Effects of Shengjiangxiexin decoction on irinotecan-induced toxicity in patients with UGT1A1*28 and UGT1A1*6 polymorphisms

Deng Bo, Jia Liqun, Tan Huangying, Lou Yanni, Li Xue, Li Yuan, Yu Lili

Abstract

OBJECTIVE: To evaluate the efficacy of Shengjiangxiexin decoction (SXD), prepared with a formula from Traditional Chinese Medicine (TCM), in reducing irinotecan-induced hematological and gastrointestinal toxicities in patients with UDP-glucuronosyltransferase (UGT)1A1*28 and UGT1A1*6 polymorphisms.

METHODS: This clinical trial included 115 patients receiving irinotecan combined with 5-fluorouracil plus l-leucovorin (FOLFIRI) treatment. All patients consented to UGT1A1*28 and *6 gene polymorphism detection prior to chemotherapy. SXD were administered from 1 day prior to chemotherapy to 6 day post chemotherapy. Chemotherapy induced adverse reactions (neutropenia, diarrhea, nausea, vomiting, anorexia and infection) were recorded, and short-term effect of chemotherapy was evaluated regularly.

RESULTS: A total of 50 patients had *1/*1 wild genotype, 58 patients had single allele variants with genotype *1/*6 or *1/*28, and 7 patients had two alleles variants with genotype *6/*6, *28/*28 or *6/*28. In *1/*6 or *1/*28 patients (high risk group), 9 patients (15.5%) developed grade 1 – 2 diarrhea and no patient developed severe diarrhea; neutropenia occurred in 19 patients (32.8%) and only 3 patients (8.6%) developed severe neutropenia. There were no significant differences in any toxic effects (neutropenia, diarrhea, nausea, vomiting, anorexia or infection) between *6 or *28 variant patients (high risk group) and wild type patients. No severe toxicity was found in high risk two alleles variants patients (*6/*6, *6/*28 or *28/*28). No significant differences were observed between UGT1A1*6/*28 polymorphisms and clinical response of chemotherapy.

CONCLUSION: SXD could significantly reduce irinotecan-induced hematological and gastrointestinal toxicities in UGT1A1*28 or *6 variant patients (high risk group), while this treatment didn’t affect clinical response of chemotherapy.

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Key words: UGT1A1 enzyme; Diarrhea; Irinotecan; Shengjiangxiexin decoction

INTRODUCTION

7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin (irinotecan) is a chemotherapeutic agent efficacious in the treatment of colorectal cancer and lung cancer. Chemotherapy with irinotecan is often accompanied by unpredictable, dose-limiting and life-threatening diarrhea and myelosuppression, attributed to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38). SN-38 is converted to an inactive
Traditional Chinese Medicine (TCM) herbs have been widely used in China to relieve side effects of chemotherapy. Previous study showed Banxiaxiexin decoction or Shengjiangxiexin decoction (SXD) was effective in preventing and controlling irinotecan-induced diarrhea. However no study to date has evaluated effects of TCM herbs in relieving irinotecan-induced hematological and gastrointestinal toxicities, combined with UGT1A1*6/*28 polymorphisms. In this study, the effects of SXD in reducing irinotecan induced hematological and gastrointestinal toxicities were evaluated in patient with UGT1A1*28 and UGT1A1*6 polymorphisms.

MATERIALS AND METHODS

Participants
This study was conducted at China-Japan Friendship Hospital. From June 2011 to February 2014, 115 malignant tumor patients treated with irinotecan combined with 5-fluorouracil plus l-leucovorin (FOLFIRI) were included in the study. All patients gave informed consent and the trial was approved by the Ethics Committee of China-Japan Friendship Hospital.

Inclusion criteria
Middle or advanced stage malignant tumor confirmed by pathological or cytological examination; patients treated with FOLFIRI; no serious heart, liver or renal dysfunction; patients of 18 to 75 years old; expected lifetime > 3 months; Eastern Cooperative Oncology Group (ECOG) score ≤ 1 point; Informed consent provided.

Exclusion criteria
Patients without pathological or cytological diagnosis; patients have contraindications of irinotecan; chronic diarrhea (> 3 times per day) before chemotherapy; patients with gastrointestinal bleeding, serious electrolyte disturbance or acid-base imbalance; patients treated by antibiotics or had been treated by antibiotics more than 1 week in 3 month before enrollment.

UGT1A1 genotyping
Genomic DNA was extracted from peripheral blood, stored at −80 °C until analysis, with the use of a QIAamp Blood Kit (QIAGEN, Valencia, CA, US). The G71R polymorphism (*6) and TATA box polymorphism (*28) were analyzed by the polymerase chain reaction-restriction fragment length polymorphism method. PCR specific primers were 5’-CTG ACC TTT GTG GAC TGA C-3’ (forward) and 5’-TGC CCG AGA CTA ACA AAA GAC T-3’ (reverse). After purification, *6 and *28 was analyzed by the direct sequencing method using ABI-3730. Genotyping result was showed in Chromas 2.31 (Technelysium Pty Ltd., Brisbane QLD, Australia). Upstream promoter and downstream sequence of UGT1A1, TA repetition in A (TA) nTA and G > A in 21 of in exon1 1 was read by a specialist.

Preparation of Shengjiangxiexin decoction
SXD was prepared with Shengjiang (Rhizoma Zingiberis Recens) 12 g, Gancao (Radix Glycyrrhiza) 9 g, Gangshen (Radix Codonopsis) 9 g, Ganiang (Rhizoma Zingiberis) 3 g, Huangqi (Radix Astragali Mongolici) 9 g, Banxia (Rhizoma Pinelliae) 9 g, Huanglian (Rhizoma Coptidis) 3 g, Dazao (Fructus jujubae) 24 g. All TCM herbs were purchased from the pharmacy of China-Japan Friendship Hospital and were identified and authenticated by the head of the department. According to Pharmacopeia of the People’s Republic of China (2010), extract amounts of component herbs were weighed according to the classic percentage and mixed well. The mixture was soaked in distilled water for 30 min and then boiled in 8 volumes of water (v/w) for 1 h and extracted twice. The supernatant was condensed to 200 mL.

Treatment
Patients were treated with l-leucovorin 200 mg/m² as a 2-h intravenous infusion and irinotecan 180 mg/m² as a 90-min intravenous infusion, followed by a bolus intravenous injection of 5-fluorouracil 400 mg/m² and a 46-h intravenous infusion of 5-fluorouracil 2400 mg/m², repeated every 2 weeks (FOLFIRI). Treatment was discontinued if grade 3 or 4 toxicity recurred in a subsequent cycle, despite dose reduction. From patients 24 h before chemotherapy, patients were treated with SXD (100 mL b.i.d. p.o.), with 7 days taken as one treatment course. Treatment was continued until disease progression, unacceptable adverse events, or withdrawal of consent by the patient.

Toxicity assessments
Toxicity was evaluated according to National Cancer Institute Common Toxicity Criteria for Adverse Events, Version 3.0, in all patients who had received FOLFIRI at least once. Complete blood counts and hepatic and renal function tests were performed before the initiation of each cycle. Patients were questioned specifically about diarrhea, nausea and vomiting, and appetite during chemotherapy.
Efficacy assessments
In patients who had received FOLFIRI for at least 2 chemotherapy periods, responses were assessed by computed tomography or magnetic resonance imaging according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria.\textsuperscript{13} Efficacy was evaluated on the basis of the overall response rate.

Complete response (CR): disappearance of all target lesions. Partial response (PR): at least a 30% decrease in the sum of the long diameter (LD) of target lesions, taking as reference the baseline sum LD. Stable disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started. Progressive disease (PD): at least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

Statistical analysis
SPSS 19.0 (IBM SPSS Statistics for Windows, Version 19.0. Released 2010, IBM Corp., Armonk, NY, USA) was used to analyzed data. c² test or Fisher’s exact test was performed to evaluate the effect between groups. \( P < 0.05 \) was regarded as statistically significant.

RESULTS

\( \text{Ugt}1\alpha1 \) genotype
Participant flow is presented in Figure 1. Downriver promoter sequence of \( \text{Ugt}1\alpha1 \), TA repetition in A (TA) nTAA was shown in sequencing peak figure (Figure 2). There were 3 genotypes, TA 6/6 (\( \text{Ugt}1\alpha1*1/*1 \)), TA 6/7 (\( \text{Ugt}1\alpha1*1/*28 \)) and TA7/7 (\( \text{Ugt}1\alpha1*28/*28 \)). Frequencies of TA6/6, TA6/7, TA7/7 genotypes for \( \text{Ugt}1\alpha1*28 \) were 73.9 \% (\( n = 85 \)), 25.2 \% (\( n = 29 \)) and 0.9 \% (\( n = 1 \)) respectively. Upriver promoter sequence of \( \text{Ugt}1\alpha1 \), 211 nucleotide substitution in exon 1 was shown in sequencing peak figure (Figure 3). There were 3 genotypes, G/G (\( \text{Ugt}1\alpha1*1/*1 \)), G/A (\( \text{Ugt}1\alpha1*1/*6 \)) and A/A

Figure 1 Participant flow
FOLFIRI: irinotecan+5-fluouracil+l-leucovorin; UGT: UDP-glucuronosyltransferase.
Table 1 UGT1A1 genotype

<table>
<thead>
<tr>
<th>Genotyping</th>
<th>No. of patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1*28</td>
<td>TA6/TA6</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>TA6/TA7</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>TA7/TA7</td>
<td>1</td>
</tr>
<tr>
<td>UGT1A1*6</td>
<td>G/G</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>3</td>
</tr>
</tbody>
</table>

Note: UGT: UDP-glucuronosyltransferase.

*6/*28 variants and patients’ sex, age, primary tumor sites, number of metastatic sites and so on. Most patients were in good overall physical condition, and all patients had a performance status of 0 or 1 according to the ECOG- scale.14

Toxicity between UGT1A1*28 polymorphisms

In this study, neutropenia occurred in 32 patients (27.8%) and delayed-onset diarrhea occurred in 15 patients (13.2%). Severe delayed-onset diarrhea occurred in 2 patient (1.7%), and 5 (4.3%) patients developed severe neutropenia. Only 1 *28/*28 (TA7) patient was detected, so it was not included in statistical analysis. In *1/*28 (TA6/7) patients (high risk group), neutropenia occurred in 8 patients (27.6%) and delayed-onset diarrhea occurred in 5 patients (17.2%). No significant difference was found between TA6/7 patients and TA6 patients (wild type) in neutropenia (27.6 % vs 28.2%, P > 0.05) or diarrhea (17.2 % vs 11.8%, P > 0.05).

No severe toxicity was found in TA7 patient. In *1/*28 (TA6/7) patients (high risk group), only 2 patient (2.4%) developed severe diarrhea and no patient developed severe neutropenia (0%). No significant difference was found between TA6/7 patients and TA6 patients (wild type) in severe neutropenia (0% vs 5.9%, P > 0.05) or severe diarrhea (2.4% vs 0%, P > 0.05, Table 2).

Toxicity between UGT1A1*6 polymorphisms

Only 3 *6/*6 (A/A) patients were detected, so they were not included in statistical analysis. In *1/*6 (G/A) patients (high risk group), neutropenia occurred in 11
patients (31.4%) and delayed-onset diarrhea occurred in 6 patients (17.1%). No significant difference was found between G/A patients and G/G patients (wild type) in neutropenia (31.4% vs 27.3%, P > 0.05) or diarrhea (17.1% vs 11.7%, P > 0.05). No severe toxicity was found in AA patients. In *1/*6
G/A) patients (high risk group), only 3 patient (8.6%) developed severe neutropenia and no patient developed severe diarrhea (0.0%). No significant difference was found between G/A patients and G/G patients (wild type) in sever neutropenia (8.6% vs 2.6%, P > 0.05) or sever diarrhea (0% vs 2.6%, P > 0.05) (Table 3).

Toxicity between UGT1A1*6*/28 polymorphisms

In two allele variants patients (*6/*6, *6/*28 or *28/*28), only 1 patient (14.3%) developed diarrhea and no patient developed neutropenia. In single allele variants patients (*1/*6 or *1/*28), 9 patients (15.5%) developed diarrhea and 19 patients (32.8%) developed neutropenia. In wild type patients (*1/*1), 5 patients (10.0%) developed diarrhea and only 13 patients (24.0%) developed neutropenia. There were no significant differences in any toxic effects in two alleles variants patients, single allele variants patients and wild type patients (P > 0.05, Table 4).

No severe toxicity was found in two allele variants patients (*6/*6, *6/*28 or *28/*28). In single allele variants patients (*1/*6 or *1/*28), only 2 patients developed severe diarrhea (3.4%) and 3 patients (5.2%) developed severe neutropenia. In wild type patients (*1/*1), no patients developed severe diarrhea and only 2 patients (4.0%) developed severe neutropenia. There were no significant differences in sever toxic effects in two alleles variants patients, single allele variants patients and wild type patients (P > 0.05, Table 5).

Efficacy

Response was assessable in 86 of the 115 patients (74.8%). Patients with PR, SD, and PD were 11, 55 and 20, respectively. No significant differences were observed between UGT1A1*6*/28 polymorphisms and clinical response (Table 6).

DISCUSSION

UGT1A1*28 polymorphism was generated by the change of TA repeats in the TATA box of UGT1A1 promoter. The frequency of UGT1A1*28 was higher in Whites than in Asians (40-50 vs 15%-20 % for TA6/TA7; about 10 vs 4%-6% for TA7/TA7). The role of UGT1A1*28 polymorphism in the development of irinotecan-induced diarrhea and neutropenia had been documented in many studies. In contrast, the polymorphism of UGT1A1*6 characterized by a single nucleotide substitution in exon 1 of UGT1A1 (211G > A; G/G, G/A, and A/A genotypes), UGT1A1*6 polymorphism occurred at a higher frequency in Asians (about 20%), but not in Whites. Although the association between UGT1A1*6 allele and diarrhea was occasionally reported, the toxicity closely related to UGT1A1*6 allele was still neutropenia. During chemotherapy, the appetite and the digestive function of the body would be affected. In SXD, Shengjiang solves the disorder of the Qi moving up and down in the digestive system to release the bloating, and disperse water and to nourish the stomach. Zingerone and Zingerol from Shengjiang (Rhizoma Zingiberis Recens) were report to attenuate colonic motility in vivo. The herb Banxia (Rhizoma Pinelliae) and Ganjiang (Rhizoma Zingiberis) are spicy and hot, so to disperse Cold, Huangqin (Radix Scutellariae Baicalensis) and Huanglian (Rhizoma Coptidis) are bitter and cold, so to dispel Fire in the body. These herbs were reported to attenuate diarrhea and vomiting in vivo. Dangshen (Radix Codonopsis), Gancao (Radix Glycyrrhizae) and Dazao (Fructus Jujubae) invigorate and strengthen the spleen and stomach, replenishing Qi. These herbs were reported to have immunostimulating property in vivo.

In this study, neutropenia occurred in 32 patients treated with SXD, which is significantly lower than in untreated patients (27.8 % vs 65.9%, P < 0.0001) reported previously. Delayed-onset diarrhea occurred in 15 patients and 43 patients developed nausea, significantly lower than in untreated patients (13.0 vs 28.6%, P < 0.01 and 37.4 % vs 71.4%, P < 0.0001) reported previously. Severe delayed-onset diarrhea occurred in 2 patient, and 5 patients developed severe neutropenia, significantly lower than in untreated patients (1.7 % vs 7.5 %, P < 0.05 and 4.3 % vs 20.3%, P < 0.0001) reported previously. In UGT1A1*28 or UGT1A1*6 variant patients (high risk group), toxicity and efficacy were same as wild type group (P > 0.05). These result indicated that SXD treatment could effectively relieved irinotecan-induced hematomical and gastrointestinal toxicities in high risk group, while this treatment didn’t affect clinical response.

Our results were consistent with previous animal experiments. SXD could significantly relieve diarrhea in a rat model of irinotecan-induced delayed-onset diarrhea.
rhea, while decreasing animal weight loss and increasing food-intake. Histological structure of intestinal and colonic mucosa was protected in SXD treated group. SXD treatment promoted the recovery of crypt cells, such as Paneth cells, endocrine cells and goblet cells were relieved. This result indicates that SXD facilitates the regeneration of intestinal cells and colonic cells, promotes crypt recovery without affecting normal cell differentiation. In addition, CD4+ and CD8+ T lymphocytes and SIgA in intestinal and colonic mucosa were increased significantly. This result indicates that the targets and mechanism of SXD treatment related to promote the recovery of intestinal mucosal immunity barrier.

In conclusion, SXD could effectively reduced irinotecan-induced hematological and gastrointestinal toxicities in UGT1A1*28 and/or UGT1A1*6 variant patients through multiple targets of action. Irinotecan-induced hematological and gastrointestinal toxicities were also reduced by SXD treatment in UGT1A1*28 and/or UGT1A1*6 variant patients. In addition, SXD didn’t affect clinical response.

REFERENCES

Deng B et al. / Clinical Study

