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# Pharmacokinetics and Blood–Brain Barrier Penetration of (+)-Catechin and (−)-Epicatechin in Rats by Microdialysis Sampling Coupled to High-Performance Liquid Chromatography with Chemiluminescence Detection

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**ABSTRACT:** (+)-Catechin (C) and (−)-epicatechin (EC), as the basic monomer units of flavanols, can be widely found in natural products or medicinal herbs. Recent pharmacological studies have revealed that C and EC exhibit good neuroprotective effects. However, there is little information about pharmacokinetic profiles in the brain and in vivo BBB penetration of C and EC. In this paper, an ultrasensitive method using high-performance liquid chromatography (HPLC) with chemiluminescence (CL) detection was developed for the analysis of microdialysis samples. The detection limits for C and EC in Ringer's solution were 1.0 and 1.2 ng/mL, respectively. The intraday and interday accuracies for C and EC in Ringer's solution ranged from −3.0 to 4.4%, and the intraday and interday precisions were below 5.2%. The mean in vivo recoveries of C and EC in microdialysis probes were 33.7% and 26.5% in blood while 38.3% and 29.1% in brain. Pharmacokinetic parameters were estimated using the statistical moment method after iv administration (C and EC, 20 mg/kg of body weight) in rats. Brain-to-blood ($AUC_{brain}/AUC_{blood}$) distribution ratios were 0.0726 ± 0.0376 for C and 0.1065 ± 0.0531 for EC, indicating that C and EC could pass through the BBB, which is further evidence of their neuroprotective effects.

**KEYWORDS:** *(+)-catechin, (−)-epicatechin, microdialysis, pharmacokinetics, blood–brain barrier*

## INTRODUCTION

(+)-Catechin (C) and (−)-epicatechin (EC) (Figure 1), as the basic monomer units of flavanols, are present in a wide range of botanical sources both as monomers or oligomers (procyanidins). The richest sources of flavanols include green tea, cocoa, red grapes, apples, red wine, and berries.[1] Catechins, namely (+)-catechin, (+)-gallocatechin, (−)-epicatechin, (−)-epicatechin-3-gallate, (−)-epigallocatechin, and (−)-epigallocatechin-3-gallate, which account for 30% of the dry weight of green tea leaves,[2] were found to have antioxidant, anticancer, antiangiogenic, antimutagenic, hypocholesterolemic, antiaging, antidiabetic, antibacterial, anti-HIV, and anti-inflammatory effects.[3] Recent pharmacological studies have revealed that C and EC exhibit good neuroprotective effects in brain.[4,5]

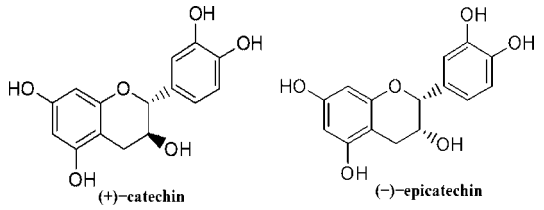


**Figure 1.** Chemical structures of C and EC.

To ascertain the neuroprotective effects of C and EC, it is necessary to understand the pharmacokinetic profiles in blood and brain, whether C and EC cross the blood–brain barrier (BBB). In vivo microdialysis is a technique for drug molecule sampling without depriving the subject of biological fluids and involving minimal disturbance of physiological functions.[6] Moreover, the dialysis membrane is only permeable to small molecules, the analytes can be separated from enzymes by the dialysis membrane, and microdialysis samples need no further cleanup and will not undergo further metabolism after collection.[7] For the pharmacokinetics study of drugs in brain, intracerebral microdialysis is the only technique offering the possibility to continuously monitor the local BBB transport of unbound drugs in tested animals, under physiological and pathological conditions.[8] However, the drug concentration of brain dialysate is usually at a trace level, and the collecting volume of dialysate sample is also limited. Therefore, microdialysis combined with a high selectivity and high sensitivity analytical technique is the ultimate solution to the search for a brain real-time biosensor.

Analytical methods employed for the quantitative determination of drugs and their metabolites in biological samples are the key determinants of pharmacokinetic study. For the assay of C and EC in biological samples, high-performance liquid chromatography (HPLC) has been shown to be suitable, in which UV,[9] electrochemical,[10−13] and mass[14−17] detections were used. In our previous work, a highly sensitive HPLC method coupled with $HAuCl_4$–luminol–$H_2O_2$ chemiluminescence (CL) detection was developed to evaluate the pharmacokinetics of phenolic compounds after oral administration of Danshen extract in rats.[18]

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A number of pharmacokinetic studies of C and EC have been reported both in humans and in animal models,[19−29] and two BBB cell lines, RBE-4 cells (immortalized cell line of rat capillary cerebral endothelial cells) and hCMEC/D3 (immortalized human cerebral microvessel endothelial cell line), were used to evaluate the transmembrane transport of C and EC across BBB.[30] However, there is little information about pharmacokinetic profiles in brain and in vivo BBB penetration of C and EC.

In this work, the HPLC−CL method was based on the chemiluminenscent enhancement by C and EC of the $HAuCl_4$−luminol−$H_2O_2$ CL system and successfully applied to the rat blood and brain pharmacokinetic studies coupled with microdialysis. To the best of our knowledge, it is the first time to provide the pharmacokinetic profiles of C and EC in brain as well as the blood-to-brain distribution ratios.

## MATERIALS AND METHODS

**Chemicals.** The reference compounds of C and EC were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The stock solutions of C and EC were prepared by dissolving them in methanol and stored in the dark at 4 °C. The working solutions of C and EC were obtained by diluting their stock solutions with aqueous 0.2% phosphoric acid. A stock solution of luminol (0.01 mol/L) was prepared by dissolving luminol (Merck, Darmstadt, Germany) in sodium hydroxide solution (0.10 mol/L) and stored at least 7 days before dilution. The buffer solutions of $NaHCO_3$−$Na_2CO_3$ (Sinopharm Chemical Reagent Co., Ltd., China) were prepared by mixing 0.1 mol/L of $NaHCO_3$ and $Na_2CO_3$ aqueous solution. The working $H_2O_2$ solution was prepared fresh daily from 30% (w/w) $H_2O_2$ (Suzhou Chemical Reagent Company, China). A stock solution (1% w/w) of $HAuCl_4$ was prepared by dissolving $HAuCl_4{\cdot}4H_2O$ (Shanghai Chemical Reagent Company, China) in water. Methanol was of HPLC grade, and all other chemicals were of analytical-reagent grade. The ultrapure water (18.3 MΩ·cm at 25 °C, Millipore, Billerica, MA) was used throughout the study. The mobile phases of HPLC were prepared fresh daily and filtered through a 0.22 μm membrane filter (Bandao, Shanghai, China). The perfusate used for blood and brain microdialysis was Ringer's solution (122.0 mmol/L NaCl, 3.0 mmol/L KCl, 0.4 mmol/L $KH_2PO_4$, 1.2 mmol/L $MgSO_4$, 25.0 mmol/L $NaHCO_3$, 1.2 mmol/L $CaCl_2$).

**Animals.** Adult, male Sprague-Dawley rats (296 ± 23 g) were supplied by the Laboratory Animal Center of Anhui Medical University, housed in an air-conditioned room (temperature, 22−25 °C; relative humidity, 55 ± 5%), and kept on a light/dark cycle of 12 h/12 h. Free access to food and drinking water was allowed throughout the study except for fasting 18 h before the experiment. Animal studies were performed according to the Guidelines for the Care and Use of Laboratory Animals approved by the Committee of Ethics of Animal Experimentation of Anhui Medical University (Hefei, China).

**HPLC−CL Assay.** The HPLC−CL detection system consisted of an HPLC system and a postcolumn CL detection system. The HPLC system is a Shimadzu LC-20A series (Shimadzu Corporation, Japan), including a quaternary pump, a vacuum degasser, a thermostatted column compartment, a prominence diode array detector (DAD), a manual sample valve injector with a 20 μL loop, and an analytical column (Shim-pack ODS, 250 mm × 4.6 mm, I.D. 5 μm; Shimadzu). The postcolumn CL detection is performed with a flow injection CL system (Remax, China), which is composed of a model IFFM-E peristaltic pump, a mixing tee, and a model IFFS-A CL detector equipped with a flat glass coil (used as reaction coil and detection cell) and a photomultiplier tube operated at −600 V. The HPLC−CL assay was performed on a column at 40 °C with isocratic elution at a flow rate of 1.0 mL/min. The mobile phase was composed of aqueous phosphoric acid (0.2%, v/v) and methanol. Detection by the DAD was set at 280 nm. The column effluent from the DAD was first mixed with $HAuCl_4$ solution via a PEEK tube and then combined with luminol and $H_2O_2$ solution in a mixing tee, respectively. All the CL solutions were delivered by the same peristaltic pump at a flow rate of 2.0 mL/min. The quantification was based on the relative CL intensity $\Delta I$, where $\Delta I = I_S - I_0$; $I_S$ and $I_0$ (blank signal) are the CL intensities in the presence and absence of analytes, respectively.

**Method Validation.** For the pharmacokinetic study, the selectivity of the HPLC−CL method was confirmed by spiking six different sources of rats' blank blood and brain microdialysates with 40.8 ng/mL of C and 48.5 ng/mL of EC. The linearity of the HPLC−CL method was evaluated by the correlation coefficients ($r$) of calibration curves obtained with seven standard mixtures in the Ringer's solution containing 0.2% phosphoric acid. An external standard method was used to establish the calibration curves of C and EC, which were constructed by plotting the logarithm of concentration (log $C$) against the logarithm of relative CL intensity (log $\Delta I$) and required to have the value of $r$ at least 0.990. The signal-to-noise ratio (S/N) of 3 was set as the threshold for calculating the detection limit.

The intraday and interday variabilities of the HPLC−CL method for the determination of C and EC were assayed at the concentrations of 40.0, 80.0, and 200.0 ng/mL on the same day and on five sequential days, respectively. The accuracy (bias) was calculated from the nominal concentration ($C_{nom}$) and the mean value of observed concentration ($C_{obs}$) as follows: bias (%) = $[(C_{obs} - C_{nom})/(C_{nom})] \times 100$. The precision (relative standard deviation; RSD) was calculated from the observed concentrations as follows: RSD (%) = [standard deviation/$C_{obs}$] × 100.

**Microdialysis Experiments.** The microdialysis system (CMA, Stockholm, Sweden) consists of a CMA/400 microinjection pump, a CMA/150 temperature controller, and a CMA/470 refrigerated fraction collector. Rats were anesthetized with chloral hydrate (300 mg/kg, ip) and remained anesthetized throughout the experimental period. The rats' body temperature was maintained at 37 °C with a heating blanket (CMA, Stockholm, Sweden). Surgical sites were shaved and cleaned with povidone−iodine. The probe for blood sampling (MAB 7.8.10, membrane length, 10 mm, molecular weight cutoff 15 kDa) was implanted within the jugular vein/right atrium and perfused with Ringer's solution at a flow rate of 3.0 μL/min. The rat was then mounted on a stereotaxic frame, and the brain microdialysis probe (CMA/12, membrane length, 4 mm, molecular weight cutoff 20 kDa) was implanted in the right hippocampus (coordinates: 5.6 mm posterior to bregma, 5.0 mm lateral to midline, and 7.0 mm lower to tip) and perfused with Ringer's solution at 1.2 μL/min. The proper placement of the probe in the right hippocampus was confirmed by making an incision at the site of probe implantation after the completion of studies. C and EC were dissolved in normal saline, and this dosing solution was prepared fresh immediately prior to the initiation of injection. The injected volume of C and EC solution depends on the body weight of rats, and rats were administered (20 mg/kg of body weight) via femoral vein after a 2 h postsurgical stabilization period. The microdialysis samples of blood and brain were collected every 10 min, respectively, for 140 min. A 2.0 μL amount of aqueous phosphoric acid (4.0%, v/v) was added to the collecting vials in advance to prevent C and EC degradation. The collected samples were kept at 4 °C and analyzed within 48 h.

**Microdialysis Probe in Vivo Calibration.** To estimate the in vivo recoveries, a retrodialysis calibration technique was utilized. The microdialysis probe was inserted into the rat's jugular vein and hippocampus under anesthesia, and then the Ringer's solution containing C, EC, and phosphoric acid (0.2%, v/v) were perfused through the probe at a flow rate of 3.0 μL/min and 1.2 μL/min, respectively. The dialysate was obtained at 0.5, 1.0, 1.5, 2, 2.5, 3, 3.5, and 4.0 h to optimize the time for administration when the recovery reached a constant value. The perfusate ($C_{perf}$) and dialysate ($C_{dial}$) concentrations of C and EC were determined by the HPLC−CL method. The in vivo relative recovery ($R_{dial}$) of C and EC across the microdialysis probe was calculated by the following equation: $R_{dial} = (C_{perf} - C_{dial})/C_{perf}$. The concentrations of C and EC were converted to free-form concentrations ($C_f$) as follows: $C_f = C_m/R_{dia}$.

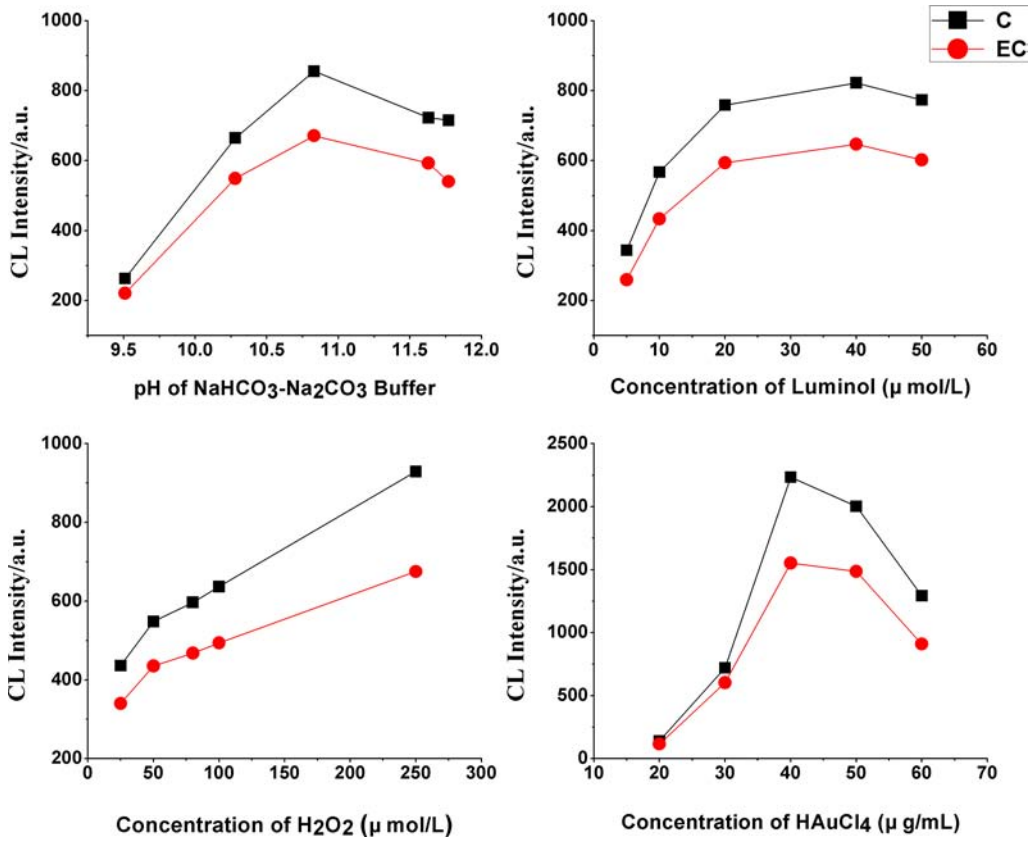


**Figure 2.** Effects of the CL reagent conditions on the $HAuCl_4$–luminol–$H_2O_2$ CL detection for HPLC. (A) Effects of pH values of $NaHCO_3$–$Na_2CO_3$ buffer solution: luminol, 20.0 $\mu$mol/L; $H_2O_2$, 50.0 $\mu$mol/L; $HAuCl_4$, 40.0 $\mu$g/mL. (B) Effects of luminol concentrations: $NaHCO_3$–$Na_2CO_3$ buffer, pH 10.83; $H_2O_2$, 50.0 $\mu$mol/L; $HAuCl_4$, 40.0 $\mu$g/mL. (C) Effects of $H_2O_2$ concentrations: $NaHCO_3$–$Na_2CO_3$ buffer, pH 10.83; luminol, 40.0 $\mu$mol/L; $HAuCl_4$, 40.0 $\mu$g/mL. (D) Effects of $HAuCl_4$ concentrations: $NaHCO_3$–$Na_2CO_3$ buffer, pH 10.83; luminol, 40.0 $\mu$mol/L; $H_2O_2$, 100.0 $\mu$mol/L. The concentration of C and EC used for the optimization experiments was 40.0 ng/mL.

**Table 1. Stability of C and EC at 100.0 ng/mL under Different Conditions**[a]

| | in Ringer's solution at 4 °C (%) | | | | | | in Ringer's solution containing 0.2% $H_3PO_4$ at 4 °C (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| analytes | 0 h | 2 h | 4 h | 8 h | 24 h | 48 h | 0 h | 2 h | 4 h | 8 h | 24 h | 48 h |
| (+)-catechin | 100 | 71.8 ± 5.3 | 52.3 ± 6.6 | 30.1 ± 3.8 | 0 | 0 | 100 | 99.9 ± 2.7 | 99.3 ± 1.8 | 98.5 ± 3.2 | 96.7 ± 2.4 | 91.4 ± 3.0 |
| (−)-epicatechin | 100 | 80.0 ± 3.1 | 60.2 ± 4.7 | 38.5 ± 5.2 | 0 | 0 | 100 | 103.4 ± 3.9 | 99.1 ± 2.3 | 98.0 ± 2.6 | 96.5 ± 1.7 | 95.7 ± 2.1 |

[a]Results are presented as stability remaining (mean ± SD, $n$ = 3).

**Data Analysis.** The drug concentration data (after being converted to free form concentrations) were processed by Drug and Statistics (DAS 2.0) (Mathematical Pharmacology Professional Committee of China, Shanghai, China) by the statistical moment method. Brain-to-blood distribution ratios were calculated by $AUC_{brain}/AUC_{blood}$.

## RESULTS AND DISCUSSION

**Optimization of the HPLC–CL System.** As for the HPLC–CL detection, the mobile phase of HPLC is not only suitable for the good separation of C and EC but is also compatible with the $HAuCl_4$–luminol–$H_2O_2$ CL reaction. It was found that acetic acid and formic acid were incompatible with the CL system, causing the quenching of luminescence, and acetonitrile caused no less baseline drift than methanol. Therefore, the mobile phase consisting of a mixture of methanol and aqueous 0.2% phosphoric acid (27:73, v/v) was chosen as the mobile phase for isocratic elution. For C and EC, the good resolution of HPLC peaks was achieved within 10 min.

The stable and maximal CL signals of C and EC were obtained when the HPLC column effluent first mixed with 40.0 $\mu$g/mL $HAuCl_4$ solution and then with 40.0 $\mu$mol/L luminol in pH 10.83 buffer solution of $NaHCO_3$–$Na_2CO_3$ and finally with 100.0 $\mu$mol/L $H_2O_2$ solution. The optimization of the CL reagent conditions of the $HAuCl_4$–luminol–$H_2O_2$ CL detection for HPLC was shown in Figure 2. The $NaHCO_3$–$Na_2CO_3$ buffer solution is a crucial factor for the optimal sensitivity of the $HAuCl_4$–luminol–$H_2O_2$ CL system. The effects of buffer pH were examined in the range of 9.5–11.8. The strongest enhanced CL intensities of C and EC were chosen at pH 10.83 (Figure 2A). The effects of luminol concentration on the relative CL intensities of C and EC were tested over the range of 5.0–50.0 $\mu$mol/L in the $NaHCO_3$–$Na_2CO_3$ medium at pH 10.83. The optimum luminol concentration was 40.0 $\mu$mol/L (Figure 2B). The effects of $H_2O_2$ concentrations were studied in the range of 25.0–250.0 $\mu$mol/L. The CL intensities increase with the increase in $H_2O_2$ concentration; however, when the $H_2O_2$ concentration is higher than 100.0 $\mu$mol/L, the baseline noise increases


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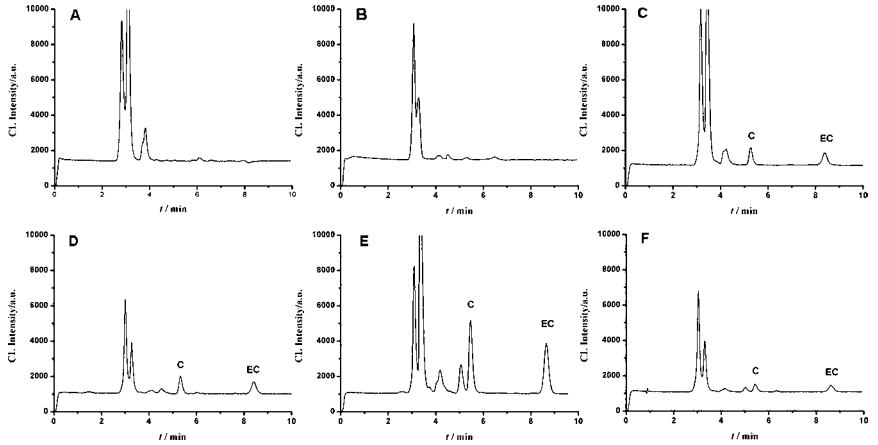


**Figure 3.** HPLC−CL chromatograms of (A) blank microdialysate from rat's blood, (B) blank microdialysate from rat's brain; (C) blank blood and (D) blank brain microdialysates spiked with C (40.8 ng/mL) and EC (48.5 ng/mL); (E) blood and (F) brain microdialysate collected 1.0 h after iv administration of C and EC (20 mg/kg of body weight).

**Table 2. Precisions and Accuracies of C and EC by the HPLC−CL Method**

| | | intra-assay | | | interassay | | |
|---|---|---|---|---|---|---|---|
| analytes | nominal concentration (ng/mL) | observed concentration[a] (ng/mL, $n$ = 25) | precision (RSD, %) | accuracy (bias, %) | observed concentration[a] (ng/mL, $n$ = 5) | precision (RSD, %) | accuracy (bias, %) |
| (+)-catechin | 204.0 | 206.7 ± 6.4 | 3.1 | 1.3 | 208.3 ± 5.9 | 2.8 | 2.1 |
| | 81.6 | 80.2 ± 4.1 | 5.1 | −1.8 | 79.8 ± 3.0 | 3.8 | −2.3 |
| | 40.8 | 41.3 ± 2.0 | 4.9 | 1.2 | 42.0 ± 1.8 | 4.4 | 2.9 |
| (−)-epicatechin | 242.4 | 238.1 ± 5.2 | 2.2 | −1.8 | 241.8 ± 6.4 | 2.7 | −0.3 |
| | 97.0 | 101.2 ± 5.0 | 4.9 | 4.4 | 99.2 ± 5.1 | 5.1 | 2.3 |
| | 48.5 | 46.8 ± 2.4 | 5.2 | 3.5 | 47.1 ± 2.4 | 5.1 | −3.0 |

[a]Concentrations are presented as the mean ± SD.

dramatically, which causes poor reproducibility of CL signal. Therefore, 100.0 $\mu$mol/L of $H_2O_2$ with the maximal S/N for C and EC was chosen for further studies (Figure 2C). The effects of $HAuCl_4$ concentrations in the range of 20.0−6.0 $\mu$g/mL on the CL intensities were examined. The CL intensities increased with an increasing $HAuCl_4$ concentration up to 40.0 $\mu$g/mL, above which the CL intensities decreased (Figure 2D). Therefore, 40.0 $\mu$g/mL of $HAuCl_4$ was selected as the optimum concentration. The concentration of C and EC used for all the optimization experiments was 40.0 ng/mL.

**Analyte Stability in the Dialysate.** The concentration of C and EC used for the optimization experiments of stability was 100.0 ng/mL. As shown in Table 1, the concentration of C and EC decreased to 30.1% and 38.5% of the original level of 100.0 ng/mL within 8.0 h in the Ringer's solution at 4 °C, indicating that C and EC were easily degraded in the Ringer's solution. Huang et al. reported that the addition of EDTA-$Na_2$ (0.04%, w/v) and L-cysteine (0.02%, w/v) to both aCSF and ACD solutions could avoid the degradation of baicalin.[7] In the present study, the degradation of C and EC were pH-dependent; when phosphoric acid (0.2%, v/v) was added to the Ringer's solution, no significant degradation of analytes was observed after 48 h at 4 °C. Therefore, the determination of C and EC in microdialysis samples could be accomplished in 48 h.

**Selectivity, Linearity, Detection Limit, Precision, and Accuracy.** C and EC were completely separated with retention times of 5.6 and 8.8 min, respectively. Representative chromatograms of blank blood and brain dialysate are shown in Figure 3A and Figure 3B. The blank blood and brain microdialysates spiked with C (40.8 ng/mL) and EC (48.5 ng/mL) are shown in Figure 3C and Figure 3D, and microdialysis samples from rat blood and brain collected 1.0 h after iv administration of C and EC are presented in Figure 3E and Figure 3F, respectively. No interference was presented in the chromatographic separation, and each target peak had good resolution.

The linear regression of C and EC displayed good linear relationships over the ranges of 5.1−408.0 and 6.1−484.8 ng/mL, respectively. The calibration equations for C and EC are $\log \Delta I = 0.9687 \log C + 10.263$ and $\log \Delta I = 0.9495 \log C + 9.918$ with the correlation coefficients ($r$) of 0.9952 and 0.9962, respectively. The detection limit (S/N = 3) of C and EC were found to be 1.0 and 1.2 ng/mL in Ringer's solution.

The accuracies and precisions of the HPLC−CL method are summarized in Table 2. The intraday RSD ranged from 2.2% to 5.2%, while the interday RSD ranged from 2.7% to 5.1%. The intraday bias varied from −1.8% to 4.4%, while the interday bias varied from −3.0% to 2.9%. The accuracy and precision values were well within the acceptance criteria of bioanalysis.

**In Vivo Recoveries of the Probes.** Figure 4 shows the correlations of microdialysis probes recoveries with different times in blood and brain. As can be seen clearly, the concentration of analytes reached equilibrium in the dialysate 2 h after implantation, leading to a relatively stable value of recoveries from 2 to 4 h. Consequently, the drug was administrated and dialysates were collected at 2 h after the probe implantation. The mean in vivo recoveries ($n$ = 5) of C and EC in microdialysis probes were 33.7% and 26.5% in blood while 38.3% and 29.1% in brain, as shown in Table 3. The recovery of the probes is not only proportional to the active

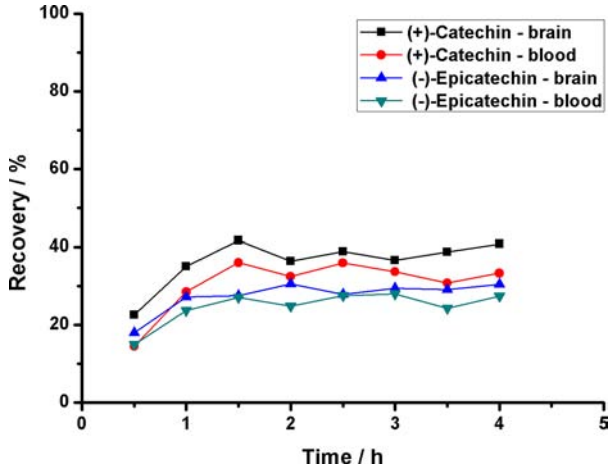


**Figure 4.** Correlations of microdialysis probes recoveries with different time points in brain and blood.

**Table 3. In Vivo Recoveries of Blood and Brain Microdialysis Probes for C and EC[a]**

| | | in vivo recovery (%) | |
|---|---|---|---|
| analytes | concentration (ng/mL) | blood probe | brain probe |
| (+)-catechin | 204.0 | 34.8 ± 2.0 | 38.7 ± 1.7 |
| | 40.8 | 32.6 ± 1.6 | 37.7 ± 3.5 |
| | average | 33.7 ± 2.0 | 38.3 ± 2.4 |
| (−)-epicatechin | 242.4 | 26.5 ± 2.0 | 29.6 ± 0.7 |
| | 48.5 | 26.5 ± 1.4 | 28.6 ± 1.6 |
| | average | 26.5 ± 1.5 | 29.1 ± 1.3 |

[a]Results are presented as the mean ± SD ($n$ = 5).

membrane length but is also related to perfusion flow rate. Higher recoveries are obtained when probes are perfused at lower flow rates because this allows more time for the analytes to diffuse from the extracellular fluid across the membrane into the perfusate. In this study, the blood and brain probe perfusion flow rate were set at 3.0 and 1.2 $\mu$L/min, respectively. Therefore, it is reasonable that the average recoveries for C and EC of brain were higher than those of blood.

**Pharmacokinetics and BBB Penetration Study.** Some literature reported the plasma pharmacokinetics of C and EC in rats, rabbit, dogs, and humans. However, no brain pharmacokinetic data of C and EC was mentioned. Only one article described that C and EC are capable of crossing the BBB cells in vitro, and the involvement of a stereoselective process during the passage of these compounds across BBB cells.[30] Recent reports showed that C and EC from *Smilacis chinae* rhizome prevent amyloid $\beta$ protein (25−35)-induced neuronal cell damage in vitro by interfering with the increase of $[Ca^{2+}]_c$ and then by inhibiting glutamate release, generation of ROS, and caspase-3 activity.[31] Moreover, C can relieve the cerebral ischemia reperfusion injury, and its mechanism may be partly related to the effects of its anti-inflammation and antioxidation.[4] Similarly, EC expressed antioxidant effects on the hippocampal toxicity caused by amyloid-$\beta$ 25−35 in rats.[5] All the above pharmacological effects in brain were based on the facts that C and EC could pass through the BBB.

C and EC are the most abundant antioxidants in the human diet such as green tea and red wine. During digestion and transfer across the small intestine and in the liver, C and EC are rapidly metabolized in phase I and II biotransformations to a diverse family of bioactive metabolites.[32,33] Renouf et al.[15] detected significant amounts of EC, epigallocatechin, and epigallocatechin gallate in human plasma after feeding 400 mL of green tea, 1.25% infusion to nine healthy subjects. EC was absorbed very quickly with a $C_{max}$ of 202.6 ± 21.1 nM around 1−2 h after ingestion, and clearance from plasma was also rapid and back to baseline 6−8 h after ingestion with a monophasic response. EC was present predominantly in plasma as conjugates, and the conjugated forms of EC were two-thirds as sulfate and one-third as glucuronide.[34] El-Hady et al. also reported that the concentrations of C and EC in human plasma after ingestion of green tea were found to be 32.0 and 11.0 ng/mL, which are about 17.4% and 1.8% of the administered dose in the 2 h plasma collection.[35]

C and EC administered via femoral vein would escape gastrointestinal metabolism but are subjected to hepatic metabolism where it can be modified to a variety of metabolites, mainly as methyl, sulfate, and glucuronide metabolites. Abd EL Mohsen et al. reported that EC glucuronide and 3′-*O*-methyl EC glucuronide were found after oral ingestion in rat brain tissue.[17] However, which has the better physiological role, free-flavanol or conjugated-flavanol? Recently it was reported that EC metabolites exhibit a greatly

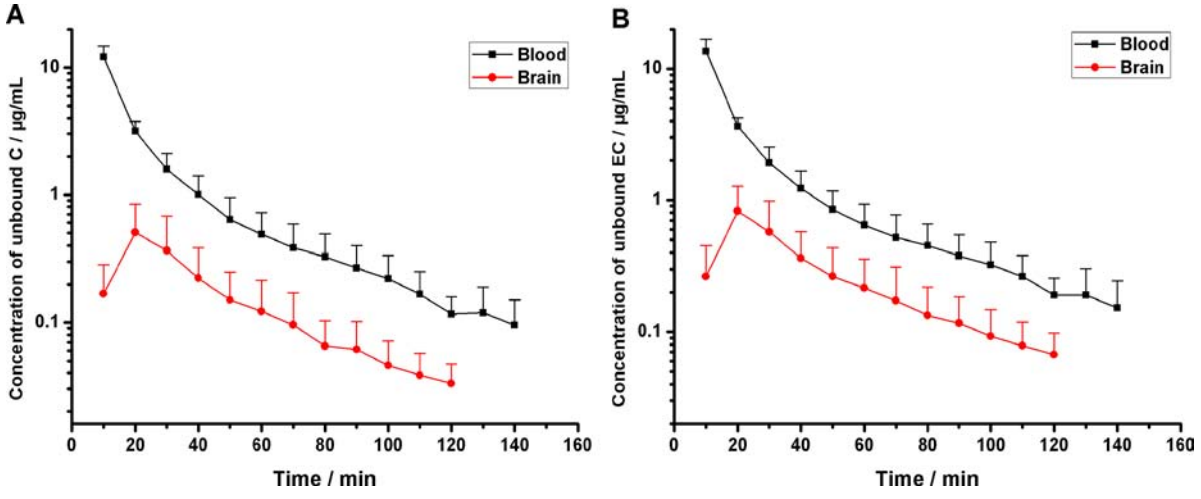


**Figure 5.** Unbound (A) C and (B) EC concentration versus time curves of blood and brain after iv administration. ($\bar{x}$ ± $S$, $n$ = 6).


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reduced ability to attenuate peroxide-mediated cell damage compared to native EC.[36] On the other hand, the likelihood that conjugated-flavanol enters the brain has been considered low, owing to the increased polarity that reduces its ability to partition and, therefore, limits its transit through the blood–brain barrier.[17] Therefore, to ascertain the substance basis of neuroprotective effects of C and EC, the parental molecules of C and EC in rats' blood and brain were quantified.

Mean unbound C and EC concentration–time profiles at a dose of 20.0 mg/kg in the rat blood and brain are presented in Figure 5. Among them, C and EC in brain samples at 130 and 140 min could not be detected because of the sensitivity of HPLC-CL analysis. The $t_{1/2}$ values of C and EC were 12.70 ± 3.76 and 13.67 ± 4.33 min for blood while 33.66 ± 3.68 and 41.67 ± 9.14 min for brain. The maximum brain concentrations of C and EC were observed after about 20 min of administration. The mean brain $C_{max}$ values of C and EC were 0.51 ± 0.34 and 0.83 ± 0.45 μg/mL. The main blood and brain pharmacokinetic parameters of C and EC estimated by the statistical moment method are given in Table 4. Both C and

**Table 4. Pharmacokinetic Parameters of C and EC in Rat Blood and Brain after 20 mg/kg of Body Weight, iv Administration[a]**

| parameters | (+)-catechin | (−)-epicatechin |
|---|---|---|
| Blood | | |
| $t_{1/2}$ (min) | 12.70 ± 3.76 | 13.67 ± 4.33 |
| $C_{max}$ (μg/mL) | 12.17 ± 2.45 | 13.61 ± 3.23 |
| $AUC_{0\text{-inf}}$ (μg min/mL) | 323.07 ± 44.26 | 369.52 ± 62.13 |
| $AUMC_{0\text{-inf}}$ (μg min/mL) | 5765.65 ± 1581.98 | 7094.78 ± 1862.39 |
| $MRT_{0\text{-inf}}$ (min) | 18.33 ± 5.95 | 19.72 ± 6.25 |
| Brain | | |
| $t_{1/2}$ (min) | 33.66 ± 3.68 | 41.67 ± 9.14 |
| $C_{max}$ (μg/mL) | 0.51 ± 0.34 | 0.83 ± 0.45 |
| $AUC_{0\text{-inf}}$ (μg min/mL) | 23.31 ± 11.92 | 39.37 ± 20.54 |
| $AUMC_{0\text{-inf}}$ (μg min/mL) | 1089.03 ± 429.85 | 2177.13 ± 950.66 |
| $MRT_{0\text{-inf}}$ (min) | 48.58 ± 5.31 | 60.13 ± 13.18 |
| Brain-to-Blood Distribution | | |
| $AUC_{brain}/AUC_{blood}$ | 0.0726 ± 0.0376 | 0.1065 ± 0.0531 |

[a]Results are presented as the mean ± SD ($n$ = 6). $t_{1/2}$, elimination half-life; $C_{max}$, maximum plasma (or brain) concentration; AUC, area under the plasma (or brain) concentration versus the time curve; AUMC, area under the first moment of the plasma (or brain) concentration–time curve; MRT, mean of residence time.

EC have similar pharmacokinetic properties after iv administration, especially for relatively short $t_{1/2}$ in blood and long $t_{1/2}$ in brain, indicating that C and EC suffer more intense biotransformation in blood than in brain. The fast decreasing concentrations of unbound C and EC in blood lead to the decrease of permeation rate across the BBB. Moreover, EC was easily modified by the liver in blood, and the main metabolites were glucuronide conjugates, which were considered difficult to cross the BBB.[17] Therefore, the brain distribution ratio ($AUC_{brain}/AUC_{blood}$) of C was calculated only to be 0.0726 ± 0.0376 while EC was 0.1065 ± 0.0531. In our previous work, the brain distribution ratio of danshensu was calculated to be 0.25 ± 0.04. The good BBB permeability of danshensu is probably due to its low molecular weight and low protein binding rate of 5%.[18] C and EC are stereoisomers, and a stereoselective process was involved in the passage of these compounds across BBB cells.[30] In this study, the brain distribution ratio of EC was higher than C, which agrees well with the previous results of C and EC acrossing BBB cells in vitro. C and EC rapidly (within 20 min) crossing the BBB and entering the extracellular fluid of the hippocampus after iv administration demonstrated a substantial basis for the therapeutic effect on brain diseases and provided scientific proof for rational administration. The BBB penetration mechanisms of C and EC (such as how or in what form they passed through the BBB) and the pharmacokinetic profiles of metabolites are under investigation in our laboratory.

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### Notes

The authors declare no competing financial interest.

## ABBREVIATIONS USED

C, (+)-catechin; EC, (−)-epicatechin; HPLC, high-performance liquid chromatography; CL, chemiluminescence; BBB, blood–brain barrier; $r$, correlation coefficients; RSD, relative standard deviation

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