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•Research article•

The effects of borneol on the pharmacokinetics and brain distribution of tanshinone IIA, salvianolic acid B and ginsenoside Rg_1 in *Fufang Danshen* preparation in rats

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[ABSTRACT] *Fufang Danshen* preparation (FDP) is consisted of Salviae Miltiorrhizar Radix et Rhizoma (*Danshen*), Notoginseng Radix et Rhizoma (*Sanqi*) and Borneolum Syntheticum (borneol). FDP is usually used to treat myocardial ischemia hypoxia, cerebral ischemia and alzheimer's disease, etc. In the treatment of cerebrovascular diseases, borneol is usually used to promote the absorption and distribution of the bioactive components to proper organs, especially to the brain. The purpose of this study is investigating the effects of borneol on the pharmacokinetics and brain distribution of tanshinone IIA (TS IIA), salvianolic acid B (SAB) and ginsenoside Rg1 in FDP. Male healthy Sprague-Dawley (SD) rats were given *Danshen* extracts, *Sanqi* extracts (*Panax notoginseng* saponins) or simultaneously administered *Danshen* extracts, *Sanqi* extracts and borneol. Plasma and brain samples were collected at different points in time. The concentration of TS IIA, SAB and Rg1 was determined by UPLC-MS/MS method. The main pharmacokinetics parameters of plasma and brain tissue were calculated by using Phoenix WinNolin 6.1 software. In comparison with *Danshen* and *Sanqi* alone, there were significant differences in pharmacokinetic parameters of TS IIA, SAB and Rg1, and the brain distribution of SAB and TS IIA when *Danshen*, *Sanqi* and borneol were administrated together. Borneol statistically significant shortened t_{max} of TS IIA, SAB and Rg1 in plasma and brain, increased the bioavaiability of Rg1, inhibited metabolism of Rg1 and enhanced the transport of TS IIA and SAB to brain. These results indicated that borneol could affect the multiple targets components and produce synergistic effects. Through accelerating the intestinal absorption and brain distribution, borneol caused the effective ingredients of *Danshen* and *Sanqi* to play a quicker therapeutic role and improved the therapeutic effect.

[KEY WORDS] Borneol; *Fufang Danshen* preparation; Tanshinone IIA; Salvianolic acid B; Ginsenoside Rg1; Pharmacokinetics
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Introduction

Fufang Danshen preparation (FDP), a well-known traditional Chinese preparation,is compounded from Salviae Miltiorrhizar Radix et Rhizoma (*Danshen*,*Salvia miltiorrhiza)*, Notoginseng Radix et Rhizoma (*Sanqi*, *Panax notoginseng*),and Borneolum Syntheticum. FDP has many effects, including treating myocardial hypoxia, ischemia reperfusion injury, cerebral ischemia, alzheimer's disease, decreasing atherosclerosis and inhibiting thrombus in clinic [1–3]. The main active constituents of FDP include quinones (including tanshinone IIA, cryptotanshinone, etc.), phenolic acids (including tanshinol, salvianolic acid B, etc.), saponins (including ginsenoside Rg_1, Rb_1, Re, etc.), etc. [4–6]. In China Pharmacopoeia, TSIIA, SAB and Rg_1 have been used as markers for quality control of the FDP, Salviae Miltiorrhizar Radix et Rhizoma and Notoginseng Radix et Rhizoma [7]. Hence, in order to study pharmacokinetics behaviors of *Danshen-Sanqi* and probable interaction between *Danshen-Sanqi* with borneol, it is necessary to select TS IIA, SAB and Rg_1 as objected drugs and measure their concentration in rat plasma and brain homogenate.

Borneol is a highly lipid-soluble bicyclic monoterpene

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component from resins of *Dryoblanops armarica*. It played a role in the treatment of cardiovascular and cerebrovascular diseases [8] and was usually acted as an adjuvant component to facilitate the absorption and distribution of other ingredients to proper organs, especially to the brain. So, in the theories of traditional Chinese medicine, borneol is always employed as "upper guiding drug" in many traditional Chinese compound medicines in clinic for the treatment for CNS illness, such as stroke, cerebral ischemia and cerebritis, etc. In recent years, many researchers found that borneol could not only enhance compounds from traditional Chinese medicine distribution in the brain, such as notoginsenoside R_1, Re, kaempferol [9-10], but also promote the accumulation of chemicals in the brain tissue such as antiepileptic drug [11–13]. Recent studies demonstrated that borneol increased the brain bioavailability of drugs by opening the blood-brain barrier (BBB), and borneol had a lot of pathways to open the BBB, including loosening the intercellular tight junctions, increasing the number of pinocytosis vesicles of the BBB cells [14], suppressing the expression and activity of efflux transporters, such as P-gp and breast cancer resistance protein [14–16], and decreasing the distribution and expression of the tight junction membrane proteins [17].

In most of the previous studies, pharmacokinetics of active components from *S. miltiorrhiza* or notoginseng and its prescription were investigated. Herb-herb interaction studies showed the effects of borneol on pharmacokinetics of TS IIA, SAB and Rg_1 in plasma [9, 18-20] and brain distribution of Rg_1 in a single time [9, 21]. However, to our knowledge, there is no report on simultaneous investigating the effects of borneol on pharmacokinetics in plasma, brain distribution and elimination of TS IIA, SAB and Rg_1 at different times. Especially, little is known about the brain distribution of SAB, which is likely due to its poor brain accumulation. The protective effect of BBB vastly limits the distribution of hydrosoluble SAB to brain [22–24]. The present work is to examine whether borneol can increase the accumulation of TS IIA, SAB and Rg_1 in brain. Therefore, from the perspective of modern science, the effect of borneol on the brain accumulation of TS IIA, SAB and Rg_1 from FDP can be used as a scientific basis for the traditional Chinese medicine theory.

In this study, sensitive and selective UPLC-ESI-MS methods were developed and validated for the determination of TS IIA, Rg_1 and SAB in rat plasma and brain homogenate. We applied these methods to study the pharmacokinetic action and brain distribution of these three compounds after a single oral administration of *S. miltiorrhiza* and notoginseng or administrating *S. miltiorrhiza*, notoginseng combined with borneol.

Materials and Methods

Chemicals and reagents

Salviae Miltiorrhizar Radix et Rhizoma were purchased from Shanxi Shangluo *Danshen* Cultivation Base (Shangluo, China). The purchased herb was authenticated by Professor LI Jin-Ping, Xiangya School of Pharmaceutical Sciences, Central South University, Changsha, China. The voucher specimen was deposited at the Third Xiangya Hospital, Central South University, Changsha, China (15121403). The extracts of *P. notoginseng* saponins (containing 39.40% Rg_1, batch number Must-15060601) were purchased from Chengdu Must bio-Tech. Co., Ltd. (Chengdu, China) and borneol was purchased from Hunan Kerui Hong Tai Pharmaceutical Co., Ltd. (Changsha, China). Authentic standards TS IIA (99.08%, batch number Must-15081916), SAB (99.08%, batch number Must-15092512) and GRg1 (98.10%, batch number Must-15042215) were purchased from Chengdu Must bio-Tech. Co., Ltd. (Chengdu, China). Dorperidone (Batch number 100304-201103) and diclofenac sodium (Batch number 100334-201506, internal standard, IS) were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and formic acid (HPLC grade) were supplied by Merck & Company, Inc. (Darmstadt, Germany). Water was bought from Wahaha Group (Hengyang, China). Ethyl acetate and hydrochloric acid were supplied by the Sinopharm chemical Reagent Co., Ltd. (Beijing, China).

Instrumentation and operation conditions

We established two analysis methods to determinate the concentration of SAB and the concentration of TS IIA, Rg_1 in the plasma and brain homogenate samples, respectively. The two chromatographic analysis methods were both performed on an ACQUITY UPLC™ system (Waters Corp., Milford, MA, USA) with a cooling autosampler and a column oven allowing control temperature of the analytical column. The chromatographic separation was carried out on an ACQUITY UPLC™ BEH phenyl column (50 mm × 2.1 mm, 1.7 μm; Waters Corp., Milford, MA, USA) at 35 °C. The mobile phase was consisted of acetonitrile (A, containing 0.1% formic acid) and 0.25% formic acid (B). When determined SAB, the elution gradient was as follows: 0−1.8 min, 35% A; 1.8−4 min, 60% A; 4−5 min, 35% A. The flow rate was set at 0.25 mL·min^{-1}. Elution gradient of the determination of TS IIA and Rg_1 was carries out according to the following program: 0−3 min, 24% A; 3−4 min, 24%−85% A; 4−6 min, 85% A; 6−7 min, 24% A. It had a flow rate of 0.2 mL·min^{-1}. The inject volume was both 10 μL.

A Waters ACQUITY™ TQD triple-quadrupole tandem mass spectrometer (Waters Corp., Manchester, UK) was connected to the UPLC system *via* an electrospray ionization (ESI) source. The ESI source was operated in negative ionization mode for SAB and IS (diclofenac sodium), and in positive ionization mode for TS IIA, Rg_1 and IS (Dorperidone). The ESI source parameters were set at: capillary 3.0 kV and Source Offset 50.0 V. The temperatures of the source and desolvation were maintained at 150 and 500 °C, respectively. Nitrogen was used as the desolvation gas (1000 L·h^{-1}) and cone gas (150 L·h^{-1}). For collision-induced dissociation (CID), argon was used as the collision gas at a flow rate of 0.12 mL·min^{-1}. The multiple reaction monitoring (MRM) mode was employed for all analytes. The precursor-to-product ion pair, the optimized Cone Voltage (CV) and Collision Energy (CE) for all analytes are described in Fig. 1. Masslynx™ NT4.1 software (Waters Corp., Milford, MA,

USA) was used for data acquisition and peak integration. Post-acquisition quantitative analysis was performed using the QuanLynx™ program (Waters Corp, Milford., MA, USA).

Pharmacokinetic and brain distribution study

Healthy male Sprague-Dawley rats weighing 180−200 g were supplied by Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China). The animals were kept in a 22−24 °C room with a light/dark cycle of 12 ∶ 12 h and 55% ± 5% relative humidity. They had free access to standard rodent food and water. The rats were fasted for 12 h before the experiments. All produces were in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republics of China.

Danshen extract was obtained as follows: temperature 85 °C, 10-fold 95% ethanol as solvent and the operation time was 1.5 h. Then, the residue of *Danshen* was refluxed with 8-fold 50% ethanol at 50 °C, with the content of TS IIA reached 46.55%. Finally, the residue was extracted with water to obtain phenolic acids extract by heating at 95 °C. The SAB content was 22.5%. All filtrate was concentrated to obtain *Danshen* extract.

One hundred and twenty SD rats were randomly divided into two groups. One group of SD rats (n = 60) named *Danshen-Sanqi* group was orally administrated with the mixture of *Danshen* extract and *Sanqi* extract, equivalent to 60, 150, 300 mg·kg^{-1} of TS IIA, Rg_1 and SAB. Another group named FDP group was orally administrated with the mixture of *Danshen* extract, *Sanqi* extract combined with borneol, equivalent to 60, 150, 300, 300 mg·kg^{-1} of TS IIA, Rg_1, SAB and borneol, respectively. Suspend all extracts or borneol in 0.5% carboxymethyl cellulose sodium salt (CMC-Na) aqueous solution just before experiment. Six individuals from each group were euthanized at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8,

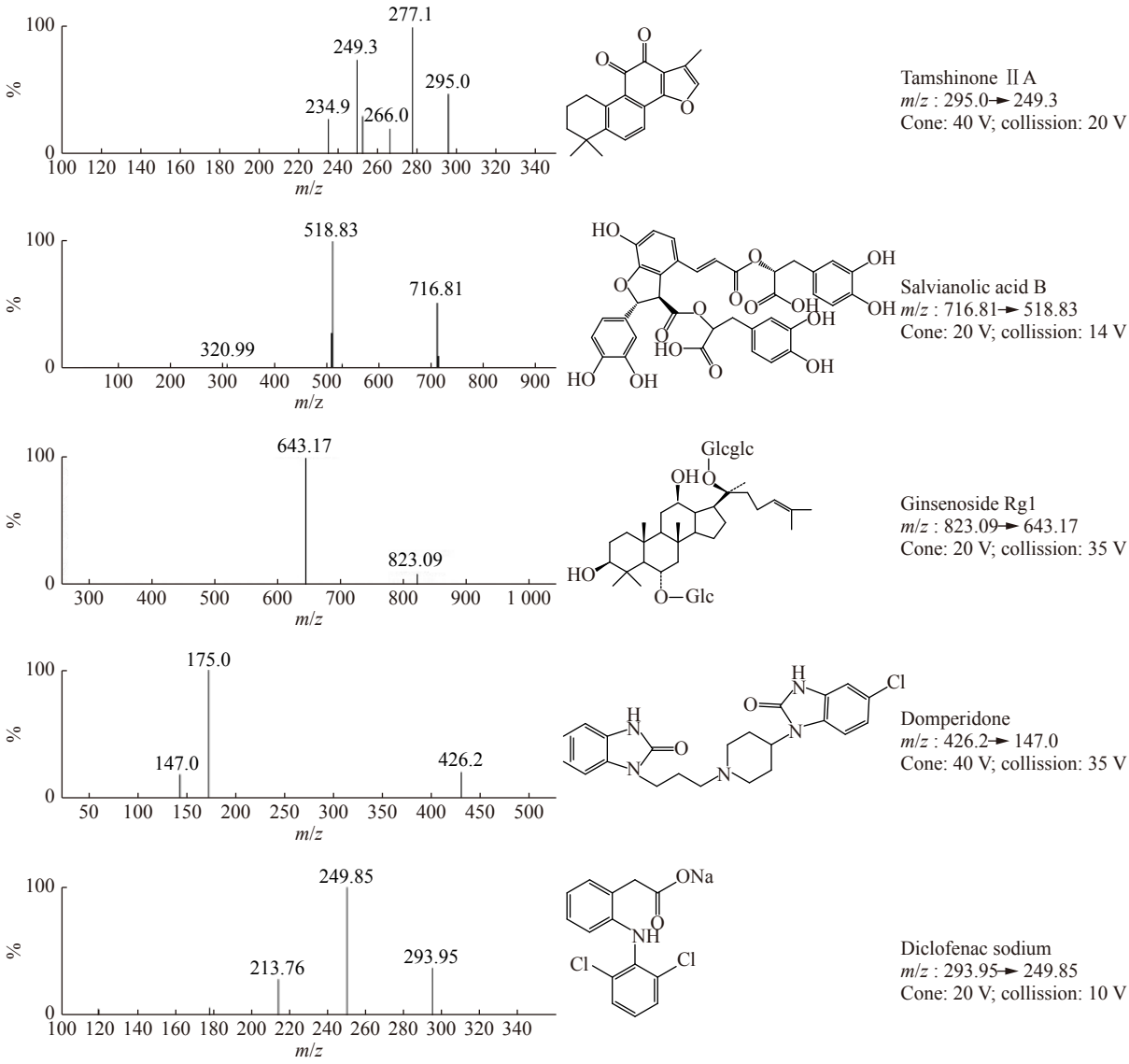

Fig. 1 The product ion scan spectrum, chemical structure, monitored transitions, cone voltage and collision energy of TS IIA, SAB, Rg_1, domperidone (IS) and diclofenac sodium (IS)

10 h following administration. For each point, we got blood samples *via* removing eyeball and harvested blood into heparinized tube. Then immediately centrifugate at 3500 r·min^{-1} for 10 min to get plasma samples. After the rat was sacrificed, brain tissue was removed. Then we washed brain with normal saline and blotted with filter paper, weighted and homogenized in normal saline to prepare 0.45 g·mL^{-1} homogenate. All these plasma and brain homogenate samples were stored at −20 °C until analysis.

Prepare plasma and brain homogenate sample

Prepare TS IIA and Rg_1 plasma samples

Add 15 μL domperidone (IS) solution (20 ng·mL^{-1}) and 300 μL acetonitrile to 100 μL plasma samples. The mixture was eddied for 10 min, and centrifuged at 13 200 r·min^{-1} for 8 min to separate the protein, then added 200 μL water to 100 μL separated supernatant. 10 μL blended clean fluid was directly injected into the UPLC-MS/MS system for analysis of TS IIA and Rg_1.

Prepare TS IIA and Rg_1 brain homogenate samples

Five microliter domperidone (IS) solution (20 ng·mL^{-1}) and 150 μL acetonitrile were added to 100 μL brain homogenate samples. The mixture was eddied for 10 min, and centrifuged at 13 200 r·min^{-1} for 8 min to separate the protein. Then, 150 μL water was added to 100 μL separated supernatant. 10 μL blended clean fluid was directly injected into the UPLC-MS/MS system for analysis of TS IIA and Rg_1.

Prepare SAB plasma and brain homogenate samples

200 μL plasma or brain homogenate samples were mixed with 10 μL diclofenac sodium (IS) solution (1 μg·mL^{-1}) and 80 μL HCl (1 mol·L^{-1}), followed by adding 2 mL acetic ether, and shook at 1500 r·min^{-1} for 15 min. After centrifuging the mixture at 4000 r·min^{-1} for 8 min, we drew the supernatant to a new tube, and evaporated it to dryness under a stream of N_2 at 40 °C. The residue was dissolved in 150 μL acetonitrile-water (50 : 50, *V*/*V*), and 10 μL clean supernatant was injected into the UPLC-MS/MS system for quantitation of SAB.

Method validation

Specificity

To investigate the specificity of the method, MRM chromatogram of blank plasma or brain homogenate from six rats, the lower limit of quantitation (LLOQ) with IS (domperidone, diclofenac sodium) and plasma or brain homogenate samples from rats after the oral administration of FDP were compared.

Linearity and LLOQ

Linearity was accessed by analyzing TS IIA standard samples over 5−1000, 0.05−10 ng·mL^{-1} concentration ranges, Rg_1 standard samples over 1−200, 0.5−100 ng·mL^{-1} concentration ranges in rat plasma and brain tissues on three consecutive days, respectively. And the linearity range of SAB in rat plasma and brain tissues were separately 5−200, and 5−200 ng·mL^{-1} on three consecutive days. The calibration curves were fitted by least-square regression using $1/x^2$ as the weighting factor of the peak area ratio of analyte to IS against the individual plasma or brain homogenate concentrations. LLOQ was defined as the lowest concentration on the calibration curve with an acceptable precision (relative standard deviation, RSD) below 20% and accuracy (RE) within ± 20%.

Precision and accuracy

Different concentrations of TS IIA, SAB and Rg1 reference solution were added to blank plasma and blank brain homogenate to prepare QC samples at low, medium and high concentrations. Treat them as the method in 2.4. There were 5 samples for each concentration and measure them with the standard curve in the same batch. The intro- and inter-day precision were assessed by determining QC samples at low, middle and high concentration levels in five replicates on the same day and on three consecutive days. Accuracy is defined as relative error (RE) which is calculated using the formula: RE (%) = [(measured value − theoretical value)/theoretical value] × 100.

Recovery and matrix effect

The recovery of TS IIA, Rg_1 and SAB from rats plasma and brain homogenate at two levels (low and high) were determined by comparing the peak areas of spiked plasma or brain homogenate samples before and after extraction. The ratio gives the percentage recovery. The matrix effect was measured by comparing the pear area ratios of the analytes in the post-extraction spiked plasma or brain homogenate samples with those of same amount of standard solutions in the mobile phase. The recovery and matrix effect of the three analytes at two QC levels (low and high) were repeated for six replicates.

Stability

The stability of the QC plasma and brain homogenate samples at two concentrations (low and high) was investigated under various storage and progress conditions. The short-term stability was evaluated by analyzing the QC samples kept at room temperature for 6 h. Post-preparative stability was determined after positioned for 24 in the auto-sampler. Freeze-thaw stability was checked after three freeze-thaw cycles. Finally, the long-term stability was performed at −20 °C for two weeks in both plasma and brain homogenate. The concentrations after storage were compared with those of freshly prepared samples at the same concentrations.

Statistical analysis

The data were fitted by fitting the non-compartment model with Phoenix winnolin 6.1 software, and the pharmacokinetic parameters of the two groups were calculated by statistical moment method. The results were presented by mean ± standard deviation and analyzed by SPSS17.0 statistical software. The group *t*-test was used to was used to analyzed dates emerging on normal distribution and the homogeneity of variance. The rank sum test was used to test with normal distribution and variance uneven ($\alpha = 0.05$).

Results and Discussion

Methodology verification

Based on the described HPLC and MS conditions, TS IIA, SAB, Rg_1, Domperidone (IS) and Diclofenac sodium (IS) were well separated and no interference peaks were observed at the retention time. The calibration curves of TS IIA, SAB and Rg_1 in the two biological matrices exhibited good

linearity within the concentration range with correlation coefficients (r) higher than 0.9922. The intra-day and inter-day precision of the investigated components showed an RSD within 9.68% and the accuracy (RE) ranged from 9.68% to 8.20% at all quality control levels. The matrix effects and recoveries of three analytes at each concentration were acceptable. The three analytes were found to be stable in the two biological matrices after short-term stability at room temperature for 6 h, post-preparative stability at autosampler for 24 h, three freeze-thaw and long-term storage stability at −20 °C for two weeks.

Pharmacokinetic study

The validated method was successfully applied to the pharmacokinetic study of SAB, TS IIA and Rg_1 in rat plasma and brain tissue after oral administration of *Danshen-Sanqi* and FDP. The pharmacokinetic parameters were estimated by Phoenix WinNolin 6.1 and are separately illustrated in Fig. 2 (The data of FDP in Fig. 2 were reported in a separate paper [25]).

The plasma and brain pharmacokinetic parameters of the FDP group were quite different from those of *Danshen-Sanqi* group. Compared with *Danshen-Sanqi* group, the t_{max} of TS IIA, SAB and Rg_1 in plasma and brain were significantly shorten after co-administrated with borneol ($P < 0.05$ or $P < 0.01$). In rat plasma, the t_{max} of TS IIA, SAB and Rg1 were reduced from 4.33 ± 0.82 to 1.58 ± 0.081 h ($P < 0.01$), from 2.00 ± 0.00 to 1.29 ± 0.21 h ($P < 0.01$), and from 4.00 ± 0.00 to 1.42 ± 0.20 h ($P < 0.01$), respectively. In rat brain, the t_{max} of TS IIA and Rg1 were shorten by 50% ($P > 0.05$) and 92.7% ($P < 0.05$) (Fig. 2B). While the c_{max} of TS IIA, SAB in brain and c_{max} of Rg_1 in brain and plasma were greatly increase (Fig. 2A).

Main pharmacokinetic parameters of TS IIA, SAB and Rg_1 in plasma

As shown in Fig. 2, in comparison with *Danshen-Sanqi* group, promoted intestinal absorption of Rg_1 was found after oral administration of FDP. The c_{max} and AUC_{0-t} of Rg_1 in rats plasma was increased by 7.29 and 1.8 times after co-administration with borneol (Figs. 2A and 2C). It has been reported that because of high water-solubility and large molecular weight of Rg1, its oral bioavailability is poor, so the absorption of Rg1 in intestine depends on the paracellular pathway. Borneol, as paracellular pathway enhancer, can open the tight connection of mesentery of gastrointestinal by contracting calcium dependent actin, thus promoting the drug absorption [26].

Though the tendency which borneol promoted the oral absorption of SAB can be viewed, no significant increase in the c_{max} and AUC_{max} of SAB in rats plasma was observed (Figs. 2A and 2C). No statistically significant results may be

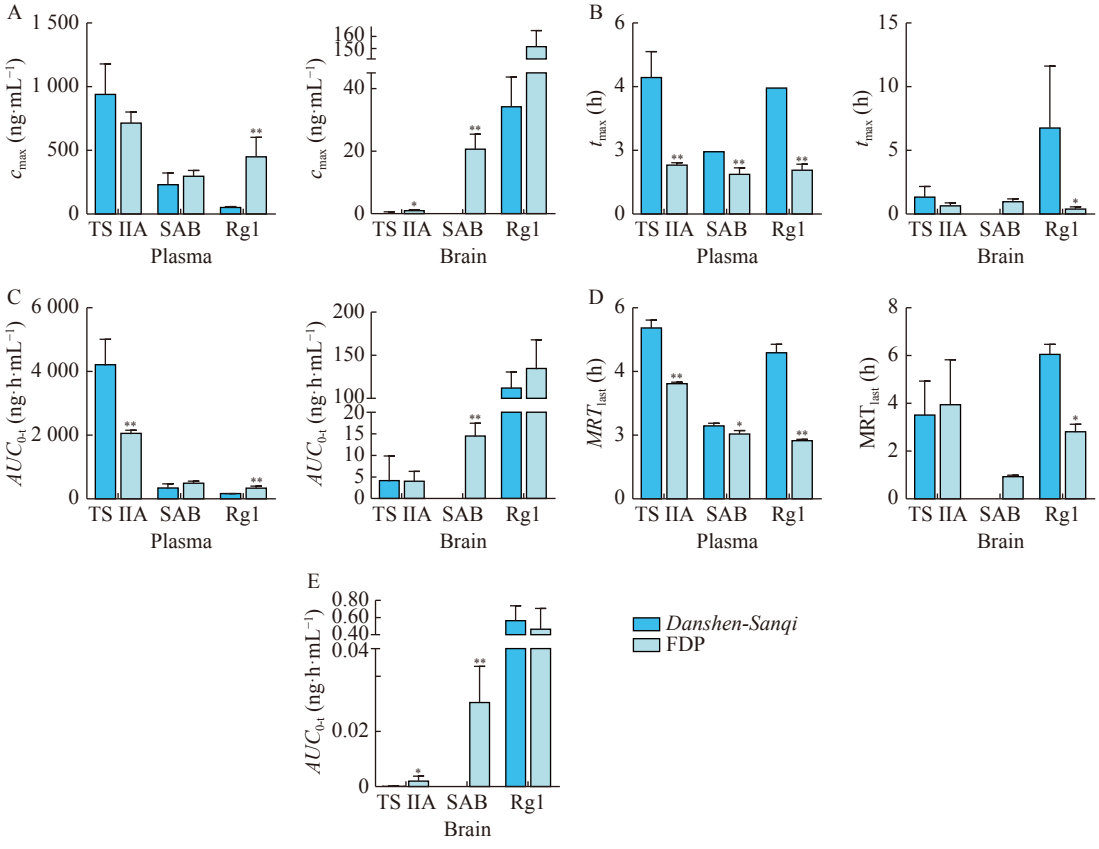

Fig. 2 Pharmacokinetic parameters of TS IIA, SAB, Rg_1 in rat blood and brain after administration of *Danshen–Sanqi* and FDP (mean ± SD, $n = 6$). (A) c_{max}, (B) t_{max}, (C) AUC_{0-t}, (D) MRT_{last}, (E) AUC_{Brain}/AUC_{Plasma}. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs Danshen-Sanqi* group

due to large standard deviation.

In contrast to *Danshen-Sanqi* group, the c_{max} and AUC_{max} values of TS IIA in rats plasma were decrease by 24% ($P > 0.05$) and 50% ($P < 0.01$), respectively, after oral administration of FDP (Figs. 2A and 2C). The reasons for this phenomenon were mainly summed up two points. On one hand, Yang *et al*. showed that the bioavailability of TS II_A didn't significantly increase after adding borneol, may be due to its small improvement in water solubility. On the other hand, borneol maybe enhance tissue distribution of TS IIA, such as lung, heart, brain, etc, which leaded to reducing the concentration of its in rat plasma. Therefore, borneol reduced the c_{max} and AUC_{max} values of TS IIA in rats plasma by accelerating its distribution and metabolism *in vivo*. TS IIA was metabolized by rat CYP2C, 3A and 2D CYP2C, 3A and 2D [27]. Meanwhile, borneol is an *in vivo* inducer of rat hepatic CYP2C6/11, CYP3A1, CYP2D and CYP2B with different regulatory mechanisms [28-32]. So in the clinical application of FDP, the decrease of TS IIA exposure may result from inducing CYPs by borneol.

In addition, shorter $MRT_{0\text{-}t}$ of TS IIA, SAB and Rg_1 were found, when *Danshen-Sanqi* was co-administrated with borneol (Fig. 2D).

Main pharmacokinetic parameters of TSIIA, SAB and Rg_1 in brain

In Fig. 2B, by comparing the main brain distribution kinetic parameters of *Danshen-Sanqi* and FDP groups, the values of t_{max} of TS IIA and Rg_1 were shortened from 1.45 ± 0.80 to 0.75 ± 0.22 h ($P > 0.05$), from 6.87 ± 4.84 to 0.50 ± 0.16 h ($P < 0.05$), respectively. The results indicated that the rates of brain distribution of TS IIA and Rg_1 were accelerated by borneol, and provided a substantial basis of FDP in the faster-acting cure for cerebral ischemia and ischemia reperfusion injury. There was also a statistically significant different in the c_{max} of TS IIA, SAB, and Rg_1 in brain between *Danshen-Sanqi* and FDP groups (*Danshen-Sanqi* and FDP groups (Fig. 2A) ($P < 0.05$ or $P < 0.01$). However, no significant different in the AUC_{0-t} of TS IIA and Rg_1 in brain were observed between two groups (Figs. 2A and 2C).

Detect SAB in brain tissue under the condition of LLOQ, SAB was not found in rats brain tissue from *Danshen-Sanqi* group, this phenomenon was identical to previous studies [24, 33-35]. After co-administration with borneol, the c_{max} and $AUC_{0\text{-}t}$ values of SAB were increased to 21.09 ± 4.85 ng·g^{-1}, 14.83 ± 3.16 ng·h mL^{-1}, respectively (Figs. 2A and 2C).

The brain-to-blood distribution ratio (AUC_{Brain}/AUC_{Plasma}) of TS IIA increased by 5 times after co-administration with borneol, but borneol didn't affect the brain-to-blood distribution ratio of Rg_1 (Fig. 2E). The advancement of borneol on the brain distribution of TS IIA and SAB, which is due to the fact that borneol can loosen the intercellular tight junctions of tissue of BBB and inhibit the activity of efflux transporter P-gp [14-16, 36]. Numerous studies reported that TS IIA is a substrate of P-gp, and P-gp inhibitor verapamil could increase the absorption of TS IIA *in vivo* and *in vitro* [27, 37]. As a substrate of P-gp, TS IIA was severely restricted by BBB and had poor brain distribution [26].

Different kinds of drugs can cause borneol to increase brain distribution to varying degrees, which was possibly caused by their different hydrophilic property and molecular weight (hydrophilic property: TS IIA > SAB > Rg_1; *Mw*: Rg_1 > SAB > TS IIA). From Fig. 2E, it was found that the brain-to-blood distribution ratio value of Rg_1 was apparently higher than TS IIA and SAB, which due to the lower protein binding of Rg_1 compared with TS IIA and SAB [23, 35, 37-39]. In general, it is assumed that only the free fraction of drugs in plasma or blood can distribute to brain. Thus, the others important factors responsible for different brain permeabilities of TS IIA, SAB and ginsenoside Rg_1 in rat should be examined.

Concentration-versus-time curve of TS IIA, SAB and Rg_1 in plasma and brain

In Fig. 3, the brain tissue concentration-time profiles of TS IIA, SAB, and Rg_1 from *Danshen-Sanqi* and FDP groups are showed. As shown in Fig. 3, TS IIA, SAB and Rg_1 can be rapidly absorbed into blood and brain after administration of *Danshen-Sanqi* and FDP. These phenomena indicated that borneol can improve the absorption rate and brain distribution rate of TS IIA, SAB and Rg_1, which were similar to previous researches [9, 18-19].

Bimodal or multi-peak phenomenon of TS IIA, SAB and Rg_1 appeared in rats after oral administration of *Danshen-Sanqi* and FDP. It were reported that a multiple peak phenomenon in unchanged TS IIA appeared in plasma concentration-time curve following oral administration of TS IIA [27], and double peak phenomenon of SAB was observed in plasma concentration-time profiles following intragastric administration of *S. miltirrhizae* and co-administration with borneol [18]. This phenomenon has also been found in the kinetic profiles of other herbal constituents, there are some possible explanations for the multipeak phenomenon at present, including non-homogeneous gastrointestinal absorption, enterohepatic circulation, tissue drug redistribution and absorption, progressive solubilization along the gastrointestinal tract and its subsequent intestinal absorption, and formulation differences [27]. For example, previous research reported that doule-peak phenomenon of Rg_1 was found in plasma concentration–time curve after administration of ginsenoside orally, but it disappeared when rats were given monomer Rg_1 [40].

Conclusion

An UPLC-MS/MS method was successfully developed and applied to characterize the effect of borneol on the pharmacokinetic and brain distribution of TS IIA, SAB and Rg_1 from Salviae Miltiorrhizar Radix et Rhizoma and Notoginseng Radix et Rhizoma in rats. According to the obtained pharmacokinetic results, that Salviae Miltiorrhizar Radix et Rhizoma and Notoginseng Radix et Rhizoma combined with borneol could improve absorption of SAB and Rg_1, promote the distribution of TS IIA and SAB in brain tissue and increase the speed of its intestinal absorption and brain distribution. These results provided an important basis for traditional Chinese medicine recipe in clinical practice. However,

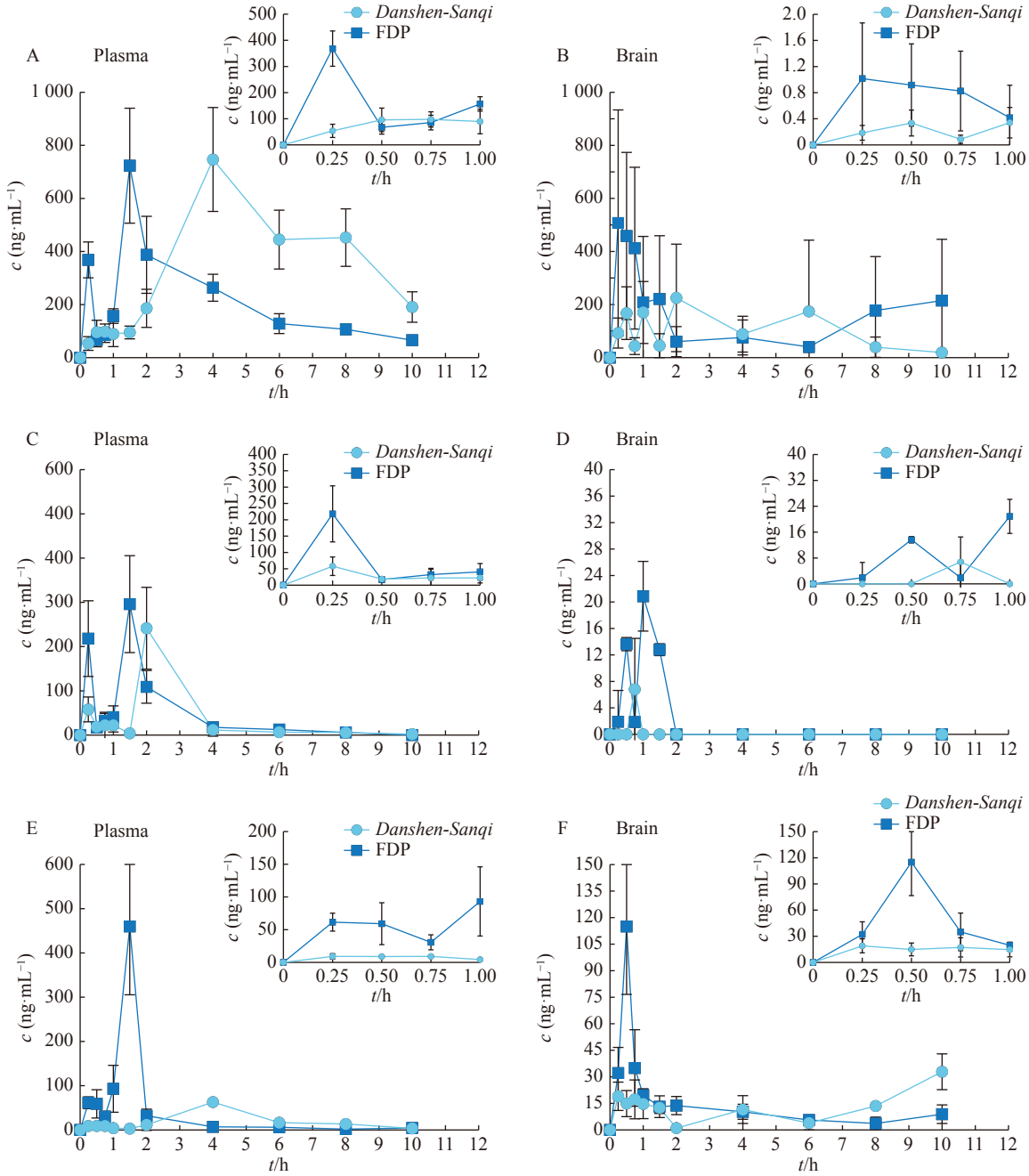

Fig. 3 Mean concentration-time profiles of (A) TSIIA (C) SAB (E) Rg_1 in rat plasma, and (B) TS IIA (D) SAB (F) Rg_1 in rat brain after administration of *Danshen-Sanqi* and FDP (mean ± SD, *n* = 6)

borneol, as an inducer of CYPs, accelerated the elimination rates of TS IIA, SAB and Rg_1 in rats plasma, whose clinical effects require further investigation in clinical trials.

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