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Pharmacovigilance of herb-drug interactions: A pharmacokinetic study on the combination administration of herbal Kang'ai injection and chemotherapy irinotecan hydrochloride injection by LC–MS/MS

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ABSTRACT

Chinese herbal drugs are often combined with chemotherapy drugs for the treatment of cancers. However, the combination administrations often do not have scientifically sound bases established on full preclinical and clinical investigations. A commonly used anti-colon-cancer herb-drug pair, irinotecan (CPT-11) hydrochloride injection and Kang'ai (KA) injection was taken as an example to investigate the possible pharmacokinetic interactions between Chinese herbal drugs and chemotherapy injections to determine the potential adverse drug reactions (ADRs). Rats were randomly divided into three groups and received 20 mg/kg CPT-11 injection 15 min after administration of 4 mL/kg saline for the CPT-11 single administration group and 4 mL/kg KA injection for the separated co-administration group, respectively. In the pre-mixed co-administration group, rats received a mixture of 20 mg/kg CPT-11 injection and 4 mL/kg KA injection. Blood samples were collected at 10 pre-determined time points between 0 and 24 h. The tissue samples were collected at 5 and 8 min after the injections, respectively. A reliable LC–MS/MS method was established for the simultaneous determination of CPT-11 and its metabolites, SN-38, SN-38 G and APC in the rat plasma and tissue samples, after full confirmation of two injections chemical and stability compatibilities. Compared to the C_0 (5129 ± 757 ng/mL) and AUC_{0-t} (7858 ± 1307 ng h/mL) of CPT-11 in the CPT-11 single administration group, the C_0 (4574 ± 371 ng/mL) and AUC_{0-t} (8779 ± 601 ng h/mL) after the separated co-administration remained unchanged, but the pre-mixed co-administration resulted with a significant increased C_0 (29,454 ± 12,080 ng/mL) and AUC_{0-t} (15,539 ± 5165 ng h/mL) ($p < 0.05$). Since the exposures of CPT-11 in most tissues in the pre-mixed co-administration group were dramatically lower than the separated co-administration group, the increased CPT-11 plasma concentration may be produced by the delayed tissue distribution because of the encapsulation by the components contained in KA injection, such as polysaccharides. Similar differences were also found in its metabolite, SN-38 G. There are obvious herb-drug interactions between CPT-11 injection and KA injection after the pre-mixed co-administration. The resulting excessive CPT-11 in the plasma may lead to many serious ADRs. Therefore, the full evaluation of herb-drug interactions is necessary and inappropriate combinations should be avoided.

1. Introduction

Chinese herbal drugs have long been recognized for their therapeutic properties. Many countries around the world have been using Chinese herbal medicines to treat a myriad of maladies for centuries. In recent years, in order to improve the efficacy of single-compound-based chemical medicines in treating complex diseases, such as cancer, Chinese herbal drugs are often co-administered with such medicines [1,2]. For example, the combination of Chinese herbal medicines and chemotherapy drugs can reduce the

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side effects of chemotherapy on cancer patients, thereby restoring impaired immune function and improving patients' quality of life [3,4]. However, the combination administrations based on incomplete clinical experiences often do not have scientifically sound bases established on full preclinical and clinical investigations. Considering that inappropriate combinations may lead to serious herb-drug interactions, the Chinese National Health Commission developed 'Basic Principles for Clinical Use of Chinese Herbal Injections' and 'National Essential Drugs Clinical Application Guideline (Traditional Chinese Medicine)' to forbid the pre-mixed co-administration of Chinese herbal injections and chemotherapy injections [5,6]. Nevertheless, these guidelines are not supported by concrete pre- and clinical evidence.

Irinotecan (CPT-11, 7-ethyl-10[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin), a semi-synthetic derivative of camptothecin (CPT) and topoisomerase I inhibitor, is hydrolyzed by carboxylesterases to produce SN-38 (7-ethyl-10-hydroxycamptothecin), a metabolite that is thousand times more cytotoxic than CPT-11 [7]. SN-38 is inactivated by the UGT1A1 (uridine diphosphate glucuronosyltransferase 1A1) to form an inactive metabolite SN-38 G (SN38 glucuronide) [8]. Moreover, CPT-11 can be oxidized by CYP3A (Cytochrome P-450 3A isoforms) to produce an inactive metabolite APC (7-Ethyl-10-(4-*N*-aminopentanoic acid)-1-piperidino) carbonyloxycamptothecin) [9]. CPT-11 is the first-line chemotherapy drug for colorectal cancer [10]. However, the dose-limiting toxicities, such as diarrhea and myelosuppression toxicity, always interfere with the clinical use of CPT-11 [11].

Kang'ai (KA) injection, a mixture of oxymatrine, *ginseng* (the root and rhizome of *Panax ginseng* C.A.Mey.) extract and *astragalus* (the root of *Astragalus membranaceus* (Fisch.) Bunge) extract, is a Chinese herbal injection authorized by China National Medical Products Administration for the treatment of tumors. In Chinese hospitals, KA injection is often combined with irinotecan or other chemotherapy drugs to treat cancers [12–17]. Researches demonstrated that KA injection can reduce the side effects and improve the therapeutic efficacy of irinotecan [14,15].

In this study, the commonly used anti-colon-cancer herb-drug pair, irinotecan hydrochloride injection and Kang'ai injection was taken as an example to investigate the possible herb-drug interactions through rats pharmacokinetic determination. The obtained results could be helpful to determine the potential adverse drug reactions (ADRs), build up reliable information on combination therapy of Chinese herbal drugs and chemical drugs and complete existing guidelines.

2. Materials and methods

2.1. Reagents and materials

Irinotecan hydrochloride injection (5 mL:100 mg) was donated by Hengrui Medicine Co. Ltd. (lot 17022731, Jiangsu, China). Kang'ai injection (10 mL) was purchased from Changbaishan Pharmaceutical Co., Ltd. (lot 01180409, Jilin, China). According to the national drug standard of Kang'ai injection formulated by China National Medical Products Administration (standard number WS-11222(ZD-1222)-2002)), the concentration of oxymatrine must be between 9.0 and 11.0 mg/mL and the total concentration of ginsenoside Rg1 and Re must be above 0.1 mg/mL. In this study, Kang'ai injection contained 9.25 mg/mL of oxymatrine, 0.08 mg/mL of ginsenoside Rg1, 0.06 mg/mL of ginsenoside Re and 0.09 mg/mL of astragaloside IV. The LC–MS/MS instrumentation and conditions and typical chromatograms of Kang'ai injection are displayed in supplementary materials. HPLC grade methanol and acetonitrile were purchased from Tedia Company Inc. (Fairfield, OH, USA). Analytical grade formic acid and ammonium formate were obtained from Nanjing Chemical Reagent Co. Ltd. (Nanjing, China). The chemical reference substance of CPT-11 was provided by Hengrui Medicine Co., Ltd. (Jiangsu, China). SN-38 and CPT were purchased from Aladdin (Shanghai, China). SN-38 G and APC were obtained from Toronto Research Chemicals (Toronto, Canada). Purified Water was purchased from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China).

Table 1
The MRM parameters for the analytes.

Compound	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
CPT-11	587.25	124.01	30
SN-38	392.33	349.01	19
SN-38G	568.51	393.06	25
APC	619.28	226.58	25
CPT	349.04	305.08	17

2.2. LC–MS/MS instrumentation and conditions

LC–MS/MS analysis was carried out on a Thermo Dionex Ultimate 3000 HPLC system combined with a TSQ Quantum Ultra AM triple quadrupole mass spectrometer (Thermo Fisher Scientific, MA, USA). The separation was performed on a Waters Symmetry C18 (150*3.9 mm, 5 μm) column at 35 °C with mobile phases consisted of a methanol-water solution (10:90, A) and a methanol solution (B) both containing 0.1 % formic acid and 0.1 % ammonium formate in linear gradient elution mode (A:B): 0 min (70:30) → 1.0 min (70:30) → 1.5 min (30:70) → 4.5 min (30:70) → 4.6 min (70:30) → 6 min (70:30). The flow rate was 0.9 mL/min and the injection volume was 10 μL. The MS/MS conditions were optimized as follows: spray voltage 4 kV, capillary temperature 350 °C, nitrogen sheath gas 275 kPa and auxiliary gas 35 kPa. The MS/MS determination was performed in the positive ionization multiple-reaction-monitoring (MRM) mode for CPT-11, SN-38, SN-38 G, APC and CPT (IS) through 0.2 Pa argon gas CID. The product ion spectra and chemical structures of the analytes are shown in Fig. 1. The ion pairs for MRM detection and their corresponding collision energy is listed in Table 1.

2.3. Physical stability

Physical stability was tested by visual inspections of the mixtures of CPT-11 and KA injections. CPT-11 injection was mixed with KA injection in volume ratios between 1:1 and 1:10 in sterilized ampoules. The mixtures were visually inspected for haziness, strands, particles, and precipitates with the unaided eye under normal fluorescent light at 0 and 12 h after sample preparation [18], respectively.

2.4. Chemical compatibility

The chemical compatibility was tested by the content assay and the degradation components test for CPT-11 in the mixture intended for the herbal-drug study of one volume of CPT-11 injection and four volumes of KA injection.

The mixture and CPT-11 injection were respectively diluted with methanol to prepare the test sample solutions in parallel with the corresponding references in methanol for the determination by the internal standard method using the above described LC–MS/MS conditions. The precursor-product ion pairs for MRM detection of CPT-11 and SN-38 carboxylate forms were *m/z* 605.20 → 543.30 and 411.20 → 347.10, respectively. The collision energy was 17 eV and 18 eV for CPT-11 and SN-38 carboxylate form, respectively. The fraction of the CPT-11 carboxylate form to lactone form was calcu-

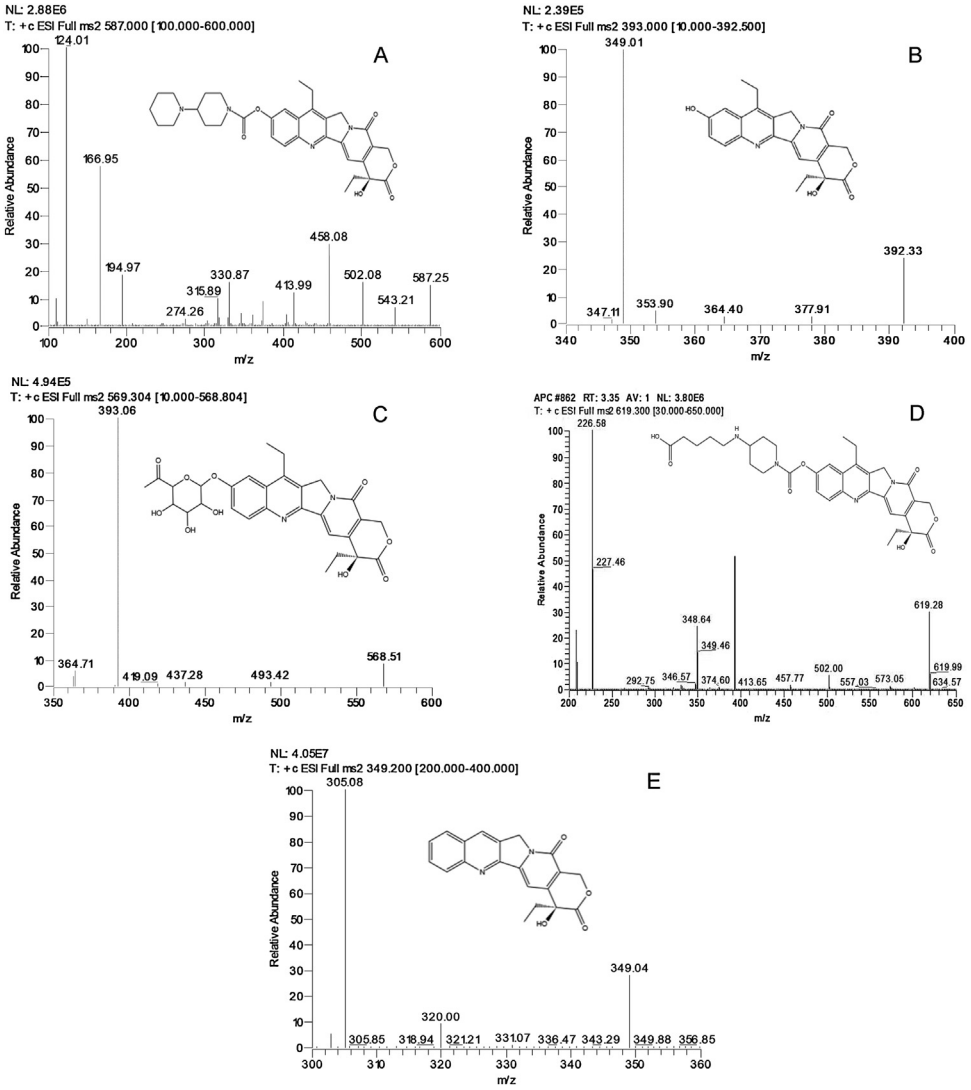

Fig. 1. The product ion spectra and chemical structures of the analytes. A.CPT-11, B.SN-38, C.SN-38 G, D.APC and E.CPT (IS).

lated by their peak areas ratio, as shown in the following equation: $(A_{\text{CPT-11 carboxylate form}}/A_{\text{IS}})/(A_{\text{CPT-11 lactone form}}/A_{\text{IS}}) \times 100\ \%$.

2.5. Herbal-drug interactions study design

The irinotecan hydrochloride injection and Kang'ai injection was taken as an example to investigate the possible herbal-drug interactions through rats pharmacokinetic determination.

Sprague-Dawley rats weighing 180–230 g, aged 8–12 weeks obtained from Shanghai SIPPR-Bk Lab Animal Co., Ltd. Rats were housed in an air-conditioned animal quarter under a standard 12 h light/dark cycle at 20–24 °C and 30–70 % humidity with free access to water and food for 7 days to adapt to the environment before the experiment. Animal studies were approved by the Animal Ethics Committee of China Pharmaceutical University (ethic approval number: 201911002) and carried out according to the Laboratory animal-guideline for ethical review of animal welfare (GB/T 35892–2018) of the Technical Committee for Laboratory Animal Sciences of the Standardization Administration of China (SAC/TC281).

Twenty-two SD rats were randomly divided into three groups:1 (6 rats), 2 (6 rats) and 3 (10 rats), each having an equal number of males and females to determine the possible pharmacokinetic interactions. Rats received a tail vein bolus intravenous injection of 20 mg/kg CPT-11 injection 15 min after intravenous administration of 4 mL/kg saline for group 1 and 4 mL/kg KA injection (presented in terms of oxymatrine, ginsenoside Rg1, ginsenoside Re and astragaloside IV were 37.0, 0.32, 0.25 and 0.35 mg/kg, respectively) for group 2, respectively; Rats in group 3 received a mixture of 20 mg/kg CPT-11 injection and 4 mL/kg KA injection via intravenous injection. The injections were diluted with saline to ensure the same volume for each intravenous injection.

About 0.2 mL blood samples were collected in heparinized tubes also spiked with 20 μL of 2 M zinc sulfate to inhibit carboxylesterase activity, from the retro-orbital venous plexus at 0, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24 h after the CPT-11 injection administration. Plasma was obtained by centrifugation at 4 °C by 1000 × *g* force for 10 min. All the plasma samples were stored at −80 °C until analysis.

Twenty-four SD rats (12 males and 12 females) were randomly divided into four groups (5–8) with six rats in each group to determine the herbal-drug interactions related tissue distributions. Rats in groups 5 and 6 received a tail vein bolus intravenous injection of 20 mg/kg CPT-11 injection 15 min after i.v. administration of 4 mL/kg KA injection; Rats in groups 7 and 8 received an intravenous injection of a mixture of 20 mg/kg CPT-11 injection and 4 mL/kg KA via the tail vein. The injections were diluted with saline to ensure the same volume for each intravenous injection. The rats in groups 5 and 7 were sacrificed 5 min after the administration, the tissue sam-

ples of heart, liver, spleen, lung, kidney, skeletal muscle, duodenum, jejunum and ileum, cecum, colon, and rectum were immediately collected and preserved at −80 °C; while those in groups 6 and 8 were collected at 8 min after the administration.

The frozen tissue samples were thawed on ice and homogenized in 5 volumes of saline with 0.2 M zinc sulfate by FSH-2A electric tissue homogenizer (Changwang Co., Ltd., Jiangsu, China). All the homogenate samples were stored at −80 °C until analysis.

2.6. Bio-assays

For the plasma samples, an aliquot of 50 μL the plasma sample was spiked with 50 μL of IS solution and 50 μL of methanol in 2 mL centrifuge tube, then 100 μL methanol containing 0.1 % formic acid to convert most of CPT-11 carboxylate form to lactone form was added. The samples were vortexed for 3 min and centrifuged at 16,000 × *g* force at 4 °C for 10 min. Then the supernatant was collected and evaporated to dryness under vacuum at 37 °C. The residue was reconstituted with 150 μL of the mobile phase and centrifuged at 16,000 × *g* force at 4 °C for 10 min again. An aliquot of 10 μL of the resulting supernatant was injected into the LC–MS/MS system for the analysis.

For the tissue samples, an aliquot of 200 μL of the homogenate tissue sample was spiked with 50 μL IS solution and 50 μL methanol before protein precipitation by 600 μL of methanol containing 0.1 % formic acid. The samples were vortex-mixed for 3 min and then centrifuged at 16,000 × *g* force at 4 °C for 10 min. Then the supernatant was collected and evaporated to dryness under vacuum at 37 °C. The residue was reconstituted with 300 μL of the mobile phase and centrifuged at 16,000 × *g* force at 4 °C for 10 min. An aliquot of 10 μL of the resulting supernatant was injected into the column for the analysis.

2.7. Calibration standards and quality control (QC) samples preparation

Stock solutions of CPT-11 (100 μg/mL) and SN-38 (50 μg/mL) were prepared separately by dissolving each reference compound a suitable amount in methanol. Stock solutions of SN-38 G (40 μg/mL) and APC (40 μg/mL) were prepared in dimethyl sulfoxide and further diluted in methanol. Working solutions of CPT-11, SN-38, SN-38 G and APC were prepared by serial dilution with methanol as their mixtures. The stock and working solutions of CPT (IS) were prepared similarly at 20 μg/mL and 1 μg/mL. The calibration standards and QC samples were prepared by spiking blank plasma or homogenate tissues with working solutions in parallel with the preparation of plasma or tissue samples. The calibration standards of CPT-11 and SN-38 were produced in the range from 9–9000 ng/mL for plasma samples, and 10–4500 ng/mL for tissue samples. The concentrations for QC samples of CPT-11 and SN-38 were set at 20, 600, 7200 ng/mL for plasma samples and 15, 300, 3600 ng/mL for tissue samples, respectively. The linear concentration range of SN-38 G and APC were prepared in the range from 3–1500 ng/mL for plasma samples and 3–750 ng/mL for tissue samples, respectively. The concentrations for QC samples of SN-38 G and APC were set at 9, 600, 1200 for plasma samples and 4.5, 300, 600 for tissue samples, respectively.

2.8. Method validation

The LC–MS/MS method was validated for selectivity, linearity, accuracy, precision, recovery, matrix effects, and stability of CPT-11 and its metabolites under determination.

Table 2
The fraction of the CPT-11 carboxylate form to lactone form.

Mobile phases pH*	CPT-11 injection (%)	Mixture of CPT-11 and KA (%)
3.60	0.19	5.00
6.50	0.31	5.70

* pH = 3.60, the pH of mobile phase A; pH = 6.50, adjusting the pH of mobile phase A to the same as the mixture of CPT-11 and KA injection.

2.9. Statistical analysis

Statistical analyses of pharmacokinetic parameters were carried out with WinNonlin 6.2 (Pharsight, St. Louis, MO, USA). Statistical significance was determined using SPSS software version 24.0 (SPSS, Inc., Chicago, IL, USA) via a two-tailed Student's *t*-test. Statistical diff ;erences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Physical stability

For up to 12 h after mixing, the mixture of CPT-11 injection and KA injection remained clear without the formation of haziness, strand, particle or precipitation. The results demonstrated that CPT-11 injection was physically compatible with KA injection.

3.2. Chemical compatibility

The concentration of CPT-11 in CPT-11 injection was 21.0 ± 1.0 mg/mL and in the mixture of CPT-11 injection and KA injection was 20.2 ± 0.6 mg/mL, respectively.

The pH of the CPT-11 injection was 3.67 ± 0.02 and the pH of the CPT-11 and KA injection mixture was 6.51 ± 0.01. The fraction of the CPT-11 carboxylate form to lactone form determined by their peak areas ratio in the two solutions are shown in Table 2. SN-38 was not found in the two solutions.

Previous research manifested that CPT-11 was mainly in its lactone form at pH < 5 and largely in its carboxylate form at pH > 8 and the two forms can be converted into each other with the variation of pH [19]. In our study, the pH increased after mixing CPT-11 injection with KA injection, however, the fraction of the CPT-11 carboxylate form to lactone form was still around 5%. It could be deduced that although the mixing of the two injections raised the pH, the lactone form remained stable as the major status. All these results indicated that CPT-11 injection was chemically compatible with KA injection.

3.3. Method validation

Selectivity: Typical chromatograms of a blank plasma sample from rats, a spiked plasma sample with CPT-11, SN-38, SN-38 G, APC and the IS, a rat plasma sample and a duodenum sample after the separated co-administration are shown in Fig. 2. There was no interference from endogenous substances and cross-talk peaks observed in this experiment.

Linearity and LLOQ: All the calibration curves were linear over the tested concentration ranges with correlation coefficients better than 0.99, as summarized in Table S1−3. The lower limits of quantification (LLOQ) sufficient for blood pharmacokinetics and tissue distribution studies were also demonstrated in Table S1−3.

Accuracy and precision: The intra- and inter-batches accuracy and precision data are demonstrated in Table S4−6. The accuracy and precision in the present assay were within the acceptable range set by the ICH for bioassay, indicating that the established method was accurate and precise.

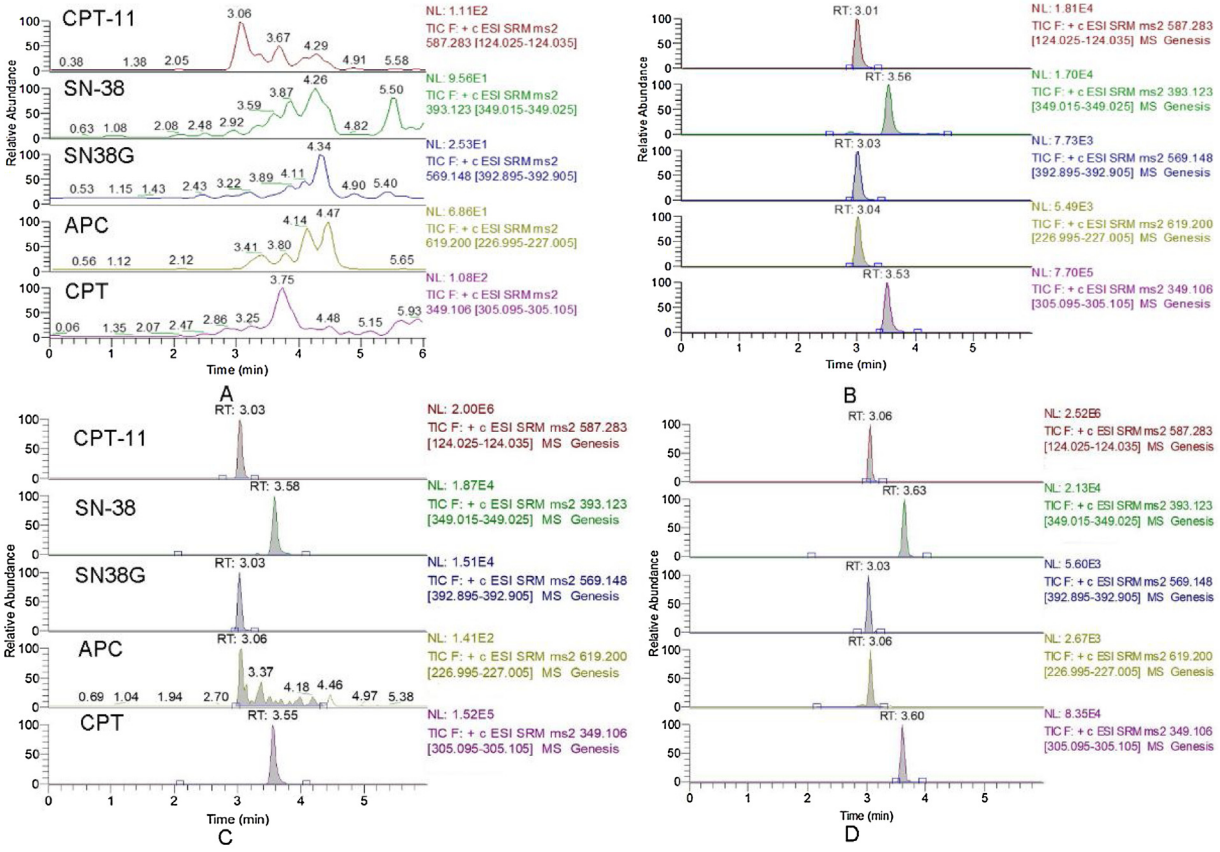

Fig. 2. Representative MRM chromatograms for the simultaneous determination of CPT-11, SN-38, SN-38 G and APC.
A. a blank plasma sample; B. a blank plasma sample spiked with CPT-11 (9 ng/mL), SN-38 (9 ng/mL), SN-38 G (9 ng/mL), APC (9 ng/mL) and IS (1 μg/mL); C. a plasma sample from a rat 5 min after the separated co-administration containing CPT-11 (4224 ng/mL), SN-38 (121 ng/mL), SN-38 G (135 ng/mL), APC (0 ng/mL); D. a duodenum sample from a rat 5 min after the separated co-administration containing CPT-11 (2187 ng/mL), SN-38 (61.2 ng/mL), SN-38 G (15.8 ng/mL), APC (7.03 ng/mL).
Retention time: CPT-11 (3.01 min), SN-38 (3.56 min), SN-38 G (3.03 min), APC (3.04 min) and IS (3.53 min).

Recovery and matrix effect: The recoveries and matrix effect of CPT-11, SN-38, SN-38 G and APC at low, medium, high QC concentrations are shown in Table S7−9. The data is represented by the mean ± standard deviation (mean ± SD). The results suggested that the recovery of the method was high and the matrix was insignificant.

Stability: The stability data of CPT-11, SN-38, SN-38 G and APC in rat plasma, intestine and liver are summarized in Table S10−12. The analytes were found to be stable in the rats plasma and tissue samples at room temperature for 4 h, after three freeze-thaw cycles, as well as stored at −20 °C for 10 days. They were stable in the autosampler at 4 °C for at least 24 h post preparation.

3.4. Herbal-drug interactions study

The possible herb-drug interactions between CPT-11 injection and KA injection were further evaluated through rats comparative pharmacokinetic study design with separated and pre-mixed co-administration. According to the drug standard and label information, the doses of CPT-11 and KA injection for human are 125 mg/m^2 and 40−60 mL per day, after conversion, 20 mg/kg CPT-11 injection and 4 mL/kg KA injection was used for rats, respectively [20,21]. There are no guidelines to specify the time interval and sequence of the separated co-administration. Therefore, after consulting local hospitals and referring to other herb-drug interaction studies, the administration of KA injection 15 min before CPT-11 injection was designed [22]. The validated LC–MS/MS method was successfully applied to determine the concentration of CPT-11, SN-38, SN-38 G and APC in rats plasma and tissue samples. The mean plasma concentration-time curves of CPT-11 and its metabolites in rats are shown in Figs. 3 and 4. The plasma pharmacokinetic parameters are summarized in Tables 3 and 4.

The results demonstrated that, for CPT-11, compared to the CPT-11 injection single administration group, no significant differences in plasma pharmacokinetic parameters were observed after the separated co-administration, however, the C_0, AUC_{0-t}, and $AUC_{0-\infty}$ of the pre-mixed co-administration group were significantly increased by 5.74, 1.98 and 1.93-fold ($p < 0.05$) and the CL was decreased by 1.94-fold ($p < 0.05$).

The drug administered by iv bolus enters the bloodstream directly, followed by rapid distribution through the circulatory system to the tissues [23]. Therefore, to study whether the pre-mixed co-administration prevented the instant distribution of CPT-11, thereby increased its plasma concentration, the tissue samples were collected at two early time points, 5 min and 8 min. Furthermore, since there are no significant differences in plasma pharmacokinetics between the single administration and the separated co-administration group, only the difference of distribution between the two co-administration groups were compared. The concentrations of CPT-11 and its metabolites in the heart, liver, spleen, lung, kidney, skeletal muscle, duodenum, jejunum and ileum, cecum, colon, and rectum at the indicated time points are shown in Fig. 5 and Tables 5 and 6.

From the result, 5 min after administration, for the pre-mixed co-administration group, the exposures of CPT-11 in most tissues such as heart, spleen, lung, kidney, skeletal muscle and colon were dramatically lower than the separated co-administration group by 1.37, 1.57, 1.51, 1.38, 1.55 and 1.28-fold, respectively ($p < 0.05$),

Table 3
The plasma pharmacokinetic parameters of CPT-11 and SN-38 after the single administration of CPT-11 injection (20 mg/kg) and the separated and pre-mixed co-administration of CPT-11 (20 mg/kg) and KA injection.

Plasma	CPT-11			SN-38		
	CPT-11 single	separated	pre-mixed	CPT-11 single	separated	pre-mixed
$t_{1/2}$ (h)	2.06 ± 0.49	1.69 ± 0.12	1.89 ± 0.47	1.79 ± 1.30	1.55 ± 0.58	2.48 ± 0.96
T_{max} (h)	NA	NA	NA	0.08 ± 0.00	0.08 ± 0.00	0.10 ± 0.05
C_0 (ng/mL)	5129 ± 757	4574 ± 371	29,454 ± 12,080*,#	NA	NA	NA
C_{max} (ng/mL)	NA	NA	NA	115 ± 32	91.0 ± 18.1	250 ± 168
AUC_{0-t} (ng h/mL)	7858 ± 1307	8779 ± 601	15,539 ± 5165*	97.3 ± 48.5	94.5 ± 38.9	125 ± 58
$AUC_{0-\infty}$ (ng h/mL)	8087 ± 1150	8847 ± 619	15,623 ± 5227*	140 ± 63	124 ± 34	188 ± 61
MRT_{0-t} (h)	1.95 ± 0.32	1.96 ± 0.09	1.37 ± 0.14*	0.91 ± 0.38	1.02 ± 0.32	1.09 ± 0.68
V_d (mL/kg)	7498 ± 2079	5534 ± 441	3483 ± 834*	NA	NA	NA
CL (mL/h/kg)	2514 ± 351	2271 ± 169	1299 ± 387*	NA	NA	NA

NA: not applicable.
* $p < 0.05$, significant difference from the CPT-11 injection single administration group.
# The samples presented in a concentration exceeding the range of quantification were diluted with the blank matrix to bring the concentrations into the range.

Table 4
The plasma pharmacokinetic parameters of SN-38 G and APC after the single administration of CPT-11 injection (20 mg/kg) and the separated and pre-mixed co-administration of CPT-11 (20 mg/kg) and KA injection.

Plasma	SN-38G			APC		
	CPT-11 single	separated	pre-mixed	CPT-11 single	separated	pre-mixed
$t_{1/2}$ (h)	6.95 ± 1.53	6.45 ± 1.90	6.05 ± 1.50	NC	NC	NC
T_{max} (h)	0.31 ± 0.16	0.22 ± 0.07	0.73 ± 1.16	1.17 ± 0.68	1.42 ± 0.66	1.40 ± 0.52
C_{max} (ng/mL)	198 ± 37	155 ± 39	185 ± 43	5.00 ± 0.78	4.73 ± 0.92	7.56 ± 4.43
AUC_{0-t} (ng h/mL)	919 ± 230	796 ± 213	1370 ± 433*	9.72 ± 5.32	10.2 ± 5.1	17.4 ± 15.5
$AUC_{0-\infty}$ (ng h/mL)	1170 ± 175	882 ± 159*	1515 ± 478	NC	NC	NC
MRT_{0-t} (h)	5.46 ± 1.70	6.54 ± 1.10	6.80 ± 1.12	1.24 ± 0.34	1.40 ± 0.43	1.58 ± 0.46

NC: not calculated.
* $p < 0.05$, significant difference from the CPT-11 injection single administration group.

Table 5
The concentration of CPT-11 and its metabolites in various tissues 5 min after the separated and pre-mixed co-administration of CPT-11 and KA injection.

Tissues	CPT-11 (ng/g)		SN-38 (ng/g)		SN-38 G (ng/g)		APC (ng/g)	
	separated	pre-mixed	separated	pre-mixed	separated	pre-mixed	separated	pre-mixed
heart	22,809 ± 1782	16,608 ± 1402*	103 ± 17	92.2 ± 19.5	14.9 ± 4.0	5.88 ± 6.51*	ND	ND
liver	18,205 ± 2858	29,956 ± 7616*	212 ± 31	219 ± 42	209 ± 45	145 ± 31*	30.5 ± 8.6	25.6 ± 9.4
spleen	13,833 ± 3667	8797 ± 1660*	52.3 ± 27.1	49.6 ± 24.5	ND	ND	ND	ND
lung	49,454 ± 10,385	32,730 ± 9999*	155 ± 22	169 ± 37	91.3 ± 19.0	68.6 ± 10.8*	ND	ND
kidney	96,517 ± 11,064	69,680 ± 8362*	717 ± 126	1092 ± 255*	724 ± 207	523 ± 92	2.76 ± 4.28	ND
skeletal muscle	12,505 ± 1002	8088 ± 1150*	44.6 ± 34.7	38.2 ± 30.0	12.4 ± 6.4	1.36 ± 3.34*	ND	ND
duodenum	10,618 ± 1096	11,348 ± 2503	309 ± 121	361 ± 71	158 ± 71	102 ± 62	77.0 ± 70.3	16.1 ± 16.4
jejunum and ileum	9393 ± 1993	7310 ± 2749	204 ± 84	278 ± 194	51.3 ± 37.3	60.1 ± 64.4	23.6 ± 25.3	7.69 ± 6.15
cecum	3986 ± 1795	3002 ± 1112	34.2 ± 40.4	12.1 ± 29.6	ND	ND	ND	ND
colon	7481 ± 948	5823 ± 1396*	34.0 ± 37.7	26.4 ± 30.9	7.75 ± 11.5	ND	4.93 ± 12.1	ND
rectum	6319 ± 1168	6380 ± 849	44.1 ± 34.7	49.8 ± 39.3	19.3 ± 15.6	6.83 ± 7.72	10.5 ± 16.5	ND

ND: not detected.
* $p < 0.05$, significant difference from the CPT-11 and KA injection separated co-administration group.

Table 6
The concentration of CPT-11 and its metabolites in various tissues 8 min after the separated and pre-mixed co-administration of CPT-11 and KA injection.

Tissues	CPT-11 (ng/g)		SN-38 (ng/g)		SN-38 G (ng/g)		APC (ng/g)	
	separated	pre-mixed	separated	pre-mixed	separated	pre-mixed	separated	pre-mixed
heart	25,716 ± 6120	19,246 ± 1834*	186 ± 44	192 ± 44	29.0 ± 3.6	22.9 ± 6.3	ND	ND
liver	64,360 ± 7501	84,169 ± 8192*	1755 ± 869	2965 ± 1094	138 ± 59	100 ± 89	67.6 ± 20.4	34.7 ± 20.4*
spleen	14,689 ± 4566	6356 ± 1760*	65.9 ± 32.4	42.8 ± 33.8	1.33 ± 3.25	ND	ND	ND
lung	60,479 ± 11,842	43,839 ± 4081*	238 ± 58	215 ± 36	98.2 ± 25.8	74.3 ± 10.0	1.51 ± 3.70	ND
kidney	122,148 ± 15,256	107,235 ± 16,898	1124 ± 272	1596 ± 360*	816 ± 254	553 ± 194	8.47 ± 6.99	6.39 ± 5.07
skeletal muscle	10,875 ± 1892	9394 ± 1332	74.3 ± 11.5	72.2 ± 19.3	13.9 ± 7.4	7.59 ± 8.41	ND	ND
duodenum	14,385 ± 3949	18,346 ± 7105	401 ± 129	578 ± 363	177 ± 119	158 ± 114	36.7 ± 25.9	12.0 ± 9.7
jejunum and ileum	12,156 ± 3566	9655 ± 4092	228 ± 106	273 ± 132	60.8 ± 57.7	67.4 ± 53.3	9.89 ± 11.4	1.60 ± 3.93
cecum	5667 ± 1594	5622 ± 1629	32.9 ± 36.8	94.1 ± 45.9*	1.52 ± 3.73	3.37 ± 8.25	ND	ND
colon	9190 ± 3028	7082 ± 1871	54.0 ± 42.3	70.6 ± 45.3	16.3 ± 12.1	1.88 ± 4.60*	ND	ND
rectum	6857 ± 736	7119 ± 506	56.1 ± 45.4	98.0 ± 29.3	35.4 ± 23.3	26.4 ± 23.4	ND	ND

ND: not detected.
* $p < 0.05$, significant difference from the CPT-11 and KA injection separated co-administration group.

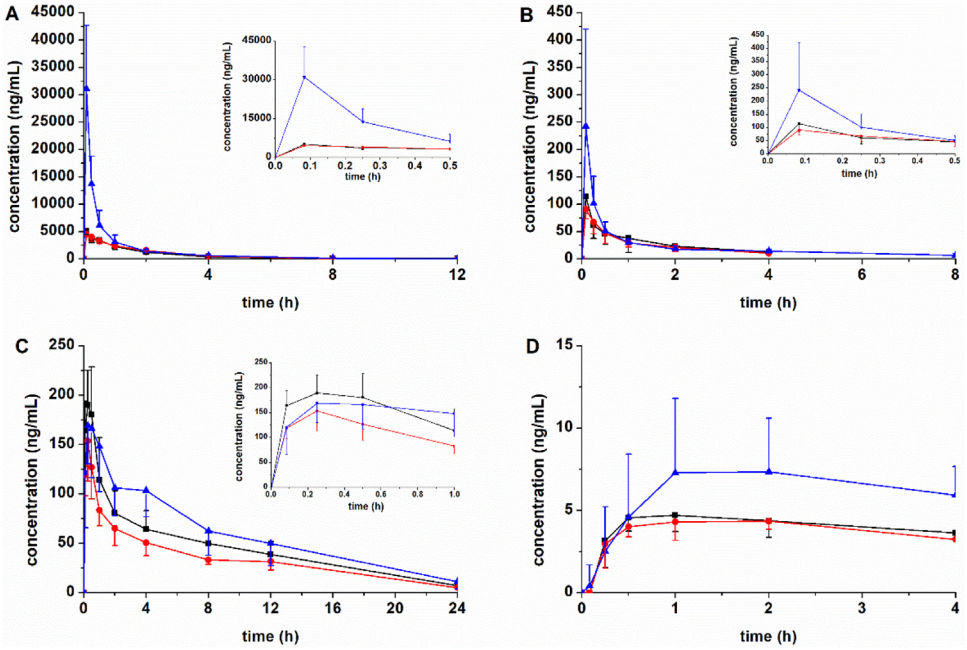

Fig. 3. The mean plasma concentration-time curves of CPT-11 and its major metabolites after the single administration of CPT-11 injection and the separated and pre-mixed co-administration of CPT-11 and KA injection.
A.CPT-11, B.SN-38, C.SN-38 G, D.APC.
CPT-11 injection single administration group.
CPT-11 and KA injection separated co-administration group.
CPT-11 and KA injection pre-mixed co-administration group.

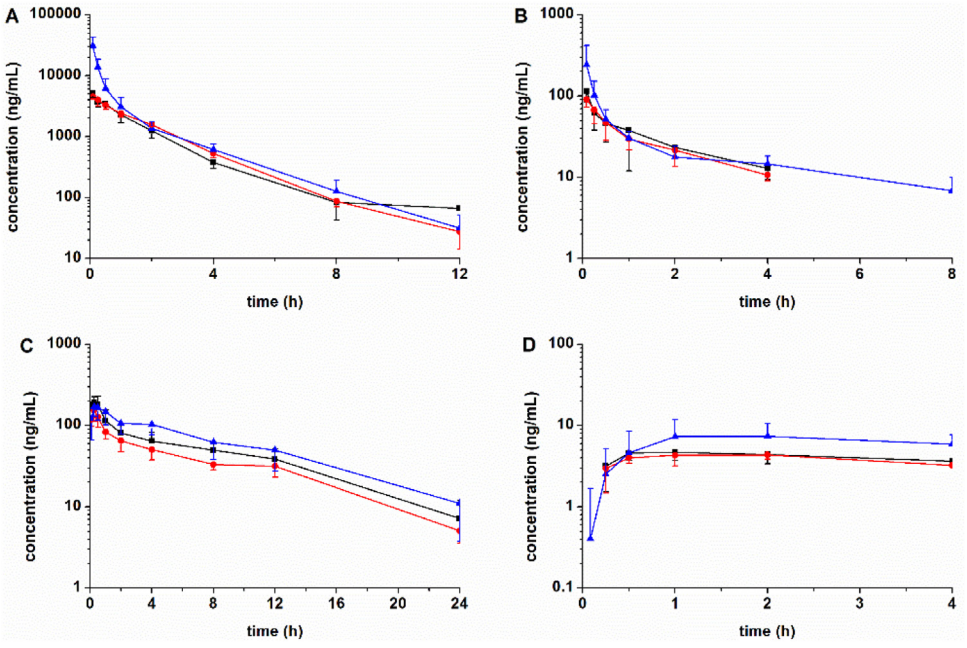

Fig. 4. The semi-log plasma concentration-time curves of CPT-11 and its major metabolites after the single administration of CPT-11 injection and the separated and pre-mixed co-administration of CPT-11 and KA injection.
A.CPT-11, B.SN-38, C.SN-38 G, D.APC.
CPT-11 injection single administration group.
CPT-11 and KA injection separated co-administration group.
CPT-11 and KA injection pre-mixed co-administration group.

except in liver was1.64-fold higher ($p < 0.05$). Such differences were gradually weakened along with the time. 8 min after pre-mixed co-administration, only the contents of CPT-11 in heart, spleen, and lung were lower by 1.33, 2.31, 1.38-fold, respectively ($p < 0.05$), and in liver were higher by 1.31-fold ($p < 0.05$).

These results demonstrated that the increased plasma concentration of CPT-11 in the pre-mixed co-administration group may be produced by the delayed distribution of CPT-11 to most tissues. It may be attributed to the complex compositions contained in the KA injection, which consists of a mixture of oxymatrine, *ginseng*

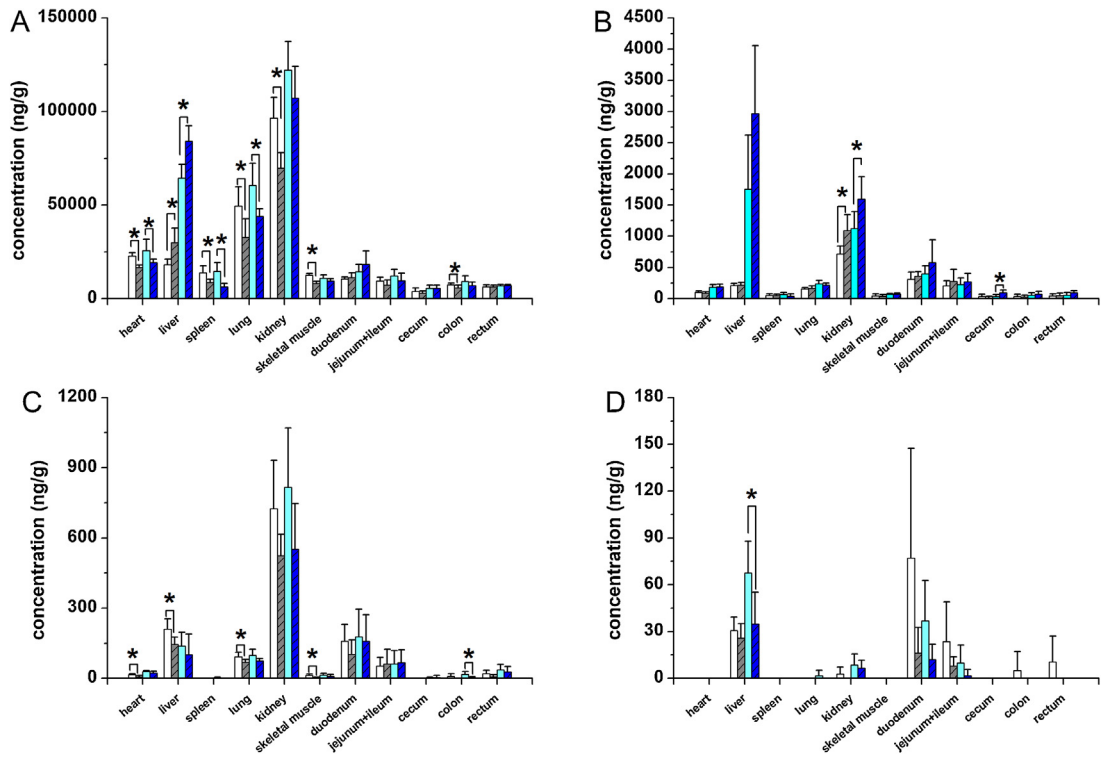

Fig. 5. The concentration-time profile of CPT-11 and its major metabolites in tissues after the separated and pre-mixed co-administration of CPT-11 and KA injection.
A. CPT-11, B.SN-38, C.SN-38 G, D.APC.
5 min after the separated co-administration of CPT-11 and KA injection.
8 min after the separated co-administration of CPT-11 and KA injection.
5 min after the pre-mixed co-administration of CPT-11 and KA injection.
8 min after the pre-mixed co-administration of CPT-11 and KA injection.
*$p < 0.05$.

extract, and *astragalus* extract. Our previous research indicated that the total solids content of KA injection was 11.1 mg/mL, except for oxymatrine, the remaining 17 % came from *ginseng* and *astragalus* extracts. According to the national drug standard of Kang'ai injection, the *ginseng* was refluxed with 90 % ethanol and *astragalus* was decocted with water at boiling temperature to prepare the *ginseng* and *astragalus* extract. Since polysaccharides are the most abundant components in *ginseng*, accounting for 40 %, and the main components of *astragalus* aqueous extract, KA injection could contain a large number of polysaccharides [24,25]. Furthermore, there are masses of oligosaccharides and glycosides with a large molecular weight in *ginseng* and *astragalus* [26–28]. The 4-piperidino-piperidine and quinoline group in CPT-11 contain basic nitrogen atoms with lone pairs of electrons, when CPT-11 injection was pre-mixed with KA injection, the glycosyl groups of glycosides, polysaccharides and oligosaccharides could form hydrogen bonds with the amine functional groups to encapsulate CPT-11. Since the large molecule compounds usually have lower vascular permeability, these glycosides and large molecule saccharides could retain CPT-11 in the blood vessels to delay its free distribution [29]. As a result, the CPT-11 level in most tissues was decreased, except the liver with discontinuous blood vessels resulting in the accumulation of macromolecules [30]. Therefore, the plasma exposure of CPT-11 in the pre-mixed co-administration group was higher. Also, the complicated competing drug-substrate or -receptor interactions between the chemical components may be another reason for this phenomenon.

For the metabolites, the plasma pharmacokinetic parameters of SN-38 and APC remained unchanged after both separated and pre-mixed co-administration, and their distribution in most of the tissues of the two co-administration groups were the same. However, similar to CPT-11, the pre-mixed co-administration dramatically increased the AUC_{0-t} of SN-38 G by 1.49-fold ($p < 0.05$) compared to the CPT-11 injection single administration group, and it decreased the exposure of SN-38 G in heart, liver, lung and skeletal muscle 5 min after administration ($p < 0.05$) compared to the separated co-administration group.

In summary, the pre-mixed co-administration of CPT-11 and KA injection resulted with a delayed tissue distribution of CPT-11, which in turn led to an increased plasma concentration, and a similar trend was observed in its metabolite, SN-38 G. However, as mentioned above, after given a separated co-administration of CPT-11 and KA injection at a 15-min interval, the rats plasma concentration of CPT-11 was as same as the CPT-11 injection single administration group. It could be deduced that the unusual distribution may not occur if the herbal and chemotherapy drugs are combined appropriately.

In addition to KA injection, there are 8 other kinds of Chinese herbal injections often prescribed for the treatment of cancers in China, most of them also contain polysaccharide-rich *ginseng* and *astragalus* extracts (Aidi injection, Shenqi Fuzheng injection and Delisheng injection) [3]. They may have similar distribution features as KA injection. Therefore, their pre-mixed co-administration with chemotherapy drugs should also be avoided. Alternatively, the separated co-administration without solvation effects could be considered.

4. Conclusion

In conclusion, this well-designed study presented solid evidence of herbal and chemotherapy drug interactions. The pre-mixed co-administration of CPT-11 and KA injection hindered the usual

distribution of CPT-11 and then led to an increased plasma concentration, because of the encapsulation by the components contained in KA injection, such as polysaccharides. The increased plasma concentration of CPT-11 may cause serious ADRs. Therefore, to exert the synergistic effects of herbs and drugs, the full evaluation of their interactions is necessary and inappropriate combinations should be avoided.

CRediT authorship contribution statement

Yanfei Chen: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing - original draft, Writing - review & editing. **Zhaoliang Hu:** Investigation, Methodology, Validation. **Wenzhu Qi:** Investigation, Methodology. **Shuxiao Gao:** Investigation, Methodology. **Jing Jiang:** Investigation, Methodology. **Shixiao Wang:** Investigation. **Lei Xu:** Investigation. **Xin Xu:** Investigation. **Min Song:** Conceptualization, Funding acquisition, Resources. **Taijun Hang:** Conceptualization, Funding acquisition, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jpba.2020.113784.

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