κB 的抑制可以减少炎性相关因素和 chemokines,23,24 因此，我们通过观察 NF-κB 在肺组织中的免疫组织化学反应来研究 NF-κB 的表达。我们的结果表明， NF-κB 的表达与 BLW 有关。此外， dexmedetomidine 可以抑制 NF-κB 的活性，这与 Lai et al. 19 的研究一致。

**Figure 5.** Immunohistochemical staining of NF-κB expression in lung tissue. Markedly thickened expression of NF-κB was observed on the bronchial epithelium and the luminal surfaces in I or IDY group compared with that of the UC group, respectively, whereas it was only slightly thickened in the ID group. The expression of NF-κB is brown-yellow colors. NF-κB expression (mean [SD]). *p < 0.05 versus I group; #p < 0.05, IDY group versus ID group.
act through regulating the activation of TLR-4/NF-κB/MAPKs pathway to exhibit its effects on regulating the endotoxin-induced up-regulation of inflammatory molecules.

Dexmedetomidine is a new and highly selective α2-adrenoceptor agonist. Several investigators have described the effects of dexmedetomidine and α2-adrenoceptor agonists on cytokines. In 1997, Straub et al. found that paminoclonidine, an α2-adrenoceptor agonist, suppressed IL-6 production in vitro. In 2008, Sud et al. reported that α2-adrenoceptor played a crucial role in the regulation of peripheral TNF production in macrophage. The results of the α2-adrenergic receptor antagonist (yohimbine) treatment significantly reversed the effect of dexmedetomidine, indicating that the therapeutic effects of dexmedetomidine might be mediated, at least in part, by α2-adrenergic receptors during PC.

In summary, the current study showed that the administration of dexmedetomidine had potential beneficial effects against blunt chest trauma–induced PC in rats. The mechanisms were likely to inhibit the NF-κB activation via α2-adrenergic receptors and attenuate the proinflammatory cytokine responses. However, we do not know the more accurate mechanisms responsible for the inhibitory effects. Therefore, further investigation is needed, including the effects of dexmedetomidine on upriver genes, the complex mechanisms involved in signaling pathway, such as Toll-like receptors or nod-like receptors, complement, IRAK, MyD88,

AUTHORSHIP
X.W., N.L., and Z.X. designed this study; collected, analyzed, and interpreted the data; wrote the paper; prepared the figures and tables; and submitted the final article. X.S., Q.M., and L.Z. interpreted the data; provided critical review of the article, its figures, and tables; and approved the final article.

DISCLOSURE
This study was supported by National Natural Science Foundation of China (no. 81000027).

REFERENCES