THE ROLE OF KETANSERIN IN MAINTAINING CIRCULATION STABILITY IN ENDOTOXIC SHOCK RATS

Wen-Yu Yang[1,2], Jin Zhang[1*], Bing-Jun Zhang[1]
[1]Department of Anesthesiology, Affiliated Shengjing Hospital, China Medical University, Shenyang 110004, China - [2]Department of Anesthesiology, Sanbo Brain Hospital Capital Medical University, Beijing 100080, China

ABSTRACT

Introduction: *To observe the effect of the serotonin 2A (5-HT2A) receptor antagonist ketanserin on hemodynamics in rats with endotoxic shock, in order to provide reference for the treatment of endotoxic shock.*

Materials and methods*: Thirty Sprague Dawley (SD) rats were randomly divided into three groups: group N, rats received normal saline + 5-HT2A receptor antagonist; group L, lipopolysaccharide (LPS) control group; group K, rats received LPS+5-HT2A receptor antagonist. The body pressure and pulmonary circulation pressure of rats in each group were monitored for six hours. The serum TNF-α and IL-1β levels in each group were detected using ELISA at the beginning of the experiment, and at 15 minutes, two hours and six hours after the injection of saline or LPS. Lung tissue hematoxylin and eosin (H&E) staining were observed under a light microscope to value the degree of inflammatory lesions in group K and in group L.*

Results*: In group L, rats presented with decreased femoral artery pressure and increased pulmonary artery pressure after receiving LPS. In group K, the femoral artery pressure of rats returned to normal levels at one hour after receiving LPS. At six hours after receiving LPS, the blood pressure was completely restored. Pulmonary artery pressure did not significantly increase at 15 minutes after receiving LPS. Thereafter up to six hours after LPS was given, pulmonary artery pressure decreased and then maintained at 28 mmHg. Furthermore, TNF-α and IL-1β levels were significantly lower in group K than in group L. Moreover, lung tissue H&E staining results revealed that the degree of inflammatory lesions in lung tissues was significantly lower in group K than in group L.*

Conclusion*: Ketanserin can improve the hemodynamic disorder caused by endotoxin, playing an anti-shock role.*

Keywords: *endotoxic shock, pulmonary hypertension, 5-HT; Ketanserin, endothelium, glycocalyx.*

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Introduction

Endotoxic shock is a common clinical critical disease, and its mortality rate can reach up to 50-80%[(1)]. Hemodynamic disorders in endotoxic shock include decreased systemic circulation pressure and increased pulmonary arterial pressure (PAP). Continuous hemodynamic disturbance is an important pathological factor for failure of multiple organs, leading to shock that is difficult to reverse[(2)]. The pathological mechanism leading to hemodynamic disturbance is very complicated. Endotoxin stimulates platelets and leukocytes to release vasoactive substances and cytokines[(3-5)], and this mechanism plays an important role in hemodynamic disturbance.

5-serotonin (5-HT) is an endogenous vasoactive factor, which is stored and released by platelets, and regulates vasoconstriction via the 5-HT receptor[(3)].

Studies have revealed that plasma 5-HT level is significantly elevated in endotoxic shock[(6)]. This suggests that 5-HT is correlated to elevated PAP in endotoxic shock. Some recent studies have revealed that the 5-HT2A receptor antagonist may be an effective drug for treating pulmonary arterial hypertension (PAH) in endotoxic shock[(4, 7)]. At present, controversies remain on whether 5-HT plays a role in hemodynamic disturbance during endotoxic shock[(8, 9)]. The present study aims to observe the effect of 5-HT2A receptor antagonist ketanserin on hemodynamics in rats with endotoxic

shock, in order to provide reference for the treatment of endotoxic shock.

Materials and methods

Major reagents

Escherichia coli (E. coli) endotoxin: E. coli lipopolysaccharide (LPS, Ecoli, 0111B4, Sigma, USA); urethane (Tianjin); ketanserin bitartrate (selective 5-HT2A receptor antagonist, Shenyang Bomei Pharmaceutical, China); rat serum tumor necrosis factor-alpha (TNF-α) ELISA kit (Shanghai Senxiong Technology Industry Co., Ltd.); rat serum interleukin-1 (IL-1β) ELISA kit (Shanghai Senxiong Technology Industry Co., Ltd.); 3% methanol-H2O2 solution (prepared with 30% H2O2 and 80% methanol solution).

Major instruments

Electronic balance: JA1003 (Shanghai Balance Instrument Factory);

MP354-channel physiological recorder: (BIOPAC, USA);

Invasive physiological monitor: Eagle3000 (USA);

Pressure transducer: TP2200P (Japanese photoelectric, Japan);

Pulmonary artery catheters: self-designed PVC catheters (Jincheng Manyao Plastic Glass Instrument Factory, China);

Intravenous infusion pump: TCI-III type (Guangxi Veryark, China);

Disposable venous detaining needle: 16G and 24G needles (Becton, Dickinson and Company, USA).

Experimental animals

A total of 30 healthy male Sprague Dawley (SD) rats were used in the present study. These rats were six months old, weighted 300-400 g, and provided by the Experimental Animal Center of China Medical University. Room temperature was maintained at 22-23°C, and the day-night cycle was set at 12 hours-12 hours. Rats were provided with food and water ad libitum. After the experiment was finished, rats were sacrificed. Then, it was roughly tested whether these rats had obvious diseases (spleen measurement and the presence of a tumor). Unhealthy rats were excluded. All experiments were carried out under the approval of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Establishment of the endotoxic shock rat model

Rats were anesthetized with an intraperitoneal injection of 20% urethane (1 g/kg body weight), endotracheal intubation was performed with a 16# vein detaining needle, and spontaneous breathing was retained. Arteriovenous detaining needles were placed at both the right femoral vein and left femoral artery, and were connected to a pressure sensor, in order to continuously monitor femoral arterial pressure. A self-designed pulmonary artery piezometric catheter was taken, directly placed in the pulmonary artery of the rat through the right jugular vein, and connected to the pressure sensor. When the pressure baseline increased and the pulmonary artery waveform appeared, this indicated that the intubation was successful.

Pulmonary arterial pressure was continuously monitored. Rats were slowly injected with 5 mg/kg of LPS through the right femoral vein. At 1.5 hours after the intravenous injection of LPS, blood pressure decreased to 75% of the baseline value, and maintained on that level for at least two hours, indicating the successful establishment of the endotoxin shock model. Rats were excluded from the present study when the blood pressure did not decrease to 75% of the baseline value within two hours after the intravenous injection of LPS.

Experimental grouping

These 30 SD rats were randomly divided into three groups (each, n=10): group N, rats received normal saline + 5-HT2A receptor antagonist; group L, LPS control group; group K, rats received LPS+5-HT2A receptor antagonist. In group K or group N, 2 mg/kg of ketanserin (dissolved in normal saline to the concentration of 2 mg/ml) or an equal volume of normal saline was injected through the jugular vein catheter at 30 minutes before a dose of LPS/equal volume of normal saline was given. Systemic pressure and pulmonary circulation pressure were continuously monitored and recorded at the beginning of the experiment, at 30 minutes after the intervention drug was given for stabilization, and at 15 minutes, one hour, two hours, three hours, four hours, five hours and six hours after LPS was given. The changes in systemic pressure and pulmonary circulation pressure after ketanserin or saline intervention were compared among groups.

Detection indices and methods

Femoral artery pressure and PAP were continuously monitored and recorded at the beginning of the experiment, and at 15 minutes, one hour, two hours, three hours, four hours, five hours and six hours after the injection of LPS/equivalent volume of saline.

The serum TNF-α and IL-1β levels in each group were detected by ELISA at the beginning of experiment, and at 15 minutes, two hours and six hours after the injection of saline/LPS. The detection rang of TNF-α is from 7.8pg/ml~500pg/ml. The lowest detection limit of TNF-α is 1.95 pg/ml. The detection rang of IL-1β is from 3.12pg/ml~200pg/ml. The lowest detection limit of IL-1β is 0.78 pg/ml.

H&E staining: The middle lobe of the right lung was clipped, rinsed with normal saline, and immersed in 10% neutral formalin for 18-24 hours. Then, this was dehydrated, routinely paraffin-embedded, sliced into 5-mm sections, stained with H&E, and observed under a light microscope.

Statistical analysis

All data were expressed as mean ± standard deviation (x ± SD). Data were analyzed using statistical software SPSS 11.00 (SPSS, Inc., Chicago, IL, USA). Intra-group comparison was conducted using paired t-test, and inter-group comparison was conducted using independent sample t-test. Multiple samples were compared using single factor analysis of variance (one-way ANOVA). $P<0.05$ was considered statistically significant.

Results

In the present study, mean arterial pressure (MAP) in groups L and K decreased to the level of shock state (<60 mmHg) after LPS was given (Figure 1). MAP was always in the shock state from this time point to six hours after LPS was given.

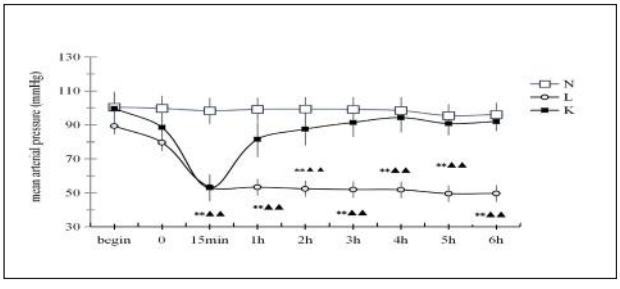

Figure 1: MAP for each group.

In group K, MAP of rats returned to normal levels (MAP>60 mmHg) at one hour after LPS was given, while at six hours after LPS was given, systemic circulation pressure remained stable (MAP>60mmHg).

For pulmonary circulation pressure (Figure 2), after LPS was given, PAP remained stable in rats in group K, while PAP significantly increased in rats in group L.

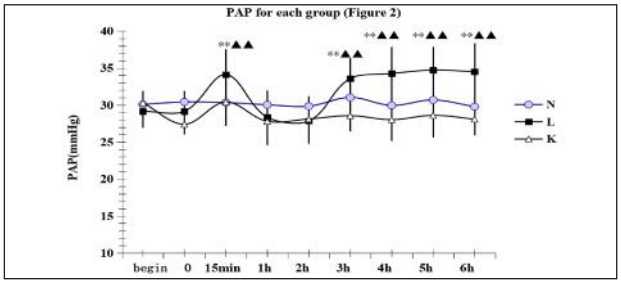

Figure 2: PAP for each group.

After LPS was given, serum TNF-α and IL-1 levels significantly increased in groups L and K. However, serum TNF-α and IL-1 levels were significantly lower in group K than in group L at two hours and six hours after LPS was given. The production of cytokines in group K was significantly inhibited (Figures 3 and 4).

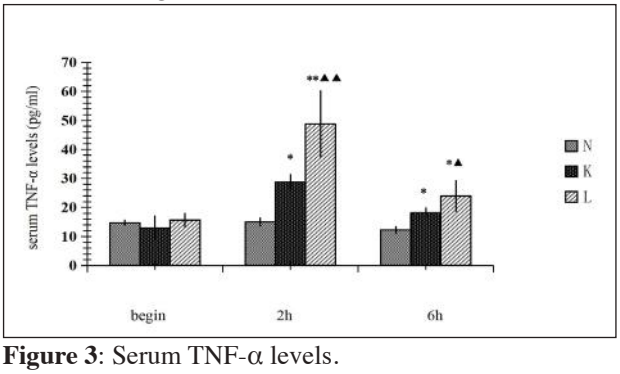

Figure 3: Serum TNF-α levels.

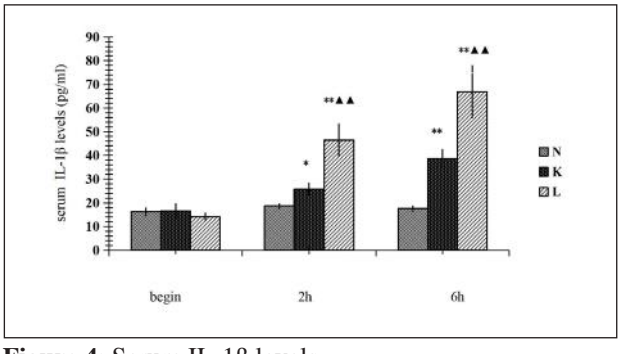

Figure 4: Serum IL-1ß levels.

The H&E staining of the lung tissue of rats revealed that in group N (Figure 5), the alveolar structures were intact, no edema was found in the

alveolar septum and no hyperemia was found in the blood vessels of the lung. In group L (Figure 6), a large number of congestion and inflammatory cell infiltration, interstitial edema and infiltration of inflammatory alveolar cells were found in the lung blood vessels and bronchi of rats.

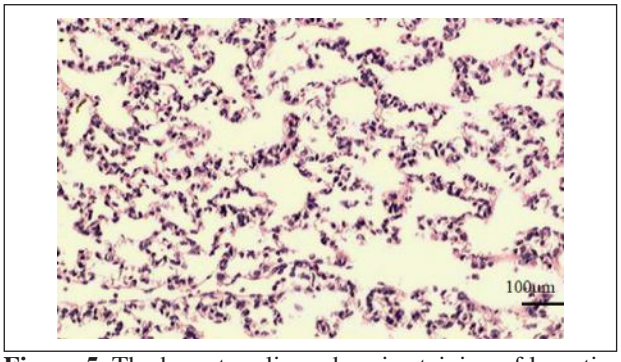

Figure 5: The hematoxylin and eosin staining of lung tissue of rats in group N.

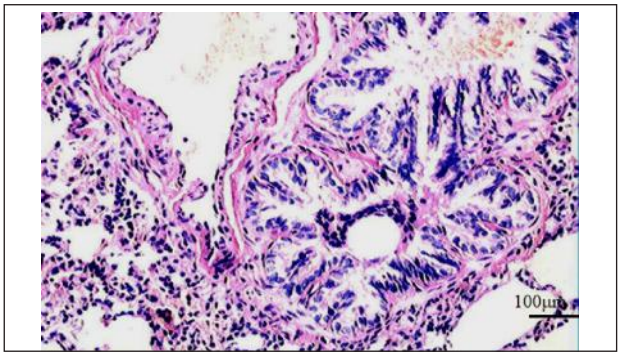

Figure 6: The hematoxylin and eosin staining of lung tissue of rats in group L.

In group K (Figure 7), the degree of inflammatory lesions in lung tissue was significantly lower, compared to that in group L, and a small amount of inflammatory cell infiltration could be observed in pulmonary capillaries, mild interstitial edema could be observed in the lungs, and a small amount of red blood cells could be observed in the alveoli.

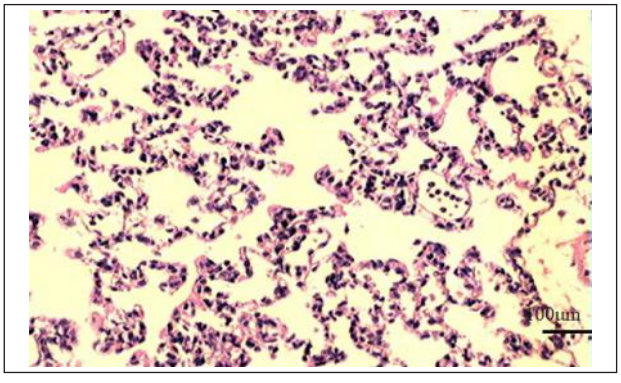

Figure 7: The hematoxylin and eosin staining of lung tissue of rats in group K.

Effects of ketanserin on PAP and femoral artery pressure in rats with endotoxic shock

The differences in MAP and PAP at the initial time among all groups were not statistically significant (P>0.05).

The comparison results of MAP among all groups are presented on Figure 1.

In group K, MAP returned to normal levels (MAP>60 mmHg) at one hour after LPS was given, and at six hours after LPS was given, the blood pressure was completely restored (MAP>60 mmHg). The difference was not statistically significant when compared with that at the initial time point of the same group (P>0.05).

The comparison of changes in PAP in rats among all groups is presented on Figure 2.

In group K, PAP did not significantly increase at 15 minutes after LPS was given, and thereafter to six hours after LPS was given, PAP decreased and then maintained at 28 mmHg. The difference was not statistically significant when compared with that at the initial time point in the same group (P>0.05). In group L, PAP significantly increased at 15 minutes and three hours after LPS was given, and the difference was statistically significant when compared with that at the initial time point in the same group (P<0.01).

Furthermore, the difference was statistically significant when compared with the corresponding time points of the other two groups (P<0.01).

Changes in serum TNF-α and IL-1β levels

The differences in TNF-α and IL-1β levels between groups L and K at the initial time point were not statistically significant (P>0.05). The change in IL-1β level among all groups is presented on Figure 3.

In groups L and K, TNF-α level was significantly increased at two hours after LPS was given, compared to that at the initial time point (P<0.05). In groups L and K, TNF-α level increased at six hours after LPS was given, compared to that at the initial time point (P<0.05). TNF-α level was significantly lower in group K than in group L at two hours after LPS was given (P<0.01). Furthermore, TNF-α level was significantly lower in group K than in group L at six hours after LPS was given (P<0.05). The changes in IL-1β level among all groups are presented on Figure 4.

In groups L and K, IL-1β level was significantly elevated at two hours and six hours after LPS was given, when compared to that at the ini-

tial time point, and the difference was statistically significant ($P<0.01$). However, serum IL-1β level was significantly lower in group K than in group L at two hours and six hours after LPS was given, and the difference was statistically significant ($P<0.01$).

The H&E staining results of lung tissues for all groups are presented in Figures 5, 6 and 7.

Discussion

Lung tissue is first attacked during endotoxin shock, which results in acute lung injury (ALI). Experimental and clinical studies have proved that PAH is one of the early manifestations of ALI in endotoxic shock. Pulmonary vascular injury caused by endotoxin-stimulated vasoconstrictor substances and inflammatory cytokines causes abnormal contraction of pulmonary artery and continuous increase in pulmonary artery pressure. Continuous PAH promotes pulmonary interstitial edema, pulmonary dysfunction, aggravates hemodynamic disorders, and leads to refractory shock.

5-HT is an endogenous vascular active substance in the human body, and includes seven receptor types, from 5-HT1 to 5-HT7. Among these, the 5-HT2A receptor is widely distributed in vascular smooth muscle cells, which mainly induces vasoconstriction and platelet aggregation. Ketanserin is a selective 5-HT2A receptor antagonist, and its binding capacity with the 5-HT2A receptor is stronger than that of the other 5-HT2A receptor antagonists[10]. It also has weak antagonism of adrenergic α1- receptor and H receptor. Ketanserin can reduce the peripheral resistance of hypertensive patients and improve the blood supply of lower limbs in patients with obstructive angiopathy. For patients with Reynolds disease, the blood perfusion of tissues can be improved and the blood flow of skin can be increased. Intravenous injection can reduce right atrial pressure, pulmonary artery pressure and pulmonary capillary wedge pressure. It is clinically used in the treatment of hypertension, demon syndrome, preeclampsia, prevention of myocardial ischemia, origin of Kangxin syndrome and diabetic foot.

In some laboratory and clinical studies of endotoxic shock, the effects of 5HT2A receptor including antagonists on platelet aggregation, thrombosis and inflammatory cytokines have been confirmed and affirmed. However, the results of these studies on the effect of ketanserin on hemodynamics in endotoxic shock vary[9, 11-15]. Ball considered that ketanserin promoted PAH induced by endotoxin[12]. Makabali considered that ketanserin could inhibit endotoxin-induced pulmonary vasoconstriction, but had no protective effect on vascular permeability. Hence, it had no effect in the treatment of endotoxic shock[7]. Vellinga et al.[15] reported that administration of ketanserin to septic humans was associated with hypotension.

However, more and more studies have revealed that the 5-HT2A receptor is closely correlated to many infection-induced pathophysiological reactions such as microcirculatory disorder[15], macrovascular contraction[16] and thermoregulatory[17]. Therefore, in the present experiment, the method of administration reported in most studies was adopted, in which ketanserin was pre-given, in order to determine whether ketanserin can prevent hemodynamic disorder induced by endotoxin.

The results revealed that in the present experiment, the hemodynamic parameters were different from those reported in previous studies:

• In rats pretreated with ketanserin, femoral artery pressure returned to normal levels at one hour after LPS was given, and continued to remain at normal levels.

• In rats pretreated with ketanserin, the increase in PAP was not significant after LPS was given, and PAP was maintained at the normal level until the end of the experiment at six hours after LPS was given. This result reveals that as a 5-HT2A receptor antagonist, ketanserin can inhibit the increase in PAP in endotoxic shock by directly antagonizing the 5-HT2A receptor in pulmonary vessels.

Cytokines produced by inflammatory reaction lead to decreased vascular responsiveness and increased vascular permeability and platelet aggregation, and are involved in endotoxin-induced vascular dysfunction[18, 19]. A large number of TNF-α produced from mononuclear/macrophage cells induced by endotoxin stimulation play an important role in acute lung injury induced by endotoxin. TNF-α reached its peak in the early stage after endotoxin injection, and subsequently decreased. However, its hemodynamic effect could last for a long time. PAH induced by the injection of TNF-α alone in animals is similar to the changes in pulmonary hemodynamics during endotoxic shock. In infectious shock, IL-1 has many similarities to TNF-α, in which it can enter into the blood circulation and interact with TNF-α to induce fever, and

mediate 5-HT to inhibit the production of inducible nitric oxide synthase, promoting the contraction of vascular smooth muscles[20].

The results obtained in the present experiment were consistent with those reported in a literature (11), in which ketanserin inhibited the production of TNF-α and IL-1 in endotoxic shock. The results of the H&E staining of lung tissue revealed that in rats treated with ketanserin, the inflammatory response of lung tissue was milder, and the interstitial edema of the lungs was significantly improved. Therefore, ketanserin inhibits the production of TNF-α and IL-1, and may alleviate the damage of cytokines to pulmonary vascular endothelial cells, reducing the effect of cytokines on the contraction of pulmonary vessels. The inhibition of inflammatory cytokines may be one of the mechanisms of ketanserin in improving hemodynamic disorder during endotoxin shock.

The present study verified that ketanserin can improve the hemodynamic disorder induced by endotoxin. This effect may be realized by antagonizing the 5-HT receptor and inhibiting the production of inflammatory factors. Ketanserin is a clinical drug, and its adverse reactions have little effect on normal physiological functions. However, there are few studies on its anti-shock effect. The present study provides reference for the treatment of endotoxic shock.

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Corresponding Author:
Jin Zhang
Department of Anesthesiology,
Affiliated Shengjing Hospital, China Medical University,
No.36, Sanhao Street, Heping District,
Shenyang, Liaoning Province, 110004
E-mail: zhangjin1822@163.com
(China)