Effective constituents in Xiexin Decoction for anti-inflammation

Bing-Liang Ma, Yue-Ming Ma, Dong-Ming Yan, Hui Zhou, Rong Shi, Tian-Ming Wang, Yang Yang, Chang-Hong Wang, Ning Zhang

**A R T I C L E   I N F O**

Article history:
Received 21 January 2009
Received in revised form 30 April 2009
Accepted 28 May 2009
Available online 6 June 2009

Keywords:
Xiexin Decoction
Nitric oxide
Lipopolysaccharide
Raw264.7

**A B S T R A C T**

**Aim of the study:** To ascertain the effective constituents in Xiexin Decoction for anti-inflammation and the interactions of these constituents at the pharmacodynamic level.

**Materials and Methods:** Rats were administered oral Xiexin Decoction 1 h before intraperitoneal lipopolysaccharide. Nitric oxide production and Xiexin Decoction constituents in venous serum samples were quantified and the correlation between nitric oxide production and each constituent in serum was calculated. Raw264.7 cells were stimulated with lipopolysaccharide and one or more Xiexin Decoction constituents; cell viability and nitric oxide production was quantified.

**Results:** Xiexin Decoction significantly decreased nitric oxide production in vivo, which correlated well with rhein, baicalin, emodin and aloe-emodin. All the typical constituents of Xiexin Decoction, with the exception of physcion and chrysophanol, dose-dependently inhibited nitric oxide production in vitro. In an orthogonal designed in vitro study, rhein was the most powerful constituent, followed by baicalin then berberine and no synergy was found among these constituents.

**Conclusions:** Rhein was the most effective anti-inflammatory constituent in Xiexin Decoction followed by baicalin; no synergy was observed between rhein, baicalin and berberine at the pharmacodynamic level in vitro.

© 2009 Elsevier Ireland Ltd. All rights reserved.

**1. Introduction**

Xiexin Decoction (XXD) is a traditional Chinese medicinal formula containing *Radix et Rhizoma Rhei* (*Rheum palmatum* L.), *Rhizoma Coptidis* (*Coptis chinensis* Franch) and *Radix Scutellaria* (*Scutellaria baicalensis* Georgi). It has been used for about 1700 years and is still widely used to treat inflammation-related diseases such as upper respiratory tract infection and gastritis. It has been reported to prevent lipopolysaccharide (LPS)-induced arterial hypotension in rats by inhibiting the expression of the inducible isomerase of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), as well as cytokine formation and prostaglandin E2 (PGE2) production (Lo et al., 2005a), and has been shown to attenuate inflammatory responses in LPS-exposed rat lungs (Lo et al., 2005b). These in vivo results were achieved by intravenous injection of XXD. In a previous study we set out to determine the in vivo anti-inflammatory effects of oral XXD. We found that oral XXD showed significant anti-inflammatory effects in four animal models of acute experimental inflammation including carrageenan and egg white-induced hind paw edema in rats, 2% acetic acid-induced inflammatory exudation in the abdominal cavity of mice and LPS-induced acute lung injury in mice. XXD also inhibited iNOS activity and reduced the production of inflammatory factors, such as nitric oxide (NO), tumor necrosis factor-α (TNF-α) and malondialdehyde (MDA), in a LPS-induced acute inflammation mouse model (Ma et al., 2006). We also found that *Radix et Rhizoma Rhei* was the most important anti-inflammatory component in XXD, followed by *Radix Scutellaria*, and that there was beneficial synergy between *Radix et Rhizoma Rhei* and *Radix Scutellaria* (Ma et al., 2007). However, the effective constituents in XXD for anti-inflammation have not been determined, which limits further research and development on this formula.

We know that, *Radix et Rhizoma Rhei* yields anthraquinones such as rhein, emodin, chrysophanol, physcion and aloe-emodin, *Rhizoma Coptidis* yields alkaloids such as berberine, coptisine, palmatine and jatrorrhizine, and *Radix Scutellaria* yields flavonoids such as baicalin, baicalein, wogonoside and wogonin (Fig. 1). All these anthraquinones, alkaloids and flavonoids can be detected in vivo after oral administration of XXD (Tan et al., 2007; Yan et al., 2007; Yan and Ma, 2007). Rhein (Wang et al., 2002), emodin (Wang et al., 2002; Li et al., 2005), aloe-emodin (Mijatovic et al., 2004), baicalin, baicalein and wogonin (Chen et al., 2001), and berber-
ine (Kuo et al., 2004; Zhang et al., 2008) have been reported to have anti-inflammatory effects in vivo and in vitro. All the constituents cited above may contribute to the anti-inflammatory effects of XXD. However, the importance of each constituent in XXD needs to be investigated in a systematic way, so that the effective anti-inflammatory constituents can be clearly determined.

Chinese medicines, including XXD, are usually prescribed as a formula. The combination of several Chinese medicines is believed to increase the curative effects and decrease side effects due to drug–drug interactions. We found that there was a beneficial synergy between *Radix et Rhizoma Rhei* and *Radix Scutellaria* in vivo (Ma et al., 2007); however, these interactions may occur at the pharmacological, pharmacokinetic or pharmacodynamic level. Therefore, if interactions among the constituents of XXD occur at the pharmacodynamic level, this may be worthy of investigation.

In this study, both in vivo and in vitro experiments were carried out to ascertain the effective anti-inflammatory constituents in XXD and the potential interactions of these constituents at the pharmacodynamic level in vitro.

2. Materials and methods

2.1. Chemicals

Herbs in XXD including *Radix et Rhizoma Rhei* (*Rheum palmatum* L.), *Rhizoma Coptidis* (*Coptis chinensis* Franch) and *Radix Scutellaria* (*Scutellaria baicalensis* Georgi) were purchased from Shanghai Kang Qiao herbal pieces Co. Ltd. Authentication of these herbs was performed by Prof. Zhi-Li Zhao, Department of Botany, Shanghai University of Traditional Chinese Medicine. Authentication was performed by a comparison with appropriate voucher specimens at the herbarium and by performing both physical and chemical properties identification according to *The Pharmacopoeia of People’s Republic of China* (2000 edition). All flavonoid, anthraquinone and alkaloid (except coptisine) standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Coptisine was purchased from the Wako Pure Chemical Ind. Ltd. (Japan). Dulbecco’s modified Eagle’s minimum essential medium (DMEM) was purchased from the Invitrogen Corporation. Fetal bovine serum (FBS) was purchased from Fumeng gene Co. Ltd. (Shanghai, China). Trypsinase was purchased from the Amresco Corporation. Culture-grade dimethyl sulfoxide (DMSO) was purchased from CalBiochem. Methanol, sodium pyruvate, penicillin, streptomycin sulfate, 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl-tertazolium bromide (MTT), *E. coli* lipopolysaccharide (LPS), sulfanilamide, phosphoric acid, naphthylethylenediamine–HCl, and HEPES free acid were purchased from the Sigma Chemical Co. (USA). Water used in this study was produced using the Milli-Q system (Millipore, Bedford, MA, USA). All other chemicals were of analytical grade or better.

2.2. Animals

Male SD rats of grade II (Certificate No. SYXK 2004–2005), weighing 250 ± 20 g, were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China). The rats were housed in an air-conditioned room at 22–24 °C with a 12 h dark/light cycle and were allowed food and water spontaneously. They were fasted for 12 h before the experiments. The animal study was performed according to the National Research Council’s guidelines.

2.3. Cell culture

Raw264.7 murine macrophage cells were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). Cells were cultured at 37 °C in DMEM medium supplemented with 10% heat-deactivated FBS, penicillin (100 U/mL), streptomycin sulfate (100 mg/mL), sodium pyruvate (1 mM) and HEPES (15 mM) in a humidified atmosphere of 5% CO2 and passed when about 85% confluence was achieved with trypsinase solution [0.25%, dissolved in phosphate buffer (PBS)].

![Fig. 1. Structures of the Xiexin Decoction constituents cited in this article (all obtained from http://sis.nlm.nih.gov/chemical.html).](http://sis.nlm.nih.gov/chemical.html)
Scutellaria and evaporated to dryness under reduced pressure at 60°C. An eightfold mass of water was added and boiled for 1 h for the first decocting. The mixture was then boiled for 1.5 h for the second decocting. Deionized water was added and boiled for 30 min. A 10-fold mass of water was added and boiled for 1 h for the second decocting. After filtration, the two decoctions were mixed and evaporated to dryness under reduced pressure at 60°C to obtain the power form of XXD (yield: 27.8%). Simultaneous quantification of the XXD constituents was performed using HPLC according to our previous report (Shi et al., 2007a, b).

2.4. XXD preparation and simultaneous quantification of twelve XXD constituents

XXD was prepared according to our previous report (Yan and Ma, 2007). Briefly, the blended mixture of Radix et Rhizoma Rhei, Radix Scutellaria and Rhizoma Coptidis, in a 2:1:1 ratio, was immersed in deionized water for 30 min. A 10-fold mass of water was added and the mixture was then boiled for 1.5 h for the first decocting, then an eightfold mass of water was added and boiled for 1 h for the second decocting. After filtration, the two decoctions were mixed and evaporated to dryness under reduced pressure at 60°C to obtain the power form of XXD (yield: 27.8%). Simultaneous quantification of eleven typical constituents of this extract was performed using HPLC according to our previous report (Shi et al., 2007a, b).

2.5. MTT assay

Cells at a density of 5 \times 10^4 cells/well were cultured in 96-well plates. The culture medium was carefully renewed after 24 h. Cells were then treated with LPS (0.25 μg/mL) and one or more of the XXD constituents. MTT solution (dissolved in PBS) was added (final concentration was 0.5 mg/mL) 24 or 30 h later and the cells were further incubated at 37°C for 4 h. The medium was carefully discarded, 200 μL DMSO was added to dissolve the generated formazan, and after 20 min vibration, the dissolved solution was diluted four times with DMSO and absorbance at 550 nm was measured using a microplate reader.

2.6. Nitrite assay

The sample (100 μL, serum or cell culture medium) was mixed with an equal volume of Griess reagent [equal volumes of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphthylethylenediamine–HCl] and incubated at room temperature for 20 min; absorbance at 550 nm was measured using a microplate reader.

2.7. Effects of XXD constituents on LPS-treated Raw264.7 cells

Raw264.7 cells were seeded in 96-well plates at a density of 5 \times 10^4 cells/well. The culture medium was carefully refreshed after 24 h. Cells were then treated with LPS (0.25 μg/mL, dissolved in culture medium) with or without one of the XXD constituents. After an additional 24 h incubation, 100 μL of the culture medium was sampled for the NO production assay, and MTT solution was added for the MTT assay.

2.8. Orthogonal designed in vitro study

Rhein, baicalin and berberine were selected for an L_9(2^4) orthogonal designed study (Table 1) to determine the most important anti-inflammatory constituents in XXD and to determine their interactions in vitro. The orthogonal design is shown in Table 1. Raw264.7 cells were seeded in 96-well plates at a density of 5 \times 10^4 cells/well. The culture medium was carefully refreshed after 24 h. Cells were treated with LPS (0.25 μg/mL, dissolved in culture medium) and various combinations of rhein, berberine and baicalin. After additional 30 h incubation, 100 μL of the culture medium was sampled for the NO production assay, and MTT solution was added for the MTT assay.

2.9. Animal studies

Rats were divided into four groups. The animals received a single dose of XXD (oral administration, 3.34 mg/kg) or 0.15 mg/kg dexamethasone (DXM) (positive control) or an identical volume of normal saline (negative control and model groups) 1 h before ip LPS (3.2 mg/kg) (for XXD, positive control and model groups) or an identical volume of normal saline (negative control group), venous blood samples were collected from the eyehole 0.25, 0.5, 2, 3, 4, 8 and 12 h later. NO production in serum was measured by the Griess reaction, anthraquinones, flavonoids and alkaloids in serum were quantified by HPLC analysis with fluorescence detection (HPLC–FLD) (Yan and Ma, 2007) or by ultraviolet detection (HPLC–UV) (Tan et al., 2007; Yan et al., 2007), respectively.

2.10. Statistical analysis

All results were expressed as mean ± S.D. Statistical differences between the groups were evaluated by one-way ANOVA; analysis of the findings from the orthogonal designed study was performed using orthogonal variance analysis; correlation between pharmacokinetic parameters of the constituents and NO production in XXD-treated rats challenged with LPS was performed by correlation analysis. A P-value of <0.05 was considered significant, P<0.01 was considered highly significant.
3. Results

3.1. Contents of anthraquinones, flavonoids and alkaloids in XXD

As shown in Table 2, baicalin was the most abundant constituent in the water extract, whereas rhein and berberine were the most abundant anthraquinone and alkaloid, respectively.

3.2. Effect of XXD on serum NO production in LPS challenged rats

NO production in rat serum was measured. The total NO production during 8–12 h after ip injection of LPS in each rat was summed. Data analysis showed that NO production in the negative control, DXM and XXD groups was significantly lower than that in the model group (Fig. 2).

3.3. Pharmacokinetic parameters of the XXD constituents in the XXD-treated rats challenged with LPS

Pharmacokinetic parameters of the XXD constituents were calculated and are listed in Table 3. The concentrations of physcion and berberine in serum were only determined at 0.5–2 h and 2–4 h after administration, respectively, therefore their pharmacokinetic parameter AUC$_{0–8}$h could not be calculated. These results showed that rhein and baicalin were the most abundant anthraquinone and flavonoid, respectively, absorbed into the blood after oral administration of XXD in rats.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>$C_{\text{max}}$ ($\mu$g/mL)</th>
<th>AUC$_{0–8}$h ($\mu$g/(mL h))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baicalin</td>
<td>2.550 ± 0.903</td>
<td>9.350 ± 4.719</td>
</tr>
<tr>
<td>Rhein</td>
<td>1.326 ± 1.833</td>
<td>3.560 ± 1.576</td>
</tr>
<tr>
<td>Wogonoside</td>
<td>0.722 ± 0.349</td>
<td>2.694 ± 0.804</td>
</tr>
<tr>
<td>Chrysophanol</td>
<td>0.120 ± 0.252</td>
<td>1.430 ± 1.096</td>
</tr>
<tr>
<td>Emodin</td>
<td>0.075 ± 0.168</td>
<td>0.216 ± 0.202</td>
</tr>
<tr>
<td>Aloe-emodin</td>
<td>0.072 ± 0.100</td>
<td>0.304 ± 0.148</td>
</tr>
<tr>
<td>Physcion</td>
<td>0.011 ± 0.020</td>
<td>NC</td>
</tr>
<tr>
<td>Berberine</td>
<td>0.004 ± 0.002</td>
<td>NC</td>
</tr>
</tbody>
</table>

“NC” implies “not calculated”; $C_{\text{max}}$: maximum plasma concentration; AUC$_{0–8}$h: the area under the plasma concentration–time curve from 0 to 8 h.

3.4. Correlation between pharmacokinetic parameters of XXD constituents and NO production in XXD-treated rats challenged with LPS

Correlations between pharmacokinetic parameters and NO production were calculated and are listed in Table 4. Significant negative correlations existed between some XXD constituents including aloe-emodin, rhein, emodin, baicalin and NO production, which showed that these constituents may be the anti-inflammatory substances in XXD.

3.5. Effects of XXD constituents on LPS-treated Raw264.7 cells

The effects of thirteen typical XXD constituents on LPS-induced NO production in Raw264.7 cells and cell viability are shown in the following figures.
Effects of the alkaloids in *Rhizoma Coptidis* on lipopolysaccharide-treated Raw264.7 cells (*x±s, n=6*). Raw264.7 cells were treated with lipopolysaccharide (0.25 μg/mL) and various concentrations of *Rhizoma Coptidis* alkaloids. After additional 24 h incubation, 100 μL of the culture medium was sampled for the nitric oxide production assay, and MTT solution was added (final concentration, 0.5 mg/mL) for the MTT assay. All data were expressed as percentage of model. C: Control, M: Model. *P*<0.05, **P**<0.01 compared with Control; *P*<0.05, **P**<0.01 compared with Model.

Figs. 3–5. In general, almost all of the constituents at all tested concentrations, with the exception of coptisine, had a dose-dependent inhibitory effect on NO production; however, all the alkaloids and anthraquinones demonstrated cytotoxicity above a certain concentration. To abolish the influence of cytotoxicity, the ratio of NO production to cell viability [expressed as "NO/cell viability" (NOCV) in this article] was calculated as modified NO production for the inducible isoform of nitric oxide synthase (iNOS) (Denlinger et al., 1996). Thus, LPS has been widely used to establish the in vitro anti-inflammatory effects of these XXD constituents. In brief, rhein (≥1 μg/mL), emodin (≥10 μg/mL), aloe-emodin (≥3 μg/mL), berberine (≥0.3 μg/mL), coptisine (≥0.3 μg/mL), palmatine (≥0.3 μg/mL), jatrorrhizine (≥1 μg/mL), baicalin (≥0.3 μg/mL), baicalein (≥0.3 μg/mL), wogonoside (≥10 μg/mL), and wogonin (≥3 μg/mL) significantly decreased NOCV, while chrysophanol (≥0.15 μg/mL) and physcion (≥0.075 μg/mL) dose-dependently increased NOCV.

### 3.6. Orthogonal designed studies in LPS challenged Raw264.7 cells

The effects of eight combinations of rhein, berberine and baicalin on LPS-induced NO production and cell viability in Raw264.7 cells are shown in Fig. 6. Variance analysis showed that rhein had the most powerful inhibitory effect on NOCV, followed by baicalin then berberine (*F* values were 77.153, 35.954 and 29.621, respectively, and *P* values were all <0.01). No synergy was observed among these constituents.

### 4. Discussion

Sustained NO overproduction can be deleterious to the host, and has been implicated in the pathogenesis of various inflammatory diseases. Bacterial LPS stimulates NO production in macrophages such as Raw264.7 cells, an Abelson virus-transformed murine macrophage cell line, by inducing transcription of the gene coding for the inducible isofrom of nitric oxide synthase (iNOS) (Denlinger et al., 1996). Thus, LPS has been widely used to establish the in vitro (Chen et al., 2001; Li et al., 2005) and the in vivo experimental model of inflammation. In this study, we aimed to ascertain the anti-inflammatory constituents in XXD and their potential interactions based on LPS-induced in vitro and in vivo experimental inflammation models.

---

**Table 4**

Correlation between pharmacokinetic parameters of the Xiexin Decoction constituents and nitric oxide (NO) production in the XXD-treated rats challenged with lipopolysaccharide.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>$\ln C_{\text{max}}$</th>
<th>NO (μM)</th>
<th>lnAUC$_{0-8\text{h}}$</th>
<th>NO (μM)</th>
<th>$r$</th>
<th>$p$</th>
<th>$r$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhein</td>
<td>−0.615 &lt;0.05</td>
<td>Rhein</td>
<td>−0.628 &lt;0.05</td>
<td>Rhein</td>
<td>−0.615 &lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baicalin</td>
<td>−0.601 &lt;0.05</td>
<td>Baicalin</td>
<td>−0.609 &lt;0.05</td>
<td>Baicalin</td>
<td>−0.601 &lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emodin</td>
<td>−0.57 &lt;0.05</td>
<td>Emodin</td>
<td>−0.619 &lt;0.05</td>
<td>Emodin</td>
<td>−0.57 &lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aloe-emodin</td>
<td>−0.541 &lt;0.05</td>
<td>Aloe-emodin</td>
<td>−0.616 &lt;0.05</td>
<td>Aloe-emodin</td>
<td>−0.541 &lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wogonoside</td>
<td>−0.463 &gt;0.05</td>
<td>Wogonoside</td>
<td>−0.524 &gt;0.05</td>
<td>Wogonoside</td>
<td>−0.463 &gt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysophanol</td>
<td>−0.359 &gt;0.05</td>
<td>Chrysophanol</td>
<td>−0.37 &gt;0.05</td>
<td>Chrysophanol</td>
<td>−0.359 &gt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physcion</td>
<td>−0.054 &gt;0.05</td>
<td>Physcion</td>
<td>NC</td>
<td>Physcion</td>
<td>NC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berberine</td>
<td>−0.050 &gt;0.05</td>
<td>Berberine</td>
<td>NC</td>
<td>Berberine</td>
<td>NC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NC* implies “not calculated”; $C_{\text{max}}$: maximum plasma concentration; $\ln C_{\text{max}}$: log-transformed $C_{\text{max}}$; $\text{AUC}_{0-8\text{h}}$: the area under the plasma concentration–time curve from 0 to 8 h; lnAUC$_{0-8\text{h}}$: log-transformed AUC$_{0-8\text{h}}$; $r$: relative coefficient; $P$: the probability value of significance test of correlation coefficient.

---

**Fig. 6.** Effects of rhein, berberine and baicalin on lipopolysaccharide (LPS)-treated Raw264.7 cells using the orthogonal design (*x±s, n=6*). Raw264.7 cells were treated with lipopolysaccharide (0.25 μg/mL) and various combinations of rhein, berberine and baicalin. After additional 30 h incubation, 100 μL of the culture medium was sampled for the nitric oxide production assay, and MTT solution was added (final concentration, 0.5 mg/mL) for the MTT assay. All data were expressed as percentage of model. C: Control, M: Model. X-axis indicates the concentrations of rhein, berberine and baicalin in each combination. *$P$*<0.05, **$P$**<0.01 compared with Control; *$P$*<0.05, **$P$**<0.01 compared with Model.
We studied the in vitro anti-inflammatory effects of thirteen typical XXD constituents in LPS-treated Raw264.7 macrophages. Our results showed that almost all the constituents, with the exception of physcion and chrysophanol, had a dose-dependent inhibitory effect on NOCV in LPS challenged Raw264.7 cells. However, our in vivo study showed that the serum concentration of coptisine, palmitine, jatrorrhizine, baicalin, wogonoside, and wogonin were all very low after oral administration of XXD and could not achieve a high enough concentration to have a significant inhibitory effect on NOCV. Furthermore, there were no significant negative correlations between these constituents and NO production, therefore these constituents, in addition to physcion and chrysophanol, were not thought to be the basic anti-inflammatory constituents in XXD. Thus, the basic effective XXD constituents responsible for the anti-inflammatory effect may be due to rhein, baicalin, berberine, emodin and aloe-emodin.

Evidence for anti-inflammatory activity in both rhein and baicalin is based on the following findings: (1) baicalin was the most abundant constituent in XXD, while rhein was the most abundant anthraquinone; (2) animal studies showed that after oral administration of XXD, baicalin and rhein were the most abundant constituents of Radix Scutellaria or Radix et Rhizoma Rhei, respectively, in serum; (3) significant negative correlations existed between serum berberine and NO production; (4) both rhein and baicalin showed very strong anti-inflammatory effects in vitro at a concentration which could be achieved after oral administration of XXD.

There were significant negative correlations between emodin, aloe-emodin and NO production in the animal studies, but these constituents were very low in serum after oral XXD, and were thought not to have direct anti-inflammatory effects. Since both emodin and aloe-emodin are metabolized to rhein in vivo (Lang, 1993; Mueller et al., 1998), it is thought that these constituents might have indirect anti-inflammatory effects.

Although the serum concentration of berberine was also very low after oral XXD and a negative correlation was not observed between serum berberine and NO production, it was the most abundant constituent in Rhizoma Coptidis in tissues, and the tissue concentration of berberine was much higher than that in blood (unpublished data). Berberine also showed very strong anti-inflammatory effects in vitro. Therefore, these three effective constituents (rhein, baicalin and berberine) were included in the orthogonal designed study to further ascertain the most important anti-inflammatory constituent in XXD and their interactions in vitro.

The variance analysis of the findings from the orthogonal designed study indicated that rhein showed the most powerful inhibitory effect on NOCV followed by baicalin then berberine. This result indicated that rhein was the most important anti-inflammatory constituent in XXD, which was in accordance with the in vivo anti-inflammatory experiment which showed that Radix et Rhizoma Rhei was the most powerful component in XXD (Ma et al., 2007). We previously reported on a beneficial synergy between Radix et Rhizoma Rhei and Radix Scutellaria in XXD (Ma et al., 2007); however, variance analysis of the findings from the orthogonal designed study showed that there was no synergy between baicalin and rhein in vitro. These results suggested that the beneficial synergy between Radix et Rhizoma Rhei and Radix Scutellaria in XXD might occur at the pharmaceutical or pharmacokinetic level. In fact, our studies have shown that the contents of five anthraquinones in the extract of Radix et Rhizoma Rhei were increased following the addition of Radix Scutellaria (Shi et al., 2007a) and the total systemic exposure level of the five anthraquinones (total AUC) was higher in the Radix Scutellaria plus Radix et Rhizoma Rhei group than in the Radix et Rhizoma Rhei only group (Yan et al., 2009).

In conclusion, these results showed that several constituents account for the anti-inflammatory effect of Xie Rin Decoction. Both rhein and baicalin were the direct anti-inflammatory constituents, and both emodin and aloe-emodin were the indirect anti-inflammatory constituents. Rhein was the most effective anti-inflammatory constituent followed by baicalin. The importance of berberine in XXD for anti-inflammation remains to be determined. No synergy was observed between rhein, baicalin and berberine at the pharmacodynamic level in vitro.

Acknowledgements

This work was financially supported by National Natural Science Foundation of China (No. 90409008) and National Major Science & Technology Project (No. 2009ZX09311-003).

References


