



Research Article

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## The effect of rhein and gallic acid on the content of IL-10, IL-1 $\beta$ and TNF- $\alpha$ in serum of rats with endotoxemia

Hui Chen, Chao-Ying Ma\*, Xi Chen, Yun Geng, Chao Yang, He-Zhong Jiang and Xing Wang

School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, China

### ABSTRACT

To observe the effect of Rhein and Gallic Acid on inflammatory cytokines in the rats with endotoxemia and discuss the material base and mechanism of Dachengqi decoction for curing endotoxemia. 40 SD rats were divided into 5 groups randomly: control group, model group, and 3 experimental groups. The experimental groups were respectively intragastric administrated 2 days with Dachengqi decoction, Rhein, Gallic before modeling. While the model group and the control group were administrated 0.5% sterile solution of Sodium salt. At the third day, the rats except control group were made models by the method of abdominal cavity infection via intraperitoneal injection of rat feces suspension, in order to induce endotoxemia. Rats were orally administrated at 1 hour after modeling and 1 hour before taking their blood. 8 hours after modeling, their blood were taken for use. At last, the content of IL-10, IL-1 $\beta$  and TNF- $\alpha$  in the serum were detected. After being made the endotoxemia model, compared with the control group, the contents of IL-10, IL-1 $\beta$  and TNF- $\alpha$  in rats' serum were risen evidently in the model group and each curing group ( $P < 0.01$ ). Compared with the model group, the content of IL-10, IL-1 $\beta$  and TNF- $\alpha$  were decreased obviously in each curing group simultaneously ( $P < 0.01$ ). It shows that Rhein and Gallic Acid are the material base as they assumed a certain role in the treatment of Dachengqi decoction on endotoxemia; the mechanism of curing the endotoxemia is related with the antagonism to the inflammatory cytokines IL-10, IL-1 $\beta$  and TNF- $\alpha$  in serum.

**Keywords:** endotoxemia-Dachengqi decoction-Rhein-Gallic Acid-inflammatory cytokines

### INTRODUCTION

There are two ways to cause endotoxemia. The one is bacteria in the blood or lesions releasing large amounts of endotoxin into blood. The other is transfusing a large number of liquid contaminated by endotoxin. Endotoxemia can trigger a series of pathophysiological changes, including sepsis, shock, disseminated intravascular coagulation (DIC), multiple organ dysfunction syndrome (MODS) and so on. Therefore, antagonizing endotoxin and preventing or diminishing the injuries mediated by it has important clinical significance (Wang et al., 2004). In recent years, many studies had shown that inflammatory cytokines, such as IL-1, IL-6, IL-10, TNF- $\alpha$ , etc., played an important role in the pathogenesis of endotoxemia. Hence, regulating the release of these inflammatory cytokines has a great research value and an important significance in controlling the development of infectious diseases.

Dachengqi decoction, which is an ancient traditional Chinese medicine recipe, is used for the treatment of endotoxemia caused by infectious disease in clinic. And it has a significant effect. But which chemical composition in each medicinal material of the prescription has anti-endotoxin effect? What are the therapeutic bases of Dachengqi decoction on treating endotoxemia? It remains to be further studied. From this perspective, this paper takes rhein and gallic acid of rhubarb in Dachengqi decoction as the research objects. We observed the effect of both components of rhein and gallic acid on inflammatory cytokines for the first time, in order to explore the therapeutic bases of rhubarb in Dachengqi decoction on treating endotoxemia.

## EXPERIMENTAL SECTION

### *Experimental animals*

40 SD rats (200±20g) of conventional level, male and female in half, were obtained from Laboratory Animal Research Center of Chengdu University of TCM, license number: SCXK (Sichuan) 2008-11.

### *Herbs and medicaments*

Rhubarb slices, dry roots of *Rheum palmatum L.* which were stir-fried with wine, origin of Qinghai; Magnolia Pieces, gingered dry cortex or husk of root and branch of *Magnolia officinalis Rehd et Wils*, origin of Sichuan; Citrus aurantium Pieces, dry young fruits of *Citrus aurantium L.*, origin of Sichuan; Glauber's salt (mirabilite), Glauber's salt contained crystal water, chemical formula  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ , origin of Sichuan. These four crude drugs were purchased from Neautus Chinese Herbal Medicine Co., Ltd., Chengdu, Sichuan, and were identified by Associate Professor Song Liangke from School of Life Science and Engineering, Southwest Jiaotong University. Dachengqi decoction was decocted according to the method recorded in 'Shanghan Lun', which was composed by Zhang Zhongjing. And the decoction was concentrated to contain 0.5g drugs each milliliter.

Rhein and gallic acid were purchased from Sciphar Biotechnology Co., Ltd. (0757-200206), Xi'an, Shanxi. Before using, rhein and gallic acid were prepared to suspension with 0.5% Sodium salt of Caboxy Methyl Cellulose solution.

### *Reagents and instruments*

Rat IL-10 ELISA Kit, rat IL-1 $\beta$  ELISA kit, rat TNF- $\alpha$  ELISA kit, produced by NeoBioscience Technology Co., Ltd. Enzyme-labeled instrument (ELX800UV), produced by BIO-TEK INSTRUMENTS.INS.

### *Animal Grouping and administration*

40 SD rats were randomly divided into 5 groups (n=8, male and female in half): control group, model group, and 3 experimental groups (Dachengqi decoction group, Rhein group and Gallic Acid group). And they were adaptively fed for 1 week. Rats in control group and model group were orally administrated with 0.5% sterile solution of Sodium salt of Caboxy Methyl Cellulose (CMCNa), fed by 1 ml/100 g BW every time. Dachengqi decoction group were orally administrated with Dachengqi decoction solution (1 ml/100 g BW every time). Rhein group were orally administrated with the CMCNa solution of rhein (1.0 mg/100 g BW every time). Gallic acid group were orally administrated with the CMCNa solution of gallic acid (0.44 mg/100 g BW every time). Each group were administrated 2 times/d, totally 3d. At the third day, each group was modeled. And they were administrated about one hour late after of modeling one hour and before taking blood sample.

### *Method of model*

In this study, we use the method of abdominal cavity infection via intraperitoneal injection of rat feces suspension causing endotoxemia (Huang et al., 2010). In order to establish the rat model of intestinal endotoxemia, the fresh feces of the rats were made into 10% homogenate with sterile physiological saline, Then the homogenate were centrifugated for 3min (1000 r/min). and the supernatant were reserved for use. After weighing and marking the rats, all rats of every group except control group were injected from their left lower abdomen into their abdominal cavity with the supernatant (1.0 mL/100 g BW). Control group were injected with sterile physiological saline (1.0 mL/100 g BW).

### *Taking blood serum sample*

8h after modeling, all rats were anaesthetized with 6% chloral hydrate solution and blood samples were collected from their hearts into 2 EP tubes with 1ml blood each. After curdled for about 2h at room temperature, the blood samples were centrifugated for 10 min (3000 r/min). The blood serum was collected and kept at -20 $^{\circ}\text{C}$  after packing.

### *Targets and methods of detection*

ELISA method (Enzyme-Linked ImmunoSorbant Assay) was used to measure the concentrations of IL-10, IL-1 $\beta$  and TNF- $\alpha$  in blood serum. The rats were operated according to the instructions of the rat ELISA kit. The density values were measured at OD 450nm by enzyme-labeled instrument.

### *Statistical analysis*

The experiments were completely randomly designed. All the data were analysed by SPSS 13.0 software analysis system and were expressed as mean  $\pm$  SD (standard deviation) ( $X \pm S$ ).

## RESULTS

*Effect of each group on the content of IL-10 in serum of rats with endotoxemia*

The content of IL-10 in serum of rats in model group, experiment group and control group were significantly different after establishing the model of intestinal endotoxemia. And the IL-10 content of model group and experiment group were increased significantly ( $P<0.01$ ). Compared with model group, the content of IL-10 in Dachengqi decoction group, rhein group and gallic acid group were reduced significantly ( $P<0.01$ ) (Table 1).

**Table1 Results of content of IL-10( $\bar{X}\pm S$ ) (n=8, pg/mL)**

Groups	$\bar{X}\pm S$
Control group	31.9048±11.9882
Model group	217.0731±47.8935 <sup>Δ</sup>
Dachengqi decoction group	82.2728±24.8497 <sup>Δ▲</sup>
Rhein group	113.9349±28.2703 <sup>Δ▲</sup>
Gallic Acid group	120.3302±30.5169 <sup>Δ▲</sup>

Compared with the control group:  $\Delta P<0.01$ . Compared with model group:  $\blacktriangle P<0.01$ .

*Effect of each group on the content of IL-1 $\beta$  in serum of rats with endotoxemia*

Table 2 showed that the content of IL-1 $\beta$  in serum of rats in model group and experiment group were increased significantly ( $P<0.01$ ) after establishing the model of intestinal endotoxemia, compared with the control group.

The same as IL-10 situation, the content of IL-1 $\beta$  in each experiment group were also increased significantly ( $P<0.01$ ). Compared with model group, the content of IL-1 $\beta$  in Dachengqi decoction group, rhein group and gallic acid group were reduced significantly ( $P<0.01$ ).

**Table2 Results of content of IL-1 $\beta$ ( $\bar{X}\pm S$ )(n=8, pg/mL)**

Groups	$\bar{X}\pm S$
Control group	12.5710±5.1403
Model group	196.920±33.170 <sup>Δ</sup>
Dachengqi decoction group	59.5369±25.0691 <sup>Δ▲</sup>
Rhein group	91.9086±22.2475 <sup>Δ▲</sup>
Gallic Acid group	95.1548±24.3087 <sup>Δ▲</sup>

Compared with the control group:  $\Delta P<0.01$ . Compared with model group:  $\blacktriangle P<0.01$ .

*Effect of each group on the content of TNF- $\alpha$  in serum of rats with endotoxemia*

Table 3 showed that the content of TNF- $\alpha$  in serum of rats in model group and experiment group were increased significantly ( $P<0.01$ ) after establishing the model of intestinal endotoxemia, compared with the control group.

The same as IL-10 situation, the content of TNF- $\alpha$  in each experiment group were also increased significantly ( $P<0.01$ ). Compared with model group, the content of TNF- $\alpha$  in Dachengqi decoction group, rhein group and gallic acid group were reduced significantly ( $P<0.01$ ).

**Table3 Results of content of TNF- $\alpha$ ( $\bar{X}\pm S$ )(n=8, pg/mL)**

Groups	$\bar{X}\pm S$
Control group	17.1446±5.5441
Model group	160.1340±36.3457 <sup>Δ</sup>
Dachengqi decoction group	59.5273±15.2208 <sup>Δ▲</sup>
Rhein group	94.1760±20.2886 <sup>Δ▲</sup>
Gallic Acid group	102.0978±23.4889 <sup>Δ▲</sup>

Compared with the control group:  $\Delta P<0.01$ . Compared with model group:  $\blacktriangle P<0.01$ .

## DISCUSSION

IL-10 is an immunosuppressive factor, which is mainly derived from the CD4 T regulatory lymphocyte, especially Th2 cells. IL-10 may act on a variety of cells. Its role is inhibiting the production of cytokines and causing cellular immune suppression. Current results showed that IL-10 was an important functional antagonist of endotoxin and inflammatory cytokines, and it was induced out earlier in inflammation (Ma et al., 2003). By taking animal experiments, Kawai et al. (Kawai et al., 1995) found that IL-10 played the role of its anti-inflammatory by inhibiting the formation from macrophage to TNF- $\alpha$ , and consequently reduced the pathological damage caused by TNF- $\alpha$ . Lehmann et al. (Lehmann et al., 1995) found that the levels of IL-10 in serum were positively correlated with the concentration of proinflammatory cytokine.

TNF is an endogenous cytokines produced by the body with endotoxemia. Endotoxin can stimulates macrophages to produce TNF, and TNF can mediate the effect of endotoxin further. TNF- $\alpha$  increases, while plasma endotoxin increases. There is a positive correlation between them(Zheng *et al.*, 1999). Studies showed that TNF was one of the important mediator cytokine of endotoxin-induced damage in the body. IL-1 is a important media of acute stress response and damage, and it is a strong pro-inflammatory factors, which can activate vascular endothelial cells and neutrophils, and enhance the expression of adhesion molecules, and promote the release of other cytokines, such as IL-6, IL-8, granulocyte-macrophage colony-stimulating factor. It cause a variety of systemic inflammatory response together with TNF- $\alpha$ . Both of them play important role in the pathological process while the genesis and development of endotoxemia(Qin *et al.*, 2009).

The results showed that: the level of IL-10, IL-1 $\beta$  and TNF- $\alpha$  in serum of rats in model group and every treatment group were increased significantly, and had significant differences from control group. The level of model group was the highest. Compared with that, the level of Dachengqi decoction group, rhein group and gallic acid group fell down significantly ( $P < 0.01$ ). However, the contents of these cytokines in rhein group and gallic acid group had faintisher decrease than Dachengqi decoction group. It showed that Dachengqi decoction, could all adjust the contents of IL-10, IL-1 $\beta$  and TNF- $\alpha$ in serum in rats. And both of rhein and gallic acid were the therapeutic bases of Dachengqi decoction on treating endotoxemia.

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