Relationships between pharmacokinetics and efficacy of Xie-xin decoction in rats with experimental ulcerative colitis

Xiang-Hui Han\textsuperscript{a,b}, Jie Zhong\textsuperscript{a}, Ji-Yuan Guo\textsuperscript{a}, Rong Shi\textsuperscript{a}, Xin-Hong Wang\textsuperscript{a}, Chang-Hong Wang\textsuperscript{c}, Kun Wang\textsuperscript{d}, Guang-Li Du\textsuperscript{a}, Yun-Hui Shen\textsuperscript{a}, Yue-Ming Ma\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a} School of Pharmacy, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China
\textsuperscript{b} Longhua Hospital, Shanghai University of Traditional Chinese Medicine, 725 Wapeng South Road, Shanghai 200032, China
\textsuperscript{c} Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China
\textsuperscript{d} Center for Drug Clinical Research, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China

A R T I C L E   I N F O

Article history:
Received 19 October 2012
Received in revised form 3 April 2013
Accepted 8 April 2013
Available online 22 April 2013

Keywords:
Xie-xin decoction
Ulcerative colitis
Pharmacokinetics
Efficacy
Material basis
Canonical correlation analysis

A B S T R A C T

Ethnopharmacological relevance: Xie-xin decoction (XXD) has been used as a classic formula in China for the treatment of gastrointestinal dysfunction such as ulcerative colitis (UC). However, no potential action mechanisms and active compounds had been systematically investigated.

Aim of the study: To explore the effectiveness and the material basis of XXD in trinitrobenzene sulfonic acid (TNBS)-induced UC rats.

Materials and methods: XXD was administered orally for 8 days at a dosage of 2 or 4 g/kg/day. Plasma pharmacokinetic properties and colon tissue concentrations of multiple compounds from XXD were detected. Tissue damage scores, production of interleukin (IL)-10 and myeloperoxidase (MPO), expression of tumor necrosis factor-alpha (TNF-\(\alpha\)) and nuclear factor-kappa Bp65 (NF-\(\kappa\)Bp65) in colon tissues were examined. Canonical correlation analysis was performed to evaluate the relationships between pharmacokinetics and efficacy of XXD.

Results: XXD promoted the recovery of colitis and inhibited the colonic inflammation damage in UC rats by reducing the level of MPO and the expression of TNF-\(\alpha\) and NF-\(\kappa\)Bp65, and increasing the production of IL-10 in colon tissues. Efficacy of XXD was positively related with AUC of plasma compounds (baicalin, berberine, wogonoside, wogonin, and rhein) and concentrations of six colon tissue compounds (cortexine, jatrorrhizine, palmatine, berberine, baicaline and emodin), respectively.

Conclusions: The multiple compounds in plasma and colon tissues from XXD might be the main material basis for therapeutic potentials in UC rats.

\(\copyright\) 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Chinese herbal formulas are the main medications of traditional Chinese medicine (TCM). Unlike chemical drugs, a formula contains multiple components and shows treatment effects of multi-targets and the organic integer synergism. It is very necessary to elucidate the bioactive compounds of formulas and their therapeutic mechanisms in modernization of TCM (Li et al., 2008). Xie-xin decoction (XXD), a classic traditional Chinese formula, has been recorded since the Han Dynasty (early 3rd century) in Synopsis of Prescriptions of the Golden Chamber. In clinics, this formula commonly used to treat the patients with chronic gastritis, peptic ulcer, acute dysentery, ulcerative colitis, or other dysfunction of the gastrointestinal tract (Zhang, 2006). Recent study demonstrated that XXD, composed of Radix et Rhizoma Rheum (\textit{Rheum palmatum} L., root and rhizome, stewing with wine, Dahuang), Radix Scutellariae (\textit{Scutellaria baicalensis} Georgi, root, steaming, Huangqin), and Rhizoma Coptidis (\textit{Coptis deltoidea} C.Y. Cheng et Hsiao, rhizome, Huangqian), could ameliorate damage of colonic mucosa and promote the recovery of colitis in ulcerative colitis (UC) rats by rectal administration (Wang et al., 2010). However, its potential action mechanism remains relatively unknown. The major chemical compounds of XXD include alkaloids, anthraquinones, and flavonoids, such as berberine, palmatine, emodin, rhein, baicalin, baicaline, etc. Although it has been found that berberine and baicalin showed obvious anti-inflammatory and immunomodulatory effects on UC models (Lee et al., 2010; Dai et al., 2012), there are few reports on other constituents of this formula having similar activities.

\* Correspondence to: Department of Pharmacology, School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China. Tel./fax: +86 21 5132 2386.
\textit{E-mail addresses:} mayueming_117@hotmail.com, mayueming_117@126.com (Y.-M. Ma).

0378-8741/– see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2013.04.008
The combination analysis of pharmacokinetics (PK) and pharmacodynamics (PD) is proposed as an available method accounting for determining the material basis of TCM formulae (Shu and Li, 2008). However, current researches concerning PK/PD of formulae mainly focus on describing the correlation between single chemical compound and single therapeutic effect (Li et al., 2002; Zhao et al., 2004), which could not reflect the internal relation between PK and PD of the whole formula. In exploring PK or PD of a formula, observational studies are often collected multiple measurements. Routine approaches such as multiple linear regressions to analyze such data are usually challenged as they are plagued by the potential issues including multicollinearity and multiple testing (Fieller, 2009). Canonical correlation analysis (CCA) is a multivariate statistical model that facilitates the study of interactions among sets of multiple independent variables and multiple dependent variables (Johnson and Wichern, 2003). It has been successfully applied to a number of biomedical data such as medical imaging (Correa et al., 2010), drug side-effect prediction (Mizutani et al., 2012), TCM syndrome assessment (Zhang et al., 2012), and so on. Since CCA assesses the correlation of two canonical variables, one representing a set of the PK variables and the other set of PD variables, it is potentially a useful method to evaluate the relationships between PK and PD variables of a formula.

Therefore, the present study was designed to explore the effects and mechanism of oral administration of XDD on trinitrobenzenesulfonic acid (TNBS)-induced UC rats. Meanwhile, the correlations between PK and efficacy of this formula were evaluated to identify active compounds using CCA.

2. Materials and methods

2.1. Plant materials and preparation of XDD

The medicinal materials of Radix et Rhizoma Rhei, Radix Scutellariae, and Rhizoma Coptidis were purchased from Shanghai Kang Qiao Chinese Cut Crude Drug Co. Ltd. (Lot. 111105) and authenticated by Professor Zhi-Li Zhao from Department of Pharmacognosy, Shanghai University of TCM. All materials were stored at room temperature in the absence of light in a well-ventilated room and the voucher specimens were deposited in School of Pharmacy, Shanghai University of TCM. Authentication was performed by comparing appropriate voucher specimens at the herbaria and identified physical and chemical property according to Pharmacopoeia of People’s Republic of China (2010 edition).

2.2. Chemicals and reagents

The standards: berberine, palmatine, jatrorrhizine, aloeemodin, rhein, emodin, baicalin, wogonin (purity > 99%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), coptisine (purity > 99%) was from the Wako Pure Chemical Industries Ltd (Osaka, Japan), baicalin and wogonoside (purity > 98%) were from Shanghai U-sea Biotech Co. Ltd (Shanghai, China). Tolbutamide and trinitrobenzene sulfonic acid was from Sigma-Aldrich Co. LLC (St. Louis, Missouri, USA). Salicylazosulfapyridine (SASP, 250 mg/tablet) was produced by Shanghai Sine-Jia Hua Pharmaceutical Co. Ltd (Shanghai, China). Rat interleukin-10 (IL-10) ELISA kit was from Biosource International, Inc. (Camarillo, USA) and myeloperoxidase (MPO) ELISA kit was from Diadone Research (Besancon, France). Polyclonal rabbit anti-rat tumor necrosis factor-alpha (TNF-α) antibody (dilution 1:100) were obtained from Abcam Biochemicals (Location Bristol, UK); nuclear factor-kappa Bp65 (NF-κBp65) antibody was from Santa Cruz Biotechnology, Inc. (California, USA). Other chemicals used were all of analytical grade.

2.3. Animals

Male Sprague-Dawley rats weighing 250–270 g from Shanghai Experimental Animal Center of Chinese Academy of Sciences were used in the study. The animals were housed in a 12-h dark/light cycle environment at a temperature of 22 ± 2 °C and a relative air humidity of 45–55% with food and water ad libitum. During the experiment, all animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institution of Health. All experimental protocols were approved by the animal research ethics committee of Shanghai University of TCM.

2.4. Induction of UC model and drug treatment

Experimental colitis was induced according to a modification of the procedure of Celisniski et al. (2010). Prior to induction, all rats were fasted overnight but given free access to water. After anesthetized with 30 mg/kg pentobarbital sodium, the rats were received a single rectal injection of TNBS/ethanol mixture (70 mg/kg TNBS diluted in 0.25 mL of 50% ethanol) slowly through a catheter with 2 mm diameter at the depth of 8 cm from rectal sphincter, and then the rats were left for 15 min in a supine Trendelenburg position with the anus clipped. Thirty-two TNBS-induced rats were randomly divided into four groups: two dosages of XDD groups (2 and 4 g/kg, respectively), SASP group (0.5 g/kg), and model group (an equal volume of saline). Additionally, eight rats used as normal control group were rectally injected with saline instead of TNBS. The animals were administrated by intragastric gavage once a day, and continuously for 8 days.

2.5. Pharmacokinetics study

The PK study was performed as described previously (Zan et al., 2011). On the 7th days of XDD treatment, serial blood samples (120 μL) were collected at 0, 0.25, 0.5, 1, 2, 4, 6, 12, and 24 h into a heparinized tube. After centrifuged at 5000 rpm for 10 min, plasma fractions were separated and then frozen at −70 °C until analysis. At 1 h after dosing on the last treatment days (the 8th day), the rats were sacrificed under anesthesia and the ulcerative colon tissues were excised, rinsed in ice-cold saline, blotted, and weighed. The tissues were homogenized in two volumes of ice-cold saline, and homogenate samples were stored at −70 °C until analysis. A validated LC-MS/MS method (Zan et al., 2011) was applied to simultaneously determine the concentration of 11 compounds in biological samples (blood plasma and tissue homogenate).
2.6. Macroscopic and histological evaluation of colonic damage

A 10-cm segment of the distal colon was removed for the morphological study. Colonic mucosa damage was assessed according to previously described macroscopic scoring system (Hara et al., 2007) as follows: 0 = normal mucosa; 1 = localized hyperemia but no erosions, ulcers, or scars; 2 = minor ulcer or scar with inflammation at one site > 2 mm but < 5 mm; 3 = two or more sites of ulceration and/or inflammation, each up to 5 mm; 4 = two or more major sites of inflammation and ulcerations > 5 mm each or one major site of inflammation extending > 1 cm along the length of the mucosa. The tissue fragments (2 × 10 mm²) were excised from the central part of the lesion of each colon, fixed in 4% polyformaldehyde prior to wax embedding, sectioning, and staining with hematoxylin and eosin (HE). Histological scores were performed by a pathologist in a blind method using the criteria of Dieleman et al. (1998). Each section was graded with a range from 0 to 4 as to depth of the lesion, extent of ulceration and with a range from 0 to 3 as to degree of inflammation. These changes were indicated according to the following scale: depth of the lesion, 0 = none, 1 = mucosa, 2 = submucosa, 3 = muscularis propria, 4 = serosa; degree of inflammation, 0 = none, 1 = slight, 2 = moderate, 3 = severe; extent of ulceration, 0 = none, 1 = mild surface (0–25%), 2 = moderate surface (25–50%), 3 = severe surface (50–75%), 4 = extensive-full thickness (more than 75%).

2.7. Enzyme linked immunosorbent assay (ELISA)

The frozen colonic tissues were homogenized in nine volumes of cold saline. The homogenate was centrifuged at 2000 rpm for 5 min, the supernatants were collected, and the production of MPO and IL-10 were measured using double antibody sandwich ELISA according to the manufacturer’s instructions.

2.8. Immunohistochemistry (IHC)

IHC stain was performed using a two-step EnVision/HRP technique (Dako Cytomation, Denmark) following the manufacturer’s protocol. The omission of primary antibodies was used as the negative control. The expression of NF-κBp65 and TNF-α was quantitatively evaluated using an Olympus BH2 microscope with computer-aided images analysis system. The digital images were archived by a digital camera (Nikon 4500, Tokyo, Japan). The positive area and optical density (OD) of positive cells were determined by measuring three randomly selected microscopic fields for each slide. The IHC index was defined as an average integral optical density (AIOD) (AIOD = positive area × OD/total area).

2.9. Data analysis

2.9.1. Pharmacokinetic analysis

Plasma PK parameters were estimated by a noncompartmental method using WinNonlin (v. 5.0.1; Pharsight Corp, Mountain View, CA) software. The $c_{\text{max}}$ and the $T_{\text{max}}$ were observed values of each individual. The area under concentration–time curve up to the last measured time point ($AUC_{\text{0→t}}$) was calculated by the trapezoidal rule. The $AUC_{\text{0→t}}$ was generated by extrapolating the $AUC_{\text{0→t}}$ to infinity using the k and the last measured concentration. The terminal half-life ($t_{\text{1/2}}$) was calculated using the relationship 0.693/k.

2.9.2. Pharmacodynamic statistical analysis

The variables used in the PD analyses included macroscopic and histological damage scores, levels of MPO and IL-10, IHC index of TNF-α and NF-κBp65. All data were expressed as mean ± SEM. ANOVA was used for multiple comparisons; Fisher’s LSD test or Games-Howell tests was used for comparison of two means. A value of $P < 0.05$ was considered to be statistically significant.

2.9.3. Pharmacokinetic/pharmacodynamic analysis

Before data mining application, we normalized the original PD data using the min-max normalization to assure the comparison of these data by different units of measures (Jain and Bhandare, 2011). The transformation formula was:

$$Y_j = \frac{Y_j - Y_{\text{min}}}{Y_{\text{max}} - Y_{\text{min}}}$$

where $Y_j$ is normalized value, $Y_j$ is original value, $Y_{\text{min}}$ is minimum value of original PD data, $Y_{\text{max}}$ is maximum value of original PD data, $j$ is the number of all the animals. Each element of original PD data was transformed to a value between 0.0 and 1.0.

Then, CCA was performed (SAS, version 6.12; SAS Institute Inc.) to analyze the relationship between PK variable ($AUC_{0→t}$, $C_{\text{max}}$, or $C_{\text{tissue}}$) of multiple compounds and multiple normalized PD variables (macroscopic and histological scores, MPO and IL-10 levels, IHC index of TNF-α and NF-κBp65). The fundamental principle of CCA is the creation of a number of canonical variables, each consisting of linear combinations for a set of independent variables ($U_i$) and a set of dependent variables ($V_j$), which have the form:

$$U_i = a_1X_1 + a_2X_2 + \ldots + a_pX_p$$

$$V_j = b_1Y_1 + b_2Y_2 + \ldots + b_qY_q$$

where X is PK parameter of multiple compounds (from 1 to p), Y is multiple PD indicator (from 1 to q), a, b is canonical weight, $U_i$ represents any linear combination of the original variables in the PK parameter of multiple compounds; $V_j$ represents any linear combination of the original variables in the PD indicators. The goal of CCA is to determine the canonical weights (a and b) that maximize the correlation between canonical variables $U_i$ and $V_j$ (Fieller, 2009). The combinations are coupled in the pairs of canonical variables ($U_i$, $V_j$), the first canonical correlation of ($U_1$, $V_1$) is the highest possible correlation, that of ($U_2$, $V_2$) being the second high correlation, etc. The maximum number of canonical correlation (i) is equal to the number of variables in the smaller set.

In this study, significance test of a canonical correlation coefficient was performed using likelihood ratio test. To limit the chance of failing to detect an effect, we set the significant level of the test at 0.10 instead of 0.05 according to the reference published (Liu et al., 2009). In addition, canonical loadings which are used interpret the importance of each original variable in the canonical variables were calculated. In canonical structure, a canonical loading represents the correlation between an original variable and its canonical variable. As a rule, an absolute value of 0.3 or greater in canonical loading was used to select the original variables that were thought to have a meaningful interpretation of the related canonical variables (Lambert and Durand, 1975). We chose a cutoff value of 0.30 to select important loadings in this study.

3. Results

3.1. Efficacy of XXD in TNBS-induced UC rats

3.1.1. Macroscopic and histological damage scores

Macroscopic observation revealed hyperemia, swelling, edema, and ulceration on the mucosal surface in model rats, and HE staining showed severe colonic inflammation with necrosis, extensive neutrophils infiltration of the gut wall, submucosal edema and thickening of muscularis mucosa in model rats. In contrast, XXD
treatment greatly relieved these lesions (Fig. 1). Compared to model group, the macroscopic and histological damage scores of colon tissue were significantly lowered in XXD-treated groups (P < 0.05). But there were no significant difference between two dosages of XXD groups (Table 1).

3.1.2. MPO and IL-10 levels in colon tissues

As shown in Fig. 2, there was an obvious increase in the production of MPO and a marked decrease in the production of IL-10 in colon tissue of model rats. In contrast, XXD at the dose of 2 and 4 g/kg significantly declined the MPO level (P < 0.01) and XXD at the dose of 4 g/kg significantly elevated the IL-10 level (P < 0.01). However, a non-significant difference in both MPO and IL-10 level was observed between two XXD-treated groups.

3.1.3. TNF-α and NF-κBp65 protein expression in colon tissues

TNBS application caused a dramatic increase in the protein expression of NF-κBp65 and TNF-α in colon tissues. In contrast, XXD or SASP treatment significantly inhibited both TNF-α and NF-κBp65 expression (Fig. 3). Consistent with the IHC observation, NF-κBp65 and TNF-α IHC index in model group were obviously higher than those in normal group (P < 0.01). Compared to model group, NF-κBp65 and TNF-α IHC index were markedly decreased in two XXD and SASP groups (P < 0.05 or P < 0.01). However, no difference in IHC index occurred between two dosages of XXD groups (Fig. 4).

3.2. Pharmacokinetics of XXD in TNBS-induced UC rats

3.2.1. Plasma PK parameters of multiple compounds from XXD

After oral administration of XXD (2 and 4 g/kg) to the UC rats for successive 8 day, nine compounds (coptisine, jatrorrhizine, berberine, palmatine, baicalin, baicalein, wogonoside, wogonin, and rhein) were detected in UC rat plasma by LC–MS/MS. Plasma concentrations of only five compounds (baicalin, berberine, wogonoside, wogonin, and rhein) were determined during the 0–24 h time interval, whereas, the other compounds were below the quantification limits of our bioanalytical methods at multiple time point. The plasma concentration–time profiles of these five compounds are illustrated in Fig. 5, and the main PK parameters were summarized in Table 2. The plasma concentrations of baicalin, wogonoside, wogonin, and berberine displayed double peaks in UC rats like those observed previously in normal rats (Zan et al., 2011). Rhein and baicalin reached the peak levels within 20 min and 1 h after dosing respectively, wogonoside, wogonin, and berberine displayed peak values at 3.0 h, indicating that these compounds were absorbed rapidly. Comparisons were made between the two dosages of XXD group. In 4 g/kg XXD group, the plasma Cmax values of baicalin, wogonoside, wogonin, and rhein were significantly higher than those observed in 2 g/kg XXD group (P < 0.05). In addition, the mean AUC0–24 of wogonoside in 4 g/kg XXD group also significantly greater than that observed in 2 g/kg XXD group (P < 0.05).

3.2.2. Colon tissue concentration of multiple compounds from XXD

The results showed that six compounds (coptisine, jatrorrhizine, palmatine, berberine, baicalein and emodin) were detected in...
ulcerative colon tissues. However, no significant difference in the tissue concentrations of these compounds was observed between the two XXD-treated groups \((P > 0.05)\). Additionally, the concentrations of coptisine, jatrorrhizine, palmatine, berberine, and baicalein in lesion tissues were much higher than those in plasma at 1 h postdose (plasma concentrations of these five compounds were \((0.61, 0.82, 1.0, 1.6, 6.4)\) ng/mL in 2 g/kg XXD group, and \((1.2, 4.5, 4.7, 5.3, 7.5)\) ng/mL in 4 g/kg XXD group, respectively), indicating high affinity of these compounds with target tissues (Table 3).
3.3. CCA between pharmacokinetics and efficacy of XXD

The first canonical correlation between AUC and PD, $C_{\text{max}}$ and PD, $C_{\text{tissue}}$ and PD were statistically significant ($F=2.17$, $P<0.05$, $F=4.07$, $P<0.05$, and $F=1.55$, $P<0.10$ respectively), the first canonical correlation coefficients were 0.9503, 0.9999 and 0.9307, respectively, indicating that the three sets of the first canonical variables ($U_i$, $V_t$) were positive correlated. Then, the original variables of canonical loadings above 0.3 were selected to be considered more important in canonical variables. As shown in Table 4, for AUC and PD, total exposure to five plasma compounds (baicalin, berberine, wogonoside, wogonin, and rhein) as a group mainly affected five PD indicators (macroscopic and histological scores, MPO and IL-10 level, TNF-α and NF-κBp65 IHC index); For $C_{\text{max}}$ and PD, peak exposure to two plasma compounds (wogonoside and wogonin) as a group mainly affected three PD indicators (macroscopic scores, MPO level, and TNF-α IHC index); For $C_{\text{tissue}}$ and PD, the concentration of six tissue compounds...
(coptisine, jatrorrhizine, palmatine, berberine, baicalein and emodin) as a group mainly affected five PD indicators ... PK and PD tests with loading 40.3 are bald.


Table 2
Pharmacokinetic parameters of multiple compounds after oral administration of Xie-xin decoction (XXD) at the dosages of 2 and 4 g/kg to UC rats, respectively (mean ± SEM, n = 8).

<table>
<thead>
<tr>
<th>PK Parameters</th>
<th>Baicalin</th>
<th>Wogonoside</th>
<th>Wogonin</th>
<th>Berberine</th>
<th>Rhein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X XD 2 g/kg</td>
<td>X XD 4 g/kg</td>
<td>X XD 2 g/kg</td>
<td>X XD 4 g/kg</td>
<td>X XD 2 g/kg</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>2.9 ± 0.2</td>
<td>2.5 ± 0.3</td>
<td>3.4 ± 0.2</td>
<td>3.3 ± 0.3</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.6 ± 0.6</td>
<td>0.8 ± 0.5</td>
<td>1.8 ± 0.8</td>
<td>2.2 ± 0.7</td>
<td>3.0 ± 0.8</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>752 ± 105</td>
<td>148 ± 247</td>
<td>283 ± 45</td>
<td>613 ± 90</td>
<td>4.9 ± 6.6</td>
</tr>
<tr>
<td>Vmax (ng/mL)</td>
<td>1050 ± 200</td>
<td>1634 ± 262</td>
<td>458 ± 85</td>
<td>596 ± 66</td>
<td>7.6 ± 9.9</td>
</tr>
<tr>
<td>AUC0–t (ng/mL)</td>
<td>6582 ± 1030</td>
<td>10686 ± 1733</td>
<td>3357 ± 425</td>
<td>5065 ± 532</td>
<td>65 ± 6</td>
</tr>
<tr>
<td>AUC∞ (ng/mL)</td>
<td>7035 ± 847</td>
<td>10713 ± 1730</td>
<td>3394 ± 424</td>
<td>5122 ± 524</td>
<td>72 ± 10</td>
</tr>
</tbody>
</table>

a P < 0.05 compared to 2 g/kg XXD group.

Table 3
Concentration of six compounds in ulcerative colon tissues after oral administration of Xie-xin decoction (XXD) at the dosages of 2 and 4 g/kg to UC rats (mean ± SEM, n = 8).

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration of colon tissues (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jatrorrhizine</td>
</tr>
<tr>
<td>2 g/kg XXD</td>
<td>17.0 ± 5.1</td>
</tr>
<tr>
<td>4 g/kg XXD</td>
<td>12.1 ± 2.8</td>
</tr>
</tbody>
</table>

Table 4
Canonical structures of the first pair of canonical variables between pharmacokinetics (PK) and pharmacodynamics (PD) of Xie-xin decoction in UC rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>U</th>
<th>Loading</th>
<th>V</th>
<th>Loading</th>
<th>Rc</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC and PD</td>
<td>Baicalin</td>
<td>0.9446</td>
<td>Scoremac</td>
<td>0.6595</td>
<td>0.9503</td>
<td>2.17</td>
<td>0.0188</td>
</tr>
<tr>
<td>Wogonoside</td>
<td>0.0692</td>
<td>Scoremax</td>
<td>0.6863</td>
<td>0.5836</td>
<td>0.1996</td>
<td>0.254</td>
<td></td>
</tr>
<tr>
<td>Wogonin</td>
<td>0.8125</td>
<td>MPO</td>
<td>0.6156</td>
<td>0.5836</td>
<td>0.1996</td>
<td>0.254</td>
<td></td>
</tr>
<tr>
<td>Rhein</td>
<td>0.8208</td>
<td>IL-10</td>
<td>0.6261</td>
<td>0.5836</td>
<td>0.1996</td>
<td>0.254</td>
<td></td>
</tr>
<tr>
<td>Berberine</td>
<td>0.6818</td>
<td>NF-xBp65</td>
<td>0.8801</td>
<td>0.5836</td>
<td>0.1996</td>
<td>0.254</td>
<td></td>
</tr>
<tr>
<td>TnF-α</td>
<td>0.8523</td>
<td>0.5836</td>
<td>0.1996</td>
<td>0.254</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax and PD</td>
<td>Baicalin</td>
<td>0.0377</td>
<td>Scoremax</td>
<td>-0.7200</td>
<td>0.9999</td>
<td>4.07</td>
<td>0.0426</td>
</tr>
<tr>
<td>Wogonoside</td>
<td>0.6049</td>
<td>Scoremax</td>
<td>-0.2217</td>
<td>0.5848</td>
<td>0.0426</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wogonin</td>
<td>0.7949</td>
<td>MPO</td>
<td>-0.2836</td>
<td>0.5848</td>
<td>0.0426</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhein</td>
<td>0.2768</td>
<td>IL-10</td>
<td>0.5848</td>
<td>0.0426</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berberine</td>
<td>0.1082</td>
<td>NF-xBp65</td>
<td>0.0993</td>
<td>0.3581</td>
<td>0.0426</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TnF-α</td>
<td>0.8523</td>
<td>0.3581</td>
<td>0.0426</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cissue and PD</td>
<td>Jatrorrhizine</td>
<td>0.3835</td>
<td>Scoremac</td>
<td>0.2827</td>
<td>0.9307</td>
<td>1.55</td>
<td>0.0947</td>
</tr>
<tr>
<td>Coptisine</td>
<td>0.6102</td>
<td>Scoremax</td>
<td>0.3366</td>
<td>0.5298</td>
<td>0.0947</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmatine</td>
<td>0.5061</td>
<td>MPO</td>
<td>0.4129</td>
<td>0.5298</td>
<td>0.0947</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berberine</td>
<td>0.7022</td>
<td>IL-10</td>
<td>0.5817</td>
<td>0.5298</td>
<td>0.0947</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emodin</td>
<td>0.5833</td>
<td>NF-xBp65</td>
<td>0.5298</td>
<td>0.0947</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baicalein</td>
<td>0.5842</td>
<td>TnF-α</td>
<td>0.7311</td>
<td>0.0947</td>
<td>0.0947</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

U: PK variables including AUC, Cmax, or Cissue of plasma compounds and concentration of ulcerative colon tissue compounds (Cissue); V: PD variables including macroscopic and histological scores (Scoremac and Scorehis), MPO and IL-10 level, TNF-α and NF-xBp65 IHC index; Rc: the first canonical correlation coefficient; PK and PD tests with loading > 0.3 are bald.

4. Discussion

Our results indicated that oral treatment with XDD for 8 days significantly promoted the recovery of colitis and inhibited the inflammatory response, which were verified by macroscopic and histological examination, MPO level assay, the enhanced level of IL-10, as well as the decreased expression of TNF-α and NF-xBp65 in ulcerative colon tissues. It showed that XDD was an effective formula for the therapeutic use of UC rats, in part by modulating the balance between pro- and anti-inflammatory cytokines during the formation of colonic inflammation. Although individual PD indicator may directly or indirectly reflect different biological responses to colonic inflammatory injury, their combinations could capture more information. Furthermore, the efficacy of a formula derives from the combined action of multiple compounds rather than the isolated compound. Therefore, further investigation of possible joint effects of these compounds on UC rats is warranted. So, in the following combination analysis of PK/PD, we respectively selected six PD indicators as one set of PD variables (V), and AUC0–t or Cmax of five compounds and Cissue of six compounds as another set of PK variables (U).
As a useful tool for multivariate analysis, CCA has gained acceptance in the study of TCM complex problem. For instance, CCA was used to assess the spectrum-effect relationships of the components from different Chinese herbs (Ni et al., 2009; Kong et al., 2009; Jiang et al., 2012). Study the compatibility of TCM formulae (Song et al., 2003; Dai et al., 2004), and explore the relationship between the toxicity-attenuating effect and the variation of chemical contents in herbs caused by processing (Wang et al., 2009). However, the method has been rarely used in correlation analysis between PK and PD of a TCM formula.

In this study, CCA used the information from all the variables in both PK and PD variable sets and maximized the estimation of the relationship between the two sets. As a result, we found that there were positive correlations between AUC and PD, Cmax and PD, Cissue and PD according to the significant first canonical correlation coefficients. Additionally, we identified the key PK and PD variables that might contribute to discovering the active compounds based on the original variable loadings to the canonical variables. That is, the plasma total exposure (AUC) to five compounds (baicalin, berberine, wogonoside, wogonin, and rhein) tended to be associated with three PD indicators (macroscopic scores, MPO level, and TNF-α IHC index), and the target tissue exposure (Cissue) to six compounds (coutisine, jatrophlorizine, palmatine, berberine, baicalin, and emodin) tended to be associated with the four PD indicators (MPO and IL-10 level, TNF-α and NF-κB IHC index). These results suggested that certain compounds in different PK variables as a group may influence certain PD variables.

TNBS-induced UC model in present study was widely adopted to mimic acute phase in human UC and assess the effects of drugs (Meng et al., 2011). It was characterized by extensive infiltration of inflammatory cells and colonic ulcers. Similar to acute phase, these major histological features also appeared in chronic phase of UC. Moreover, the changes of pro- (TNF-α, NF-κB, IL-6, IL-8) and anti-inflammatory cytokine (IL-10, IL-4) levels in this model were consistent with observation in clinic UC cases (Zhou et al., 2006). Considering the above, this acute UC model was selected to evaluate the therapeutic effects of XXD. The relationships between PK and PD of XXD in chronic UC model can for further investigation.

In summary, XXD has a benefit therapeutic effect in TNBS-induced rats and its potential mechanism might be related to modulate the imbalance between pro- and anti-inflammatory cytokines. The material basis of XXD correlated to the efficacy of five compounds in plasma or six compounds in target tissues were determined using CCA evaluation. This study provides a meaningful access for PK–PD assessment of TCM formulae.

Acknowledgments

The authors are grateful for the financial support from the National Natural Science Foundation of China (No. 81273658), Program for Shanghai Innovative Research Team in University (2009), and 085” First-Class Discipline Construction of Science and Technology Innovation (085ZY1205).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2013.04.008.

References