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# Effects of traditional Chinese medicine “Yimu Shenghuatang” on cytochrome P450 in cow inflammatory endometrial cells

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**Abstract** To investigate the metabolism mechanism of traditional Chinese medicine “Yimu Shenghuatang” on dairy cow endometrial cells, the primary cultured endometrial cells in dairy cows were isolated by a collagenase digestion method, and an inflammatory model of the endometrial cells by lipopolysaccharide (LPS) was made. The cells were treated with gradient concentrations of LPS, the cultured supernatants were collected in different periods, and the contents of TNF- $\alpha$ , IL-1 $\beta$  were determined by Enzyme-linked immunosorbent assay (ELISA). The results showed that 100 ng·mL<sup>-1</sup> LPS was the optimal concentration to induce the inflammation of cultured endometrial cells. The inflammatory endometrial cells were treated with traditional Chinese medicine “Yimu Shenghuatang” and its main components during the period of 24–72 h, and the expression of cytochrome P450 in cow endometrial cells was determined by Western blot. Our result lays a foundation for treating cow endometritis by using traditional Chinese medicines.

**Keywords** dairy cow, inflammatory endometrial cell, CYP450, traditional Chinese medicine “Yimu Shenghuatang”

## 1 Introduction

Endometritis is a common postpartum disease in dairy cows (LeBlanc et al., 2002), which can cause a serious

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economic loss in animal husbandry. As, in veterinary clinics, antibiotics and hormones are popularly and even more extensively used for treatment of cow endometritis, the drug resistance to bacteria appeared. Besides, the drug residues are retained in meat and milk, which cause hazards to human health. Therefore, to find new and effective drugs to replace antibiotics or hormones is necessary. The published data of drug-treated cow endometritis (Jia and Qi, 2005; Hu et al., 2007; Li et al., 2010) mainly focused on observing drug effects, neglecting the active mechanism of the drugs.

In our study, the cow endometrial cells were isolated and cultured. LPS was used for simulating the cow endometrial cell inflammatory model. The inflammatory endometrial cells were treated with traditional Chinese medicine “Yimu Shenghuatang”, and the expression of cytochrome P450 in cow endometrial cells was determined by Western blot. The metabolic mechanism of this traditional Chinese herb prescription at the cellular and molecular level was explored, which provided a theoretical basis for the cow endometritis clinical treatment.

## 2 Materials and methods

### 2.1 Reagents

LPS (Sigma), DMEM/F-12 and Collagenase I (Invitrogen), FBS (Hangzhou Sijiqing Company), DMSO (BIO-SHOP), Bovine IL-1 $\beta$  and TNF- $\alpha$  Quantitative ELISA kit (Shanghai Lanji Biologic Company), and CYP450 antibody (ADL) were used in our experiment.

### 2.2 Methods

#### 2.2.1 Isolation and culture of endometrial cells in cows

Isolation and culture of endometrial cells in cows were

conducted by rinsing the uterus into D-Hanks, scraping endometrium, and adding  $1 \text{ g} \cdot \text{L}^{-1}$  collagenase I to digest for 8–10 min at  $37^\circ\text{C}$  water bath. Thereafter, the 100–150 g filtrate was centrifuged for 5 min for three times and suspended in DMEM/F-12 media containing 15% fetal bovine serum, and the viability was determined by trypan blue exclusion. Endometrial cells were plated onto a collagen-coated culture plate at a density of  $6 \times 10^5 \cdot \text{well}^{-1}$  and cultured for 18 to 24 h at a  $\text{CO}_2$  incubator.

### 2.2.2 Inflammation model establishment

Endometrial cells were treated with gradient concentration LPS incubated at the  $\text{CO}_2$  incubator for 48 h, and for extra for 6 h after adding  $20 \mu\text{L}$  MTT of  $5 \text{ mg} \cdot \text{mL}^{-1}$ , and shook for 5 min after adding  $100 \mu\text{L}$  DMSO, with the absorbance measured at 570 nm to determine the best LPS concentration, which was used to treat the endometrial cells for 24–72 h. Finally, the contents of IL-1 $\beta$  and TNF- $\alpha$  were detected by double-antibody sandwich ABC-ELISA method.

### 2.2.3 Treatment of the endometrial cells by “Yimu Shenghuatang”

The endometrial cells were treated using the compound preparation of traditional Chinese medicine “Yimu Shenghuatang” for 24–72 h. The cell proteins were lysed, and the protein concentration was detected by Bradford Protein Assay Kit.

### 2.2.4 Western blot analysis

The cell lysate was subjected to centrifugation at  $12000 \times g$  for 15 min at  $4^\circ\text{C}$  to remove insoluble precipitates, resolved using 12% SDS-PAGE in a mini-gel apparatus and transferred electrophoretically to nitrocellulose membranes. After non-specific binding sites were blocked with 5% non-fat milk, the blots were incubated with CYP450 antibody that was subsequently localized with IgG conjugated with AP. The expression of CYP450 was detected by NBT/BCIP.

## 3 Results

### 3.1 Screen assay of LPS concentration

It shows that the nearest  $\text{IR} \leq 10$  is the  $100 \text{ ng} \cdot \text{mL}^{-1}$  group, so the optimal LPS concentration is  $100 \text{ ng} \cdot \text{mL}^{-1}$ .

### 3.2 Inflammation model establishment

During the incubation period, the secretion of IL-1 $\beta$  and TNF- $\alpha$  in the control group was very low. However, in the

**Table 1** Inhibitory action of LPS on cow endometrial cell growth ( $n = 6$ )

concentration/( $\text{ng} \cdot \text{mL}^{-1}$ )	absorbance value	inhibition rate/%
control	$0.1704 \pm 0.0067$	0
1	$0.1706 \pm 0.0035$	0
10	$0.1658 \pm 0.0051$	2.6
50	$0.1622 \pm 0.0024$	4.8
100	$0.1526 \pm 0.0059$	10.4
200	$0.1356 \pm 0.0062$	20.4
500	$0.1282 \pm 0.0056$	24.8

model groups, along with the time extension of LPS induction, the two cytokines secretion increased. The contents of IL-1 $\beta$  and TNF- $\alpha$  in the 48-h and 72-h model groups were significantly higher than those in the control group ( $P < 0.01$ ); meanwhile, the contents of IL-1 $\beta$  and TNF- $\alpha$  in the 72-h group were even significantly higher than those in the 48-h group ( $P < 0.01$ ) (Table 2).

**Table 2** Effects of LPS on the contents of TNF- $\alpha$  and IL-1 $\beta$  in endometrial cells ( $n = 6$ )

time/h	TNF- $\alpha$		IL-1 $\beta$	
	control group	LPS group	control group	LPS group
24	$21.96 \pm 4.70$	$22.63 \pm 2.29$	$21.08 \pm 6.52$	$35.78 \pm 7.18$
48	$22.16 \pm 4.43$	$41.44 \pm 8.74$	$21.54 \pm 5.21$	$90.17 \pm 8.38^\blacktriangle$
72	$20.20 \pm 3.89$	$63.18 \pm 6.08^\#$	$23.16 \pm 8.56$	$136.77 \pm 9.13^\blacktriangle$

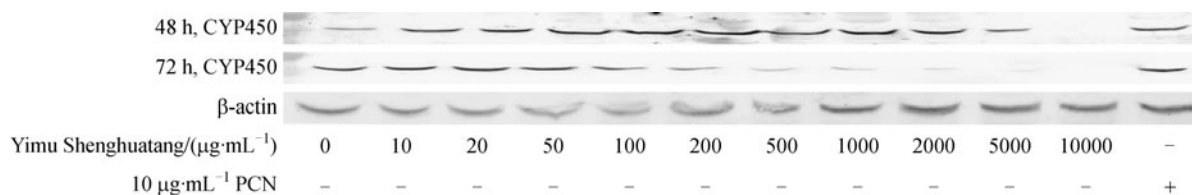
Note: #) means significant difference at  $P < 0.01$  compared with control group in the same stage;  $\blacktriangle$ ) means significant difference at  $P < 0.01$  compared with LPS groups.

### 3.3 CYP450 expression in dairy cow endometrial cells induced by “Yimu Shenghuatang”

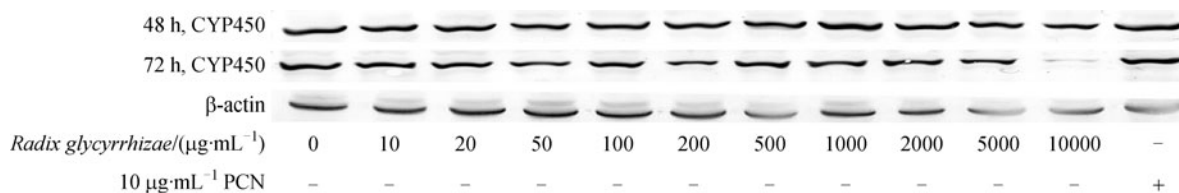
The inflammatory cells were treated with the herbal medicine prescription “Yimu Shenghuatang” at gradient concentration, and the expression of cytochrome P450 in cow endometrial cells increased gradually for the 48-h treatment. While at  $5000 \mu\text{g} \cdot \text{mL}^{-1}$  concentration, the expression of CYP450 decreased. When at  $10000 \mu\text{g} \cdot \text{mL}^{-1}$  concentration, the expression of CYP450 disappeared. As for the 72-h treatment, the expression of CYP450 increased at first but decreased later. The best dose was  $2000 \mu\text{g} \cdot \text{mL}^{-1}$  for the 48-h treatment (Fig. 1).

### 3.4 CYP450 expression in dairy cow endometrial cells induced by *Radix Glycyrrhizae*

When the endometrial cells were treated with *Radix glycyrrhizae* for 48 h and 72 h, the expression of CYP450 had no significant change (Fig.2). However, when treated with  $10000 \mu\text{g} \cdot \text{mL}^{-1}$  *Radix glycyrrhizae* for 72 h, the expression of CYP450 decreased.



**Fig. 1** CYP450 expression in dairy cow endometrial cells induced by “Yimu Shenghuatang”



**Fig. 2** CYP450 expression in dairy cow endometrial cells induced by *Radix glycyrrhizae*

### 3.5 CYP450 expression in dairy cow endometrial cells induced by *Angelica sinensis*

When the inflammatory cells were treated with *Angelica sinensis* for 48 h and 72 h, the CYP450 expression of endometrial cells decreased as the concentration of *Angelica sinensis* increased and the action time prolonged (Fig. 3).

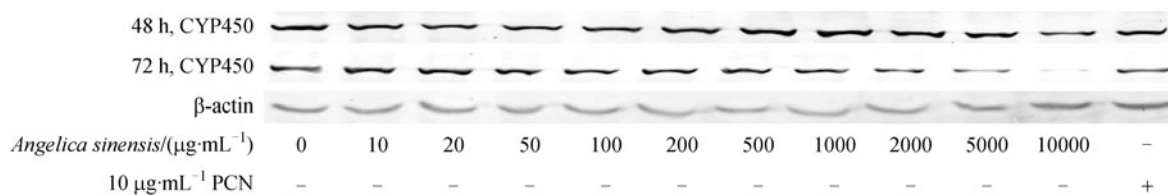
### 3.6 CYP450 expression in dairy cow endometrial cells induced by *Motherwort herb*

The inflammatory cells were treated with *Motherwort herb* at various concentrations when the expression of CYP450 increased gradually as the concentration of *Motherwort herb* increased. At the same gradient

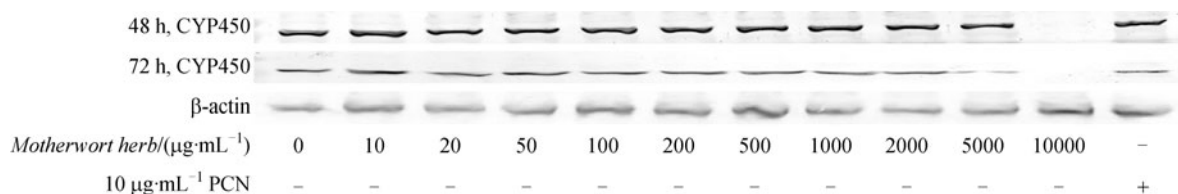
concentration, the longer the action time is, the more obvious the expression of CYP450 induced by *Motherwort herb*. The expression of CYP450 disappeared when treated with 10000  $\mu\text{g}\cdot\text{mL}^{-1}$  *Motherwort herb* (Fig. 4).

### 3.7 CYP450 expression in dairy cow endometrial cells induced by *Honeysuckle*

The inflammatory cells were treated with *Honeysuckle* at gradient concentration for 48 h and 72 h, resulting in the gradually increased expression of CYP450 with the increase in concentration of *Honeysuckle*. At the same gradient concentration, the longer the action time, the more obvious the expression of CYP450 induced by *Honeysuckle*. However, when treated with 10000  $\text{g}\cdot\text{mL}^{-1}$  *Honeysuckle* for 72 h, the expression of CYP450 decreased (Fig. 5).



**Fig. 3** CYP450 expression in dairy cow endometrial cells induced by *Angelica sinensis*



**Fig. 4** CYP450 expression in dairy cow endometrial cells induced by *Motherwort herb*

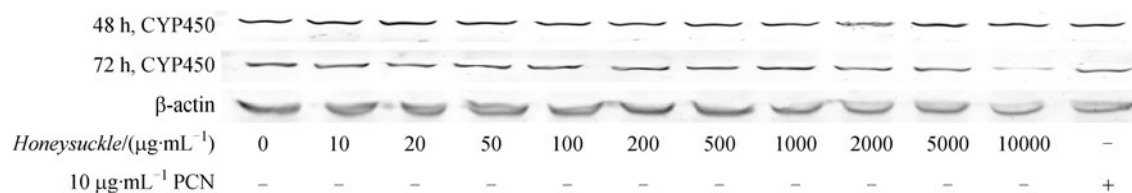


Fig. 5 CYP450 expression in dairy cow endometrial cells induced by *Honeysuckle*

## 4 Discussion

LPS was the key cytotoxic factor causing inflammation. It could induce inflammatory cytokines such as granule cells, endometrial epithelial cells, mesenchymal cells, vascular endothelial cells and so on (Ishikawa et al., 2007; Abdel-Salam et al., 2008; Banu et al., 2008). In this experiment, the most primitive stimulus LPS-induced cells were used to simulate the clinical endometritis pathogenesis. The early acute inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  were indicators to measure whether the inflammatory model was made successfully. The results showed that the cells grew well by 100 ng·mL<sup>-1</sup> LPS treatment, and the contents of TNF- $\alpha$  and IL-1 $\beta$  at 48 h and 72 h were significantly higher than those in the control group ( $P < 0.01$ ). This indicated that the endometrial inflammatory model was made successfully. This model could be used for the cow endometrial inflammatory response mechanism (Leung et al., 2001; Azawi, 2008; LeBlanc, 2008) and the drug metabolism for these inflammatory cells.

At present, there were a few methods for the treatment of dairy cow endometritis, but they were not very effective. Molecular biologic methods to study the mechanism of drug metabolism were also seldom used. In this experiment, the inflammatory endometrial cells were treated with the herbal prescription “Yimu Shenghuatang” at different concentrations, and the expression of CYP450 which related with the drug metabolism was measured. The results showed that the expression of CYP450 in endometrial cells increased with the concentration increasing of “Yimu Shenghuatang.” The best dose was at 2000  $\mu\text{g}\cdot\text{mL}^{-1}$  drug concentration for 48 h. When the drug concentration reached 10000  $\mu\text{g}\cdot\text{mL}^{-1}$ , the expression of CYP450 disappeared, which indicates that too higher concentration may cause a serious injury of the endometrial cells.

In our study, an interesting phenomenon was found. When the monomer compositions *Radix glycyrrhizae*, *Angelica sinensis*, *Motherwort herb*, *Honeysuckle* were used to treat the inflammatory cells separately, the expressions of CYP450 were different, and some were even on the contrary. However, the expression of CYP450 increased by the prescription made up of these monomer compositions, which illustrates the complexity and

rationality of the herbal medicine compatibility. Therefore, the herbal medicine “Yimu Shenghuatang” is supposed to be an effective prescription for clinic curing endometritis of dairy cows.

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