Breviscapine Reduces the Acute Lung Injury Induced by Left Heart Ischemic Reperfusion in Rat through inhibition of the Expression of IL-6 and ICAM-1

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Abstract: Objective To explore the effect of breviscapine on expression of IL-6 and ICAM-1 in rat with acute lung injury induced by left heart ischemic reperfusion， and the mechanism of breviscapine protecting respiratory function. **Methods** 60 rats were divided into 2 equal groups randomly: group 1 is treatment group (TG),group 2 is control group(CG).All rats were made left heart ischemia-reperfusion model by ligaturing the anterior descending branch 30min and loosing. Rats in this group were intravenous injected breviscapine ($10mg \cdot kg^{-1}$) when the myocardial ischemia had been for 10 minutes. While，the rats of CG were treated with normal saline. The same amount of rats in 2 groups were killed at 3 point in time: 30min after ligaturing (T1), 30min after loosing(T2) and 60min after loosing (T3).All rats were recorded and observed respiration curve with BL-420 biological signal collect and analysis system, measured the expression of interleukin 6 (IL-6)and inflammatory cell adhesion molecules-1 (ICAM-1) with Immunohistochemistry, measured the expression of IL-6 in peripheral blood and bronchial alveolarlavage fluid with Enzyme Linked Immunosorbent Assay methods, and measured the activity of myeloperoxidase (MPO) in lung tissue with colorimetry. **Results** In T1, T2 and T3, the level of the expression of IL-6 and ICAM-1 in CG were higher than those in TG, the activity of myeloperoxidase in lung tissue of CG was stronger than that of TG, the expressions of IL-6 of CG were higher than those of TG in peripheral blood and bronchial alveolarlavage fluid, and the amplitude and duration time of respiration curve of TG was higher than those of CG. The comparisons were great. **Conclusions** Breviscapine can inhibit the expression of ICAM-1, which means that leukocyte adhesiveness/aggregation and release reaction of can be reduced in the pulmonary circulation, the expression of IL-6 can be decreased, and inflammatory cascade response will be reduced to protect respiratory function.

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Key words： breviscapine; left heart ischemic reperfusion； acute lung injury； inflammatory cell adhesion molecules 1; interleukin 6

1. Introduction

Breviscapine is a commonly used drug in clinical medicine for various types of coronary heart disease presently. The correlation studies (LIU et al., 2010) of breviscapine pharmacological effect are mostly related to the following aspects: the inhibition of adhesion of platelet and erythrocyte, the decrease of the blood viscosity, and the dilating vessel. But the effect of breviscapine has not been systematically explored on expression of inflammatory factors in acute lung injury induced by left heart ischemic reperfusion. In our study, rat model of acute lung injury induced by left heart ischemic reperfusion is established, and being treated with breviscapine, to investigate the effect of breviscapine on expression of interleuki 6(IL-6) and inflammatory cell adhesion molecules 1(ICAM-1), to reveal the mechanism of breviscapine protective role of respiratory function against acute lung injury induced by left heart ischemic reperfusion, and to provide theoretical basis for the clinical application of breviscapine to treat acute respiratory dysfunction reduced by left ventricular dysfunction.

2. Materials and Methods

2.1 Laboratory animal

Sixty healthy rats of both sexes, weighting 250~350g, obtained from Experimental Animal Center of Zhengzhou University, were randomly divided into 2 groups: the first group (n=30) is treatment group (TG), these rats in TG were treated by breviscapine.The second group is control group(CG), these ones in CG were not treated by breviscapine.

2.2 Main reagents and instruments

Breviscapine injection (Feixia Pharmacology Company, Harbin, China). BL-420 biological signal collect and analysis system (Chengdu TME

Technology Company, Chengdu, China). IL-6 ELISA Kit for Detecting, IL-6 poly-clonal antibody, ICAM-I poly-clonal antibody, SP kit (Zhongshan Company, Beijing). Myeloperoxidase kit (Jiancheng Bioengineering Institute, Nanjing).

2.3 Methods

Experimental operation according to Reference(JI et al.,2011) : external jugular vein cannula was placed after the rats were anesthetized with 4% chloral hydrate （ 1ml·100g^{-1} ） , and then left anterior descending artery was ligated for 30min, then blood supply was restored, then left heart ischemia reperfusion injury was caused, and the rats of TG were treated with breviscapine injection （ 10mg·kg^{-1} ） through external jugular vein cannula when the myocardial ischemia had been for 10 minutes, however, the rats of CG were treated with normal saline. Rats' diaphragm was exposed before chest experimental operations were carried, and the diaphragm was connected with BL-420 biological signal collect and analysis system through needle electrode for recording respiratory curve. These respiratory curve data was retained to comparative analysis. 5ml Peripheral Blood were sampled through external jugular vein cannula in 30min after ischemia (T1), 30 min after reperfusion (T2) and 60min after reperfusion (T3), then rats were sacrificed, their lungs were took out, bronchial pulmonary alveolus was lavaged immediately, then 5ml bronchoalveolar lavage fluids (BALF) were obtained, the level of IL-6 were measured in BALF by Enzyme Linked Immunosorbent Assay methods. Inferior lobe of right lung tissures were divided into two parts, one part was fixed with 4% formaldehydum polymerisatum, and embedded by paraffin and sliced into 4 μm continuous sections for immunohistochemical staining to test the expression of IL-6 and ICAM-1, the other was homogenized to test the activity of Myeloperoxidase by colorimetric method. All data was retained and analysed using SPSS 11.0 statistical software, statistical methods include rank sum test and Independent t-test (size of test α=0.05).

3. Results

3.1 Comparision of expression IL-6 and ICAM-1 between two groups

The immune response products of IL-6, which are brown-yellow particles, were mainly located in the cytoplasm, these positive expressions of IL-6 protein in TG were obviously lower than those in CG at T1, T2 and T3 by rank sum test; The immune response products of ICAM-1, which are brown-yellow particles, were mainly located in the cytoplasm or membrane, these positive expressions of ICAM-1 protein in TG were obviously lower than those in CG at T1, T2 and T3 by rank sum test. The above results were showed in Tab.1 ~ Tab.2 and Fig.1~ Fig.2 (pictures of expression of IL-6 and ICAM-1 at T2 were selected and showed).

Table 1. Comparison of the expression of IL-6 between two groups(n=10)

Time	n	IL-6				*Z*	*P*
		-	+	++	+++		
T1							
TG	10	6	3	1	0	1.972	0.039
CG	10	4	3	3	0		
T2							
TG	10	5	3	1	1	2.161	0.027
CG	10	2	3	3	1		
T3							
TG	10	4	3	1	2	1.984	0.046
CG	10	2	2	4	2		

Table 2. Comparison of the expression of ICAM-1 between two groups(n=10)

Time	n	ICAM-1				*Z*	*P*
		-	+	++	+++		
T1							
TG	10	5	3	2	1	1.985	0.042
CG	10	3	3	3	1		
T2							
TG	10	4	4	1	1	1.962	0.047
CG	10	2	3	4	1		
T3							
TG	10	4	3	2	1	2.216	0.031
CG	10	2	3	3	2		

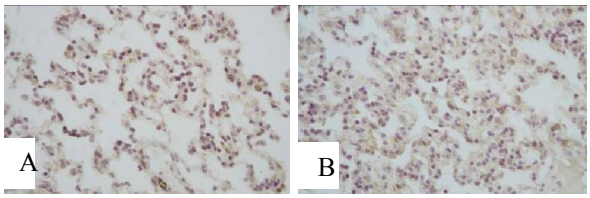

Figure 1. Comparison of expression of ICAM-1 in lung tissues (immunohistochemical chemical staining, ×400). A: expression of ICAM-1 in lung tissue at reperfusion 30min of TG rats.B: expression of ICAM-1 in lung tissue at reperfusion 30min of CG rats.

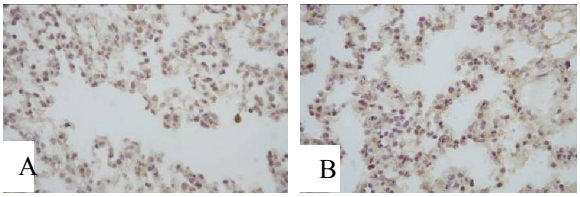

Figure 2. Comparison of expression of IL-6 (immunohistochemical chemical staining, ×400). A: expression of IL-6 in lung tissue at reperfusion 30min of TG rats. B: expression of IL-6 in lung tissue at reperfusion 30min of CG rats.

3.2 Comparison of the activity of pulmonary MPO between two groups The activity of pulmonary MPO in TG was significantly lower than that in CG at T1, T2 and T3 by independent t-test, which was showed in tab.3.

3.2 Comparison of the activity of pulmonary MPO between two groups

The activity of pulmonary MPO in TG was significantly lower than that in CG at T1, T2 and T3 by independent t-test, which was showed in tab.3.

3.3 Comparison of the level of IL-6 in peripheral blood and bronchial alveolarlavage fluid between two groups

Rats was obviously lower than that of CG rats at T1, T2 and T3 by independent t-test, which were showed in Table 4. The level of IL-6 in peripheral blood and BALF of TG was compared.

Table 3. Comparison of the activity of pulmonary MPO between two groups(n=10, $U \cdot g^{-1}$)

Group	MPO		
	T1	T2	T3
TG	54.27±4.12	63.62±3.27	67.64±4.36
CG	68.13±2.79	89.46±7.23	94.75±5.13
t	12.126	16.131	13.271
p	0.000	0.000	0.001

Table 4. Comparison of IL-6 in peripheral blood and bronchial alveolarlavage fluid between two groups (n=10，$pg \cdot ml^{-1}$)

Group	IL-6 in peripheral blood			IL-6 in BALF		
	T1	T2	T3	T1	T2	T3
TG	29.37±2.14	32.42±3.23	43.64± 4.31	11.21±1.04	14.89±2.53	19.25±3.19
CG	42.83±3.03	56.48±4.24	63.62±11.43	17.86±3.12	28.78±3.34	36.21±4.37
t	11.479	12.541	13.649	15.329	14.153	11.306
p	0.001	0.000	0.000	0.000	0.000	0.000

3.4 Comparison of respiration curve between two groups

The amplitude of respiration curve in TG was significantly higher than that in CG at T1,T2 and T3, the duration time of respiration curve in TG was obviously higher than that in CG at T1,T2 and T3, which were showed in tab.5.

Table 5. Comparison of respiration curve between two groups

Group	Amplitude(mv)			duration time (ms)		
	T1	T2	T3	T1	T2	T3
TG	159.93±4.31	147.65±7.12	141.36±3.45	375.00± 42.37	228.56±24.21	225.82±23. 16
CG	109.82±3.01	82.45±4.21	65.67±3.13	165.00±17.33	98.00±14.31	76.54± 9.14
t	15.160	22.735	19.273	18.017	14.524	15.182
p	0.001	0.001	0.000	0.000	0.000	0.000

4. Discussions

ICAM-1 protein is mainly located in the surface of endothelial cells (Yokomura et al., 2001), which was hardly expressed on most tissues of human under physiological condition(Porter et al., 2009). When the expression of ICAM-1 was increased because of pathologic factors on endothelial cells, interactions between ICAM-1 and integrin located on surface of granulocyte cell occurred, which caused aggregation, adhesion and release of leucocytes, and the cytokines were released immediately (Sun et al., 2011). The above process was molecular biological basis of inflammatory reaction(Lawson and Wolf, 2009). In our study, the expression of ICAM-1 in lung cells of TG rats was less than that of CG rats, which suggested that breviscapine might reduce the expression of ICAM-1.

MPO is the unique and stable reductase of neutrophil (Prokopowicz et al., 2012). The activity of MPO per unit weight of lung tissues, which is analyzed by colorimetric method, can reflect the number of neutrophils indirectly (Pawlus et al., 2010). The aggregation and adhesion of neutrophils result in inflammatory effectiveness in pulmonary circulation, which is considered as characteristic markers of early inflammatory injury (Gustapane et al., 2011). IL-6, which is mainly released by mononuclear macrophages, plays an important role in defense function, immune response, acute phase response, hematopoietic response, etc. In this study, the MPO activity of lung tissues of TG rats was significantly lower than that of CG rats, and the levels of IL-6 in peripheral blood and BALF of TG rats were obviously lower than those of CG rats, which suggested that

breviscapine might reduce the aggregation and adhesion of neutrophils, and the expression of pro-inflammatory cytokines IL-6. It is well known that the level of IL-6 in peripheral blood was considered as a marker to reflect tissues damage, so it is thought that breviscapine might reduce lung tissue damage after left heart ischemic reperfusion, and protect respiratory function (Ramirez et al., 2009). The above conclusion was supported by the experimental results that the amplitude and duration time of respiration curve of TG rats were significantly higher than those of CG rats in this study.

In a word, the mechanism of the effcet of breviscapine on acute lung injury induced by left heart ischemic reperfusion is that breviscapine can reduce the expression of ICAM-1, thus reduces the aggregation and adhesion of neutrophils, which reduces the expression of IL-6, and finally reduces the inflammatory injury in lung, and then respiratory function is protected.

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