Systemic and cerebral exposure to and pharmacokinetics of flavonols and terpene lactones after dosing standardized *Ginkgo biloba* leaf extracts to rats via different routes of administration

Feng Chen, Li Li, Fang Xu, Yan Sun, Feifei Du, Xutao Ma, Chenchun Zhong, Xiuxue Li, Fengqing Wang, Nating Zhang and Chuan Li

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- Supplemental Methods -

GBE50 and ShuXueNing injection

GBE50, a standardized proprietary extract of *G. biloba* leaves with a Chinese SFDA ratification no. Z20000049 for the manufacturer Shanghai XingLing Sci. & Tech. Pharmaceuticals (Shanghai, China), was a 200:3 solid extract, i.e., 200 kg of dried leaves were used to make 3 kg of the final solid extract. Preparation of GBE50 required a six-step extraction/purification process, including 60% aqueous ethanol-based extraction, concentration under reduced pressure, precipitation with water, chromatography on a porous polymer column and then on a polyamide column, further elimination of ginkgolic acids with cyclohexane, and reduction by dry-spraying to dryness (Xie *et al.*, 2000). ShuXueNing injection was a solution of GBE50 available as a sterile, nonpyrogenic parenteral dosage form for intravenous or intramuscular injection, which was manufactured by Shanghai Asia Pioneer Pharmaceuticals (Shanghai, China) with a Chinese SFDA ratification no. Z20043734. Each milliliter of ShuXueNing injection was prepared from 3.5 mg of GBE50. For preparation of ShuXueNing injection, it requires a multiple-step process including dissolution of GBE50, boiling, filtration, treatment with weakly basic anion-exchange resins, ultrafiltration, sterilization, dilution with glucose injection and 30% ethanol, adjustment to pH 5.4–5.6, ultrafiltration, sterilization, etc. Table 1 shows the content levels of flavonols and terpene lactones present in GBE50 and ShuXueNing injection.

Table 1

Content levels of ginkgo compounds in ShuXueNing injection or GBE50.

Compound	ShuXueNing injection ($\mu mol \cdot mL^{-1}$)	GBE50 ($\mu mol \cdot 10\ mg^{-1}$)
Terpene lactone		
Bilobalide (**1**)	0.0002	0.954
Ginkgolide A (**2**)	0.289	0.551
Ginkgolide B (**3**)	0.100	0.325
Ginkgolide C (**4**)	0.184	0.316
Ginkgolide J (**5**)	0.091	0.105
Flavonol		
Quercetin 3-*O*-dirhamnosyl-glucoside (**10**)	0.072	0.270
Quercetin 3-*O*-(*p*-coumaroyl)-glucosyl-*O*-rhamnoside (**11**)	0.096	0.617
Quercetin 3-*O*-rhamnosyl-*O*-glucoside (**12**)	0.174	0.660
Quercetin 3-*O*-glucosyl-*O*-rhamnoside (**13**)	0.065	0.354
Quercetin (**16**)	0.003	0.088
t-Quercetin	**0.397**	**2.149**
Kaempferol 3-*O*-dirhamnosyl-glucoside (**19**)	0.089	0.425
Kaempferol 3-*O*-(*p*-coumaroyl)-glucosyl-*O*-rhamnoside (**20**)	0.080	0.707
Kaempferol 3-*O*-rhamnosyl-*O*-glucoside (**21**)	0.162	0.655
Kaempferol 3-*O*-glucosyl-*O*-rhamnoside (**22**)	0.072	0.474
Kaempferol (**26**)	0.004	0.095
t-Kaempferol	**0.441**	**2.378**
Isorhamnetin 3-*O*-dirhamnosyl-glucoside (**27**)	0.002	0.018
Isorhamnetin 3-*O*-rhamnosyl-*O*-glucoside (**29**)	0.088	0.559
Isorhamnetin 3-*O*-glucosyl-*O*-rhamnoside (**30**)	0.0003	0.004
Isorhamnetin (**32**)	0.001	0.014
t-Isorhamnetin	**0.127**	**0.665**

The ginkgo compounds are given ID numbers in parentheses, which are consistent with those for GBE50 described in our earlier publication (Li *et al.*, 2012). Hydrochloric acid-based hydrolysis was applied to the sample to convert the flavonol glycosides present into their aglycone forms before measurement of the content levels of t-quercetin, t-kaempferol, and t-isorhamnetin in ShuXueNing injection and GBE50.

Experimental animals

All rat studies were conducted in compliance with the Guidance for Ethical Treatment of Laboratory Animals (The Ministry of Science and Technology of China, 2006; www.most.gov.cn/fggw/zfwj/zfwj2006) and the experimental protocols were approved by the Institutional Animal Care and Use Committee at the Shanghai Institute of Materia Medica (Shanghai, China). Male Sprague-Dawley rats (260–300 g; Sino-British SIPPR/BK Laboratory Animal,

Shanghai, China) were maintained in individually ventilated cages (42 × 24 × 24 cm^3, three rats per cage; Suzhou-Fengshi, Suzhou, China) at 20–24°C and relative humidity (30%–70%) with a 12 h light/dark cycle. All rodents had free access to filtered tap water. The rats were provided commercial rat chow *ad libitum*, except for the overnight period before dosing. Rats were acclimated to the facilities and environment for 7 days before use. For blood sampling, rats were anaesthetized with pentobarbital (40 $mg \cdot kg^{-1}$, i.p.), and then polyurethane cannulae (BC-2P; Access Technologies, Skokie, IL, USA; pre-filled with 25 $U \cdot mL^{-1}$ heparinized saline) were inserted into the femoral arteries after an incision of the left groins. After surgery, the femoral artery-cannulated (FAC) rats were housed individually and allowed to regain the preoperative body weights before experimental use. Unlimited access to food and water was permitted during the recovery period. For bile sampling, the bile ducts and duodena of rats under pentobarbital anaesthesia were exposed via a midline laparotomy incision. The bile duct of each rat was cannulated with two connected polyurethane catheters for bile collection and duodenum infusion. During the rat recovery period (4–5 days), the drinking water was supplemented with 5% glucose, 0.9% sodium chloride and 0.5% potassium chloride and the two cannulae were connected with a union to re-infuse bile into the duodenum. During the bile collection period, the two cannulae of the bile duct-cannulated (BDC) rat were detached and a sodium taurocholate solution (pH 7.4) was infused into the duodenum at 1 $mL \cdot h^{-1}$. All used rats were euthanatized with CO_2 gas.

Plasma PK studies

The FAC rats were randomly divided into three groups of four rats each to assess the systemic exposure to and plasma pharmacokinetics of flavonols and terpene lactones after a 15 min i.v. infusion dose of ShuXueNing injection at 1, 2 or 4 $mL \cdot kg^{-1}$. Serial blood samples (~110 μL; 0, 5, 15, 30 min and 1, 2, 4, 6, 8, 11 and 24 h) were collected into heparinized tubes via femoral artery cannula. The blood samples were centrifuged to obtain the plasma fractions that were frozen at −70°C until analysis. A similar experiment was also performed on FAC rats that received a single p.o. dose of GBE50 at 10, 30 or 90 $mg \cdot kg^{-1}$ via gavage. Before use, GBE50 was suspended in 0.5% w/v sodium carboxymethylcellulose. ShuXueNing injection was given to rats through the rat tail vein for i.v. infusion. In addition, a 15 min i.v. infusion dose of bilobalide at 1 $mg \cdot kg^{-1}$ (0.5 $mg \cdot mL^{-1}$; dissolved in an isotonic glucose injection that contained 0.5 $mg \cdot mL^{-1}$ Cremophor EL) was given to FAC rats and the blood samples were collected according to the preceding time schedule.

In addition, a subchronic PK study was implemented for seven consecutive days with four FAC rats received ShuXueNing injection at 1 $mL \cdot kg^{-1} \cdot day^{-1}$ and other four FAC rats received GBE50 at 30 $mg \cdot kg^{-1} \cdot day^{-1}$. Serial blood samples were collected, according to the preceding time schedule, on days 1 and 7 and were centrifuged to yield plasma fractions.

Brain microdialysis

A brain microdialysis study was performed in surgically-modified conscious rats according to a protocol modified from our earlier method (Sun *et al.*, 2009). In brief, FAC rats were anaesthetized with pentobarbital (50 $mg \cdot kg^{-1}$, i.p.) and each rodent was placed in a stereotaxic frame with incisor bar to achieve a horizontal skull. A CMA/150 temperature controller (CMA/Microdialysis, Solna, Sweden) was used to provide supplemental heat to the rat. A CMA/11 guide cannula holding a shaft dummy was implanted and aimed at the hippocampus [AP −5.8, ML +5.0, DV −7.0 mm; according to the atlas of rat brain (Paxinos and Watson, 1998)], and was then cemented to the skull surface. The surgically-modified rat was housed singly and was allowed to recover postoperatively before use. Before microdialysis, the shaft dummy was replaced by a CMA/11 microdialysis probe (cuprophane membrane of 4 mm in length, 6 kD cut-off). The probe was perfused with an artificial cerebrospinal fluid (125 mM sodium chloride, 0.5 mM sodium dihydrogen phosphate, 2.5 mM disodium hydrogen phosphate, 1.2 mM calcium chloride, 2.5 mM potassium chloride and 1.0 mM magnesium chloride, pH 7.4) at 2 $\mu L \cdot min^{-1}$. The rat was allowed to equilibrate for 1 h prior to initiation of sample collection. After the blank control dialysate sample was collected, the rodent received a 15 min i.v. infusion dose of ShuXueNing injection at 4 $mL \cdot kg^{-1}$ or a p.o. dose of GBE50 at 90 $mg \cdot kg^{-1}$ followed by a 6 h dialysate sampling. The dialysate samples were collected in 10 min intervals using a CMA/142 microfraction collector, and a 6 min delay was incorporated into the sampling to compensate for the dead volume between the active membrane and the sample collection outlet. Meanwhile, blood sampling was also collected at 0, 5, 15 and 30 min and 1, 2, 4 and 6 h after dosing. The concentrations of ginkgo terpene lactones in brain extracellular fluid (bECF) were calculated from that in the corresponding dialysate sample using the following equation:

$$C_{bECF} = C_d / R_{in\ vivo} \quad (1)$$

where C_{bECF} is the bECF concentration; C_d is the measured concentration in the dialysate sample. $R_{in\ vivo}$ is the *in vivo* recovery by retrodialysis, which was 16%, 13%, 11%, 9% and 11% for bilobalide, ginkgolides A, B, C and J respectively.

Excretion studies

Rats had not undergone any surgery were randomly assigned to two groups (four rats/group) and housed singly in Nalgene metabolic cages (Nalgene Co., Rochester, NY, USA). The urine and feces collection tubes of the metabolic cages were frozen at −15°C during sample collection. Urine and feces samples were collected before and 0 to 8, 8 to 24 and 24 to 32 h and weighed after a 15 min i.v. infusion dose of ShuXueNing injection at 4 $mL \cdot kg^{-1}$ or a p.o. dose of GBE50 at 90 $mg \cdot kg^{-1}$. The faeces were homogenized in ninefold volumes of ice-cold saline. The fecal homogenates and urine samples were stored at −70°C until analysis.

Three BDC rats received a 15 min i.v. infusion dose of ShuXueNing injection at 4 $mL \cdot kg^{-1}$, while other three

BDC rats received a p.o. dose of GBE50 at 90 mg·kg^{-1}. Bile samples were collected before and 0 to 10, 10 to 20, 20 to 40, 40 to 50 min and 0.8 to 1.2, 1.2 to 1.8, 1.8 to 2.2, 2.2 to 3, 3 to 3.8, 3.8 to 4.2, 4.2 to 5.8, 5.8 to 6.2, 6.2 to 7.8, 7.8 to 8.2, 8.2 to 10.8, 10.8 to 11.2, 11.2 to 23.8 and 23.8 to 24.2 h after dosing and weighed. The bile samples were stored at −70°C until analysis.

Tissue distribution study

Rats under isoflurane anaesthesia were killed by bleeding from the abdominal aorta (~10 mL of blood) at 0, 15 (i.v. only), 30 min, 1 (i.v. only), 2 (p.o. only), 4 and 8 h (p.o. only) after a 15 min i.v. infusion dose of ShuXueNing injection at 4 mL·kg^{-1} or a p.o. dose of GBE50 at 90 mg·kg^{-1}. Three rats were used per time point. The heart, lungs, brain, liver and kidneys were excised and then rinsed in ice-cold saline before gently blotting on absorbent paper. The tissue samples were weighed and homogenized in fourfold volumes of ice-cold saline. The resulting homogenates were stored at −70°C until analysis.

Analysis of ginkgo flavonols and terpene lactones in biological samples

An LC-MS/MS system, consisted of a Thermo Fisher TSQ Quantum mass spectrometer (San Jose, CA, USA) interfaced via an electrospray ionization probe with an Agilent 1100 liquid chromatograph (Waldbronn, Germany), was used to measure concentrations of the ginkgo flavonols and terpene lactones in various hydrochloric acid-hydrolyzed rat samples. Analytical assays were performed as described by Zhao *et al.* (2008). In brief, the rat plasma, excreta, dialysate or tissue homogenate samples (20 μL) were added with 10 mM aqueous ascorbic acid solution (freshly prepared) and then treated with 20 μL of 4 M hydrochloric acid at 80ºC for 30 min to release the flavonol aglycones from their glycosides and conjugated metabolites. Accordingly, the measured flavonol levels are expressed as concentrations of total quercetin (t-quercetin), total kaempferol (t-kaempferol) and total isorhamnetin (t-isorhamnetin). After the treatments, the samples were extracted with ethyl acetate. The chromatographic separation was achieved using a Phenomenex Gemini 5μm C_{18} column (50 × 2.0 mm i.d.; Torrance, CA, USA) with a water-acetonitrile mobile phase modified with formic acid at 0.2 mM and delivered at 0.3 mL·min^{-1}. The gradient programme was 0–1.5 min at 5% (v/v) acetonitrile, 1.5–1.6 min from 5% to 63% acetonitrile, 1.6–2.8 min at 63% acetonitrile, 2.8–2.9 min from 63% to 5% acetonitrile and 2.9–7.0 min at 5% acetonitrile. The MS/MS measurement was performed in the negative ion mode and the precursor-to-product ion pairs used for selected reaction monitoring of t-quercetin, t-kaempferol, t-isorhamnetin, bilobalide, ginkgolides A, B, C and J were *m/z* 301→151 (the optimal collision energy, 28 V), 285→187 (37 V), 315→300 (28 V), 325→163 (26 V), 453→351 (27 V), 423→367 (20 V), 439→383 (19 V) and 469→423 (18 V), respectively, with a scan time of 0.1 s for each ion pair. Only LC eluent flow over a period of 4.5 to 6.3 min was introduced to the ion source for data acquisition. Matrix-matched calibration curves (0.8–2000 ng·mL^{-1}) were constructed for the eight analytes using weighted (1/*X*) linear regressions of the analyte response (peak area; *Y*) against the corresponding nominal plasma concentrations of analytes (*X*, ng·mL^{-1}), which showed good linearity ($r^2 > 0.99$). Assay validation was carried out according to the US Food and Drug Administration guidance on bioanalytical validation (2001; www.fda.gov/cder/guidance/index.htm) to demonstrate that the bioanalytical assays were reliable for the intended applications.

To assess brain penetration of the ginkgo compounds, the rat brain dialysate samples were centrifuged for 10 min at 16 060× *g*. A high-speed, rapid ultrafiltration method described by Guo *et al.* (2006) was used to isolate unbound ginkgo compounds in plasma for measurement of the unbound plasma concentration and plasma protein binding.

The glycosides and metabolites of ginkgo flavonols in rat plasma, bile, and urine were also measured and profiled using an AB-SCIEX API 4000 Q Trap mass spectrometer (Toronto, Canada) interfaced via a Turbo V ion source with a Waters Acquity UPLC separation module (Milford, MA, USA). The samples collected from the rats received a 15 min i.v. infusion dose of ShuXueNing injection at 4 mL·kg^{-1} or a p.o. dose of GBE50 at 90 mg·kg^{-1} and pooled according to the collecting time, i.e., 15 min or 6 h after dosing for the plasma samples and 0–8 h after dosing for the urine sample or the bile sample. These rat samples (100 μL; pretreated without hydrochloric acid) were precipitated with 300 μL of methanol. After centrifugation, the resulting supernatants were reduced to dryness using a centrifugal concentrator under reduced pressure. The residues were reconstituted in 40 μL of 50% methanol and were centrifuged before UPLC-MS/MS analysis. The chromatographic separation was achieved using a Waters 1.7 μm BEH C_{18} column (100 × 2.1 mm i.d.; Dublin, Ireland). A water-methanol mobile phase modified with 1 mM formic acid was delivered at 0.4 mL·min^{-1} and 45°C. A binary gradient method was used, i.e., 0–8 min from 2% to 80% methanol, 8–9 min at 80% methanol, 9–12 min at 2% methanol. The MS/MS measurement was performed in the negative ion mode, and the precursor-to-product ion pairs used for selected reaction monitoring of quercetin 3-*O*-dirhamnosyl-glucoside (**10**)/quercetin 3-*O*-(*p*-coumaroyl)-glucosyl-*O*-rhamnoside (**11**), glucuronidated quercetin 3-*O*-(*p*-coumaroyl)-glucosyl-*O*-rhamnoside (**M10$_{G}$**), sulfated quercetin 3-*O*-(*p*-coumaroyl)-glucosyl-*O*-rhamnosides (**M10$_{S-1}$**/**M10$_{S-2}$**), glucuronidated quercetin 3-*O*-(*p*-coumaroyl)-glucosyl-*O*-rhamnoside sulfate (**M10$_{G-S}$**), quercetin 3-*O*-rhamnosyl-*O*-glucoside (**12**)/quercetin 3-*O*-glucosyl-*O*-rhamnoside (**13**), glucuronidated quercetin 3-*O*-rhamnosyl-*O*-glucosides/quercetin 3-*O*-glucosyl-*O*-rhamnosides (**M12/13$_{G-1}$**–**M12/13$_{G-4}$**), sulfated quercetin 3-*O*-rhamnosyl-*O*-glucosides/quercetin 3-*O*-glucosyl-*O*-rhamnosides (**M12/13$_{S-1}$**–**M12/13$_{S-4}$**), glucuronidated quercetin 3-*O*-rhamnosyl-*O*-glucosides/quercetin 3-*O*-glucosyl-*O*-rhamnoside sulfates (**M12/13$_{G-S-1}$**–**M12/13$_{G-S-3}$**), quercetin (**16**), quercetin 3-*O*-glucuronide (**M16$_{G-1}$**), quercetin glucuronides (**M16$_{G-2}$**–**M16$_{G-4}$**), quercetin sulfates (**M16$_{S-1}$**–**M16$_{S-3}$**), glucuronidated quercetin sulfates (**M16$_{G-S-1}$**–**M16$_{G-S-3}$**), quercetin diglucuronide (**M16$_{2G}$**), quercetin disulfate (**M16$_{2S}$**), sulfated quercetin diglucuronide (**M16$_{2G-S}$**), glucuronidated quercetin disulfate (**M16$_{G-2S}$**), kaempferol

3-*O*-dirhamnosyl-glucoside (**19**)/kaempferol 3-*O*-(*p*-coumaroyl)-glucosyl-*O*-rhamnoside (**20**), glucuronidated kaempferol 3-*O*-(*p*-coumaroyl)-glucosyl-*O*-rhamnoside (**M20$_{G}$**), sulfated kaempferol 3-*O*-(*p*-coumaroyl)-glucosyl-*O*-rhamnosides (**M20$_{S-1}$**/**M20$_{S-2}$**), glucuronidated kaempferol 3-*O*-(*p*-coumaroyl)-glucosyl-*O*-rhamnoside sulfates (**M20$_{G-S-1}$**–**M20$_{G-S-3}$**), kaempferol 3-*O*-rhamnosyl-*O*-glucoside (**21**)/kaempferol 3-*O*-glucosyl-*O*-rhamnoside (**22**), glucuronidated kaempferol 3-*O*-rhamnosyl-*O*-glucosides/kaempferol 3-*O*-glucosyl-*O*-rhamnosides (**M21/22$_{G-1}$**–**M21/22$_{G-5}$**), sulfated kaempferol 3-*O*-rhamnosyl-*O*-glucosides/kaempferol 3-*O*-glucosyl-*O*-rhamnosides (**M21/22$_{S-1}$**–**M21/22$_{S-4}$**), glucuronidated kaempferol 3-*O*-rhamnosyl-*O*-glucosides/kaempferol 3-*O*-glucosyl-*O*-rhamnoside sulfates (**M21/22$_{G-S-1}$**–**M21/22$_{G-S-3}$**), kaempferol (**26**), kaempferol 3-*O*-glucuronide (**M26$_{G-1}$**), kaempferol glucuronides (**M26$_{G-2}$**/**M26$_{G-3}$**), kaempferol sulfates (**M26$_{S-1}$**/**M26$_{S-2}$**), glucuronidated kaempferol sulfate (**M26$_{G-S}$**), kaempferol diglucuronides (**M26$_{2G-1}$**–**M26$_{2G-3}$**), kaempferol disulfates (**M26$_{2S-1}$**/**M26$_{2S-2}$**), sulfated kaempferol diglucuronide (**M26$_{2G-S}$**), glucuronidated kaempferol disulfate (**M26$_{G-2S}$**), isorhamnetin 3-*O*-rhamnosyl-*O*-glucoside (**29**)/isorhamnetin 3-*O*-glucosyl-*O*-rhamnoside (**30**), glucuronidated isorhamnetin 3-*O*-rhamnosyl-*O*-glucosides/tamarixetin 3-*O*-rhamnosyl-*O*-glucosides (**M29$_{G-1}$**–**M29$_{G-4}$**), sulfated isorhamnetin 3-*O*-rhamnosyl-*O*-glucosides/ tamarixetin 3-*O*-rhamnosyl-*O*-glucosides (**M29$_{S-1}$**/**M29$_{S-2}$**), glucuronidated isorhamnetin 3-*O*-rhamnosyl-*O*-glucosides/tamarixetin 3-*O*-rhamnosyl-*O*-glucoside sulfates (**M29$_{G-S-1}$**/**M29$_{G-S-2}$**), isorhamnetin (**32**), isorhamnetin glucuronides (**M32$_{G-1}$**–**M32$_{G-3}$**), isorhamnetin sulfates (**M32$_{S-1}$**/**M32$_{S-2}$**), glucuronidated isorhamnetin sulfates (**M32$_{G-S-1}$**/**M32$_{G-S-2}$**), isorhamnetin diglucuronides (**M32$_{G-1}$**–**M32$_{G-4}$**), isorhamnetin disulfate (**M32$_{2S}$**), sulfated isorhamnetin diglucuronide (**M32$_{2G-S}$**) and glucuronidated isorhamnetin disulfates (**M32$_{G-2S-1}$**/**M32$_{G-2S-2}$**) were *m/z* 755→300, 931→755, 835→755, 1011→755, 609→300, 785→609, 689→609, 865→609, 301→151, 477→301, 477→301, 381→301, 557→301, 653→301, 461→301, 733→301, 637→301, 739→284, 915→739, 819→739, 995→739, 593→285, 769→593, 673→593, 849→593, 285→187, 461→285, 461→285, 365→285, 541→285, 637→285, 445→285, 717→285, 671→285, 623→315, 799→623, 703→623, 879→623, 315→300, 491→315, 395→315, 571→315, 667→315, 475→315, 747→315 and 651→315, respectively, with a scan time of 0.02 s for each ion pair. Only LC eluent flow over a period of 2 to 10 min was introduced to the ion source for data acquisition.

Assessment of blood to plasma ratios

Rat blood-to-plasma ratios (B/P ratios) were determined for terpene lactones (bilobalide and ginkgolides A, B, C and J), flavonol glycosides (**10**–**13**, **19**–**22**, **27**, **29** and **30**) and the aglycone conjugates (**M16$_{G-1}$**–**M16$_{G-4}$**, **M16$_{S-3}$**, **M26$_{G-1}$**–**M26$_{G-3}$**, **M32$_{G-1}$**–**M32$_{G-3}$** and **M32$_{S-2}$**). Blood samples (~600 μL) were collected from rats 15 and 30 min that received a 15 min i.v. infusion dose of ShuXueNing injection at 4 mL·kg^{-1} or 15 min, 4 and 6 h that received a p.o. dose of GBE50 at 90 mg·kg^{-1}. The blood samples were centrifuged at 2 270× *g* and 6°C for 8 min to yield the plasma and erythrocyte fractions. The plasma samples (100 μL) were precipitated with 300 μL of methanol to measure the concentrations of ginkgo compounds. Meanwhile, equal volumes of water were added to the erythrocyte samples before lysis by sonication on ice. The lysed erythrocyte samples (100 μL) were treated with 300 μL of methanol and the concentrations of ginkgo compounds were measured. The B/P ratio was calculated using the following equation:

$$\text{B/P ratio} = (0.44 \times C_E + 0.56 \times C_P)/C_P \quad (2)$$

where C_E and C_P are erythrocyte and plasma concentrations, respectively. The mean hematocrit value of the rats used was 0.44 ± 0.02.

PK data analysis

Plasma PK parameters were estimated by a noncompartmental method using a Thermo Kinetica software package (Philadelphia, PA, USA). The maximum concentration (C_{max}) and the time taken to achieve the peak concentration (T_{peak}) were obtained directly from the data with no interpolation. The area under concentration-time curve up to the last measured or measurable time point (AUC_{0-t}) was calculated by the trapezoidal rule. The nonvascular bioavailability (F) was calculated using the following equations:

$$F = (AUC_{0\text{-}24h(p.o.)} \times dose_{i.v.})/(AUC_{0\text{-}24h(i.v.)} \times dose_{p.o.}) \quad (3)$$

for the total flavonols and

$$F = (AUC_{0\text{-}\infty(p.o.)} \times dose_{i.v.})/(AUC_{0\text{-}\infty(i.v.)} \times dose_{p.o.}) \quad (4)$$

for the terpene lactones. The terminal elimination half-life ($t_{1/2}$) was calculated using the relationship 0.693/k. The total plasma clearance ($CL_{tot,p}$) for i.v. dosing was estimated by dividing the administered dose by the AUC_{0-t}. The apparent volume of distribution at steady state (V_{SS}) for i.v. dosing was estimated by multiplying the $CL_{tot,p}$ by the mean residence time (MRT). The biliary clearance (CL_B) and renal clearance (CL_R) were calculated from the cumulative amount excreted ($Cum.A_e$) into bile and urine, respectively, divided by the plasma AUC_{0-t}. Dose proportionality assessment of AUC_{0-t} for total flavonols and terpene lactones from ShuXueNing injection or GBE50 was conducted by the regression of log-transformed data (the power model) with criteria that were calculated according to the method described by Smith *et al.*, (2000). To assess the extent of brain penetration, a K_p value was calculated using the following equation:

$$K_p = AUC_{bECF}/AUC_{u,p} \quad (5)$$

where $AUC_{u,p}$ is the area under the unbound plasma concentration-time curve.

All results were expressed as arithmetic mean ± standard deviation (SD).

Chemicals and reagents

Bilobalide, ginkgolides A, B, C quercetin 3-*O*-rhamnosyl-*O*-glucoside (**12**), quercetin (**16**), kaempferol (**26**) and

isorhamnetin (**32**) were obtained from the National Institutes for Food and Drug Control (Beijing, China). Ginkgolide J and kaempferol 3-*O*-glucuronide (**M26$_{G-1}$**) were from Tauto Biotech (Shanghai, China). Isorhamnetin 3-*O*-rhamnosyl-*O*-glucoside (**29**) and quercetin 3-*O*-glucuronide (**M16$_{G-1}$**) were from Extrasynthese (Genay, France). Quercetin 3-*O*-(p-coumaroyl)-glucosyl-*O*-rhamnoside (**11**), kaempferol 3-*O*-(*p*-coumaroyl)-glucosyl-*O*-rhamnoside (**20**) and kaempferol 3-*O*-rhamnosyl-*O*-glucoside (**21**) were kindly donated by Prof. J-J. Chen (Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China). The preceding compounds purity exceeded 98%. Organic solvents of HPLC grade, sodium carboxymethycellulose, sterile saline, sodium heparin and isoflurane were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Pentobarbital was from Shanghai Westang Biotechnology Co. Ltd. (Shanghai, China). Cremophor EL was purchased from Sigma-Aldrich (St Louis, MO, USA).

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