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Extraction of baicalin from *Scutellaria baicalensis* Georgi and its alleviative effect on acute respiratory distress syndrome in rats

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Abstract

In this study baicalin was extracted from *Scutellaria baicalensis* Georgi and applied to alleviate the acute respiratory distress syndrome (ARDS) in rats. The baicalin with 89.53% purity was successfully extracted from *Scutellaria baicalensis* Georgi. Sixty-five rats were randomly divided into control, model and low-, middle- and high-dose baicalin groups. The oleic acid-induced ARDS model was established in model and baicalin groups. The low-, middle- and high-dose baicalin groups were treated with 100, 200 and 400 mg/kg baicalin, respectively. After 3 h from treatment, compared with model group, in baicalin groups the arterial oxygen partial pressure and oxygenation index were obviously enhanced, the left lung ratio wet weight to dry weight and number of neutrophil, total protein content, tumor necrosis factor α, interleukin 1β and interleukin 6 levels in bronchoalveolar lavage fluid were significantly decreased, and the lung tissue high-mobility group box-1 (HMGB1) and nuclear factor kappa B (NF-κB) p65 protein expression levels were significantly decreased. In conclusion, baicalin may alleviate the ARDS in rats by reducing the inflammatory response via inhibiting the HMGB1/NF-κB signal pathway.

Keywords: baicalin; acute respiratory distress syndrome; inflammatory response; HMGB1; NF-κB.

Practical Application: Baicalin is extracted from *Scutellaria baicalensis* Georgi and applied to alleviate acute respiratory distress syndrome in rats.

1 Introduction

Many natural products have the anti-inflammatory activities (Amaral et al., 2017; Felhi et al., 2017; Lee et al., 2019; Costa et al., 2020). Baicalin ($C_{21}H_{18}O_{11}$) is one of the effective components of *Scutellaria baicalensis* Georgi, a herbal medicine commonly used in China. Modern scientific research has proved that, baicalin has the obvious anti-inflammatory (Shen et al., 2003) activity. It is reported that, baicalin can ameliorate the lipopolysaccharide-induced acute lung injury in mice by suppressing oxidative stress and inflammation via the activation of the nuclear erythroid factor 2-mediated heme oxygenase-1 signaling pathway (Ranieri et al., 1999).

Acute respiratory distress syndrome (ARDS) is a pulmonary inflammatory response caused by excessive release of inflammatory factors. How to inhibit the inflammatory response for alleviating ARDS has become a research hotspot (Reid & Donnelly, 1996). Previous studies have shown that the lung injury is related to the activation of high-mobility group box-1 (HMGB1). HMGB1 can further activate the nuclear factor kappa B (NF-κB) signal pathway, so as to release a large number of inflammatory mediators and cause the lung injury (Lan et al., 2017). This study extracted baicalin from *Scutellaria baicalensis* Georgi. Then, the oleic acid-induced ARDS model of rats was established, and the alleviative effect of baicalin on ARDS and its relation with HMGB1/NF-κB signal pathway were explored. The objective was so as to provide the data support for the clinical practice of baicalin.

2 Materials and methods

2.1 Extraction of baicalin from Scutellaria baicalensis Georgi

Five hundred gram of dry *Scutellaria baicalensis* Georgi root powder was placed in the ultrasonic extractor, and 1000 mL normal-temperature water was added, followed by ultrasonic extraction for 30 min. After filtering, the filtrate was obtained. The concentrated hydrochloric acid was added to the filtrate until pH = 2, followed by standing at 80 °C in water bath for complete precipitation. After filtering, the precipitate was taken, and 5 times (volume to mass) of distilled water was added to the precipitate. Under full stirring, 10 mol/L NaOH aqueous solution was added until pH = 7 to make the precipitate dissolve completely. Equal volume (to mass) of 95% ethanol was added to the precipitate, followed by stirring at 80 °C in water bath for 1 h. After filtering, the filtrate was obtained. The concentrated hydrochloric acid was added to the filtrate until pH = 2, followed by standing at 50 °C in water bath for complete precipitation. After filtering, the precipitate was obtained, and washed with distilled water to neutral pH. After drying at 50 °C, the crude

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baicalin product was obtained. A 20 times (volume to mass) of 95% ethanol was added to the crude baicalin product, followed by refluxing-heating for 30 min. After filtering, the filtrate was obtained. After ethanol reclamation, the solution stood until the yellow crystals were completely precipitated. The crystals were collected. After drying, the refined baicalin product with 89.53% purity was obtained.

2.2 Establishment of model and treatment

Sixty-five healthy adult male Wistar rats (200-250 g) were randomly divided into control group, model group and low-, middle- and high-dose baicalin groups, 13 rats in each group. After anesthetized with ether, the rats in model group were injected with oleic acid (0.1 mL/kg) via femoral vein combined with intraperitoneal injection of dimethyl sulfoxide solution. The rats in the low-, middle- and high-dose baicalin groups were injected with oleic acid (0.1 mL/kg) via femoral vein combined with intraperitoneal injection of 100, 200 and 400 mg/kg baicalin (Xi'an Cuizhijian Biotechnology Co., Ltd., Xi'an, China) dimethyl sulfoxide solution, respectively. The rats in the control group were injected with 0.9% NaCl solution via femoral vein combined with intraperitoneal injection of dimethyl sulfoxide solution.

2.3 Blood gas analysis

After 3 h from the treatment, the rats in each group were anesthetized by intraperitoneal injection of 20% urethane. A 0.5 mL of blood was taken from the left carotid artery. After shaking evenly, the blood gas analysis was performed using the blood gas analyzer. The arterial oxygen partial pressure (PaO_2) and oxygenation index (OI) were recorded.

2.4 Detection of bronchoalveolar lavage fluid indexes

The rats were sacrificed, and the thoracic cavity was opened immediately. The right main bronchus was separated and ligated. The left lung was lavaged slowly with 2 mL of cold phosphate buffer saline for three times. The bronchoalveolar lavage fluid (BALF) was collected. After centrifuging at 3000 rpm for 15 min, the supernatant was obtained. The total number of cells in BALF was determined using the cell counter. Then, the BALF were smeared on the glass piece, and the residual liquid was shaken off use the slitter. The number of neutrophils was determined under high-power microscope. In addition, the total protein content in BALF was detected by bicinchonininc acid method. The levels of tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6) were detected by enzyme-linked immunosorbent assay. The detection procedures were according to the instructions of kits.

2.5 Determination lung wet-dry weight ratio

After the BALF was taken, the pulmonary artery was ligated. The left lung tissue was taken, and the redundant tissue was trimmed off. The water on the surface of lung tissue was sucked dry. The left lung wet weight was measured using the electronic analytical balance. Then, the lung tissue was placed in a 60 °C incubator for 48 h. After the lung tissue was completely dried, it was weighed to obtain the dry weight. The left lung ratio wet weight to dry weight (W/D) was calculated.

2.6 Determination of HMGB1 and NF-κB p65 protein expressions in lung tissue

The right lung tissue of rats was taken. The cytoplasmic proteins were extracted using the tissue protein extraction kits. The protein concentration was measured by bicinchonininc acid method. The lung tissue HMGB1 and NF-κB p65 protein expressions were determined using the western blot assays. The detection procedures were according to the instructions of kits. The relative expression of target protein was determined by the ratio its strip integrated optical density to internal reference integrated optical density.

2.7 Statistical analysis

All data were expressed as mean ± standard deviation. Statistical analysis was carried out using GraphPad Prism 4.0 software. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $P < 0.05$ were regarded as statistically significant.

3 Results

3.1 Effect of baicalin on PaO_2 and OI of rats with ARDS

After 3 h from the treatment, the PaO_2 and OI of rats in model and low-, middle- and high-dose baicalin groups were obviously lower than those in control group, respectively ($P < 0.05$). When comparing with model group, the PaO_2 in middle- and high-dose baicalin groups and OI in high-dose baicalin group were obviously enhanced, respectively ($P < 0.05$) (Table 1).

3.2 Effect of baicalin on left lung W/D of rats with ARDS

As shown in Figure 1, after 3 h from the treatment, the left lung W/D of rats in control, model and low-, middle- and high-dose baicalin groups were 3.42 ± 0.42, 5.21 ± 0.45, 5.14 ± 0.30, 4.90 ± 0.28 and 4.56 ± 0.31, respectively. When comparing with control group, the left lung W/D in model and low-, middle- and high-dose baicalin groups was significantly increased, respectively ($P < 0.05$). When comparing with model group, the left lung W/D in middle- and high-dose baicalin groups was significantly decreased, respectively ($P < 0.05$).

Table 1. Comparison of PaO_2 and OI in five groups.

Group	n	PaO_2 (mmHg)	OI
Control	13	97.67 ± 12.26	477.50 ± 40.21
Model	13	$65.33 \pm 12.72^{\#}$	$312.32 \pm 33.44^{\#}$
Low-dose baicalin	13	$69.81 \pm 10.14^{\#}$	$346.16 \pm 42.25^{\#}$
Middle-dose baicalin	13	$75.20 \pm 8.09^{\#\$}$	$357.42 \pm 46.50^{\#}$
High-dose baicalin	13	$82.14 \pm 11.92^{\#\$\%}$	$395.88 \pm 32.18^{\#\$\%\&}$

Data were expressed as mean ± standard deviation. $^{\#}P < 0.05$ *vs.* control group; $^{\$}P < 0.05$ *vs.* model group; $^{\%}P < 0.05$ *vs.* low-dose baicalin group; $^{\&}P < 0.05$ *vs.* middle-dose baicalin group. PaO_2 = arterial oxygen partial pressure; OI = oxygenation index.

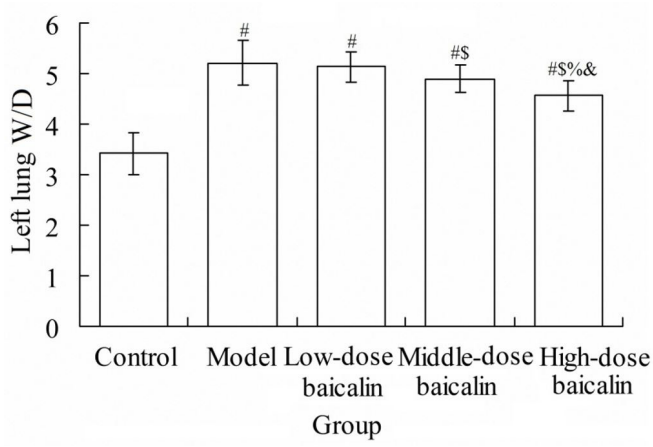

Figure 1. Comparison of left lung W/D in five groups (n =13). Data were expressed as mean ± standard deviation. #P < 0.05 *vs.* control group; $P < 0.05 *vs.* model group; %P < 0.05 *vs.* low-dose baicalin group; &P < 0.05 *vs.* middle-dose baicalin group. W/D, ratio wet weight to dry weight.

3.3 Effect of baicalin on total number of cells, number of neutrophils and total protein content in BALF of rats with ARDS

After 3 h from the treatment, the total number of cells, number of neutrophils and total protein content in model and low-, middle- and high-dose baicalin groups were obviously higher than those in control group, respectively (P < 0.05). When comparing with model group, the total number of cells and total protein content in low-, middle- and high-dose baicalin groups and number of neutrophils in middle- and high-dose baicalin groups were obviously decreased, respectively (P < 0.05) (Table 2).

3.4 Effect of baicalin on TNF-α, IL-1β and IL-6 levels in BALF of rats with ARDS

Table 3 showed that, after 3 h from the treatment, when comparing with control group, the TNF-α, IL-1β and IL-6 levels in BALF in model and low-, middle- and high-dose baicalin groups were significantly increased, respectively (P < 0.05). When comparing with model group, the TNF-α level in middle- and high-dose baicalin groups and IL-1β and IL-6 levels in low-, middle- and high-dose baicalin groups were significantly decreased, respectively (P < 0.05).

3.5 Effect of baicalin on lung tissue HMGB1 and NF-κB p65 protein expressions in rats with ARDS

The western blot assays showed that, the lung tissue HMGB1 and NF-κB p65 protein expression levels in model and low-, middle- and high-dose baicalin groups were obviously higher than those in control group, respectively (P < 0.05). When comparing with model group, the HMGB1 protein expression level in middle- and high-dose baicalin groups and NF-κB p65 protein expression level in low-, middle- and high-dose baicalin groups were obviously decreased, respectively (P < 0.05) (Table 4).

Table 2. Comparison of total number of cells, number of neutrophils and total protein content in BALF in five groups.

Group	n	Total number of cells (×10^5/mL)	Number of neutrophils (×10^5/mL)	Total protein content (mg/mL)
Control	13	0.33 ± 0.05	0.05 ± 0.01	0.20 ± 0.01
Model	13	4.19 ± 0.56$^{\#}$	2.20 ± 0.34$^{\#}$	4.83 ± 0.56$^{\#}$
Low-dose baicalin	13	3.78 ± 0.72$^{\#\$}$	2.01 ± 0.45$^{\#}$	3.29 ± 0.32$^{\#\$}$
Middle-dose baicalin	13	3.15 ± 0.50$^{\#\$\%}$	1.83 ± 0.26$^{\#\$}$	2.89 ± 0.40$^{\#\$\%}$
High-dose baicalin	13	2.52 ± 0.43$^{\#\$\%\&}$	1.52 ± 0.28$^{\#\$\%\&}$	1.92 ± 0.17$^{\#\$\%\&}$

Data were expressed as mean ± standard deviation. #P < 0.05 *vs.* control group; $P < 0.05 *vs.* model group; %P < 0.05 *vs.* low-dose baicalin group; &P < 0.05 *vs.* middle-dose baicalin group. BALF = bronchoalveolar lavage fluid.

Table 3. Comparison of TNF-α, IL-1β and IL-6 levels in BALF in five groups.

Group	n	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)
Control	13	50.37 ± 10.56	38.90 ± 45.38	78.77 ± 17.88
Model	13	488.90 ± 67.22$^{\#}$	163.62 ± 27.21$^{\#}$	256.25 ± 47.21$^{\#}$
Low-dose baicalin	13	452.32 ± 82.75$^{\#}$	125.35 ± 19.94$^{\#\$}$	187.90 ± 31.35$^{\#\$}$
Middle-dose baicalin	13	305.40 ± 45.27$^{\#\$\%}$	93.80 ± 14.57$^{\#\$\%}$	159.04 ± 27.42$^{\#\$\%}$
High-dose baicalin	13	216.77 ± 29.60$^{\#\$\%\&}$	62.41 ± 16.52$^{\#\$\%\&}$	115.33 ± 15.93$^{\#\$\%\&}$

Data were expressed as mean ± standard deviation. #P < 0.05 *vs.* control group; $P < 0.05 *vs.* model group; %P < 0.05 *vs.* low-dose baicalin group; &P < 0.05 *vs.* middle-dose baicalin group. TNF-α = tumor necrosis factor α; IL-1β = interleukin 1β; IL-6 = interleukin 6; BALF = bronchoalveolar lavage fluid.

Table 4. Comparison of lung tissue HMGB1 and NF-κB p65 protein expression levels in five groups.

Group	n	HMGB1/GAPDH	NF-κB p65/GAPDH
Control	13	0.12 ± 0.01	0.24 ± 0.04
Model	13	0.45 ± 0.05$^{\#}$	0.88 ± 0.12$^{\#}$
Low-dose baicalin	13	0.43 ± 0.06$^{\#}$	0.76 ± 0.14$^{\#\$}$
Middle-dose baicalin	13	0.38 ± 0.04$^{\#\$}$	0.55 ± 0.11$^{\#\$\%}$
High-dose baicalin	13	0.29 ± 0.02$^{\#\$\%\&}$	0.42 ± 0.06$^{\#\$\%\&}$

Data were expressed as mean ± standard deviation. #P < 0.05 *vs.* control group; $P < 0.05 *vs.* model group; %P < 0.05 *vs.* low-dose baicalin group; &P < 0.05 *vs.* middle-dose baicalin group. HMGB1 = high-mobility group box-1; NF-κB = nuclear factor kappa B; GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

4 Discussion

ARDS, as a common clinical respiratory critical disease, mainly refers to the lung parenchyma injury caused by the joint action of inflammatory mediators and effector cells (Sulkowski et al., 1997). At present, there is no especially effective treatment for ARDS in clinical practice, so it is imperative to seek better prevention and treatment measures. PaO_2 and OI are the important indexes reflecting the lung function (Turner et al., 1993). In this study, the oleic acid-induced ARDS model of rats was established, and the

protective effect of baicalin on ARDS was investigated. Results showed that, after 3 h from the treatment, when comparing with model group, the PaO_2 and OI in high-dose baicalin group were obviously enhanced. This suggests that, the baicalin can improve the lung function of rats with ARDS.

ARDS is caused by the imbalance of inflammatory factors. It is reported that, in ARDS, a large number of inflammatory cells, mainly neutrophils, gather in the lung tissue. These inflammatory cells produce cytokines and inflammatory mediators. The inflammatory signals are amplified step by step, resulting in the microcirculation disturbance, increased permeability of alveolar epithelial cells, and damage of pulmonary capillary endothelium (Chen et al., 1993). This eventually leads to the alveolar hemorrhage and pulmonary edema, resulting in the increased lung W/D and increased protein content in the BALF (Tsao et al., 1999; Elfeky et al., 2018). TNF-α, IL-1β and IL-6, as important factors involved in the inflammatory cascade, play an important role in inducing the cell infiltration and accelerating the process of respiratory diseases (Fu et al., 2016). Results of this study showed that, after 3 h from the treatment, compared with model group, in middle- and high-dose baicalin groups the left lung W/D was significantly decreased, the number of neutrophils and total protein content in BALF were obviously decreased, and the TNF-α, IL-1β and IL-6 levels in BALF were significantly decreased. This indicates that, baicalin can reduce the inflammatory response, thus alleviating the ARDS in rats.

HMGB family includes HMGB1, HMGB2 and HMGB3. The understanding of HMGB1 is the most comprehensive. HMGB1 can only be detected 24 h after the inflammation occurrence, so it is called the late inflammatory mediator (Li et al., 2008). In ARDS, HMGB1 can cause the protein exudation from lung tissue, which is the main reason for the loss of alveolar function (He et al., 2016). NF-κB widely exists in all kinds of cells. In the resting state, NF-κB combines to its inhibitor to form an inactive trimer. Under the stimulation of inflammation, NF-κB is activated and its expression will be significantly increased, so it is a marker factor in the inflammatory response (Li et al., 2008). HMGB1 can bind its ligand RAGE to causes the NF-κB nuclear translocation, and induces the increased expression and release of other inflammatory factors, resulting in the deterioration of inflammatory response (He et al., 2016). In our study, after 3 h from the treatment, when comparing with model group, the lung tissue HMGB1 and NF-κB p65 protein expression levels in middle- and high-dose baicalin groups were obviously decreased. Therefore, it is suggested that the protective effect of baicalin on ARDS in rats may be related to its inhibition of HMGB1/NF-κB signal pathway.

5 Conclusion

Baicalin with 89.53% purity was successfully extracted from *Scutellaria baicalensis* Georgi. It can alleviate the ARDS in rats by reducing the inflammatory response via inhibiting the HMGB1/NF-κB signal pathway. This study can provide a reference for the preparation of baicalin and its clinical application. However, due to the complexity of the pathogenesis of ARDS, the specific role and regulatory mechanism of baicalin in the occurrence and development of ARDS need to be further explored.

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