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Tissues Distribution of R-(–)- and S-(+)-*m*-Nisoldipine after Single Enantiomer Administration in Rats

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Rapid, sensitive, and selective high-performance liquid chromatography methods were developed and validated for determination of *m*-nisoldipine enantiomers in rat tissues. All of the samples were prepared based on a simple and efficient liquid–liquid extraction method. After validating that no racemation occurred by ULTRON ES-OVM (Japan), *m*-nisoldipine enantiomers were determined, respectively, on a reverse-phase C_{18} column (5 μm, 250 × 4.6 mm). This method was applied to study tissue distribution of *m*-nisoldipine enantiomers in rats after a single administration of *m*-nisoldipine enantiomers. By the two-sample *t* test, there were basically no significant differences between the two enantiomers in each tissue ($p > .05$), which indicates that they may have the same potency in rats. In small intestine, lung, liver, and spleen, the concentrations of R-(–)- and S-(+)-*m*-nisoldipine were high at 30 and 150 min than that at 90 min, which showed that *m*-nisoldipine enantiomers may have the phenomenon of hepatoenteral circulation. A small quantity of the prototype of R-(–)- and S-(+)-*m*-nisoldipine in brain showed that they can cross the blood–brain barrier to arrive at the brain tissue. The high quantity of distribution in lung and brain implied that the lipophilicity of the drug was powerful.

Keywords *m*-nisoldipine; enantiomers; tissues distribution; high-performance liquid chromatography

INTRODUCTION

Calcium channel antagonists (CCAs) are used today as first-line therapy drug for hypertension. The 1,4-dihydropyridines (1,4-DHPs) are the largest class of CCAs. The characteristic skeleton of this important group among the CCAs is the 1,4-DHP structure exhibiting phenyl substitution in position 4. The structural variations of this prototype is primarily due to the ester functions and variations in phenyl substitution as well as changes in position 2. R-(–)- and S-(+)-*m*-nisoldipine (Figure 1) (1,4-dihydro-2,6-dimethyl-4-[3-nitrophenyl]-3, 5-pyridinedicarboxylate methyl-2-methylpropyl esters), as new DHP calcium ion antagonists, were firstly composed and chirally separated in School of Pharmacy, Hebei Medical University (Li, Wang, Wang, & Du, 2006; Wu, Yan, Nie, & Yuan, 1989). *m*-Nisoldipine enantiomers are relatively more stable than other 1,4-DHPs under lighting condition, for example, nisoldipine enantiomers almost lost activity under lighting condition for 30 min (Heinig, Muschalek, & Ahr, 1994; Marques, Santos, Coelho, Bonato, & Lanchote, 2001; Zimmer & Muschalek, 1994).

Compared with nisoldipine, *m*-nisoldipine increased cardiac output and cardiac index significantly and had an equal effect on rabbits in decreasing mean arterial blood pressure and increasing regional blood flow (Gao, Li, & Fu, 1990a; Zhang et al., 1994). Meanwhile, the negative inotropic effects of *m*-nisoldipine on myocardium were dramatically lower than that of nisoldipine. As a result, *m*-nisoldipine has relatively higher selectivity on the thoracic aorta than nisoldipine (Gao, Li, & Fu, 1990b).

The drug target macromolecules, usually proteins, are often selective for the spatial arrangement of the small drug molecule. Therefore, different enantiomers of *m*-nisoldipine may show the stereoselectivity regarding their pharmacokinetic and pharmacodynamic properties. Recently, chirality has caused great attention in many fields, such as pharmaceutical, biological

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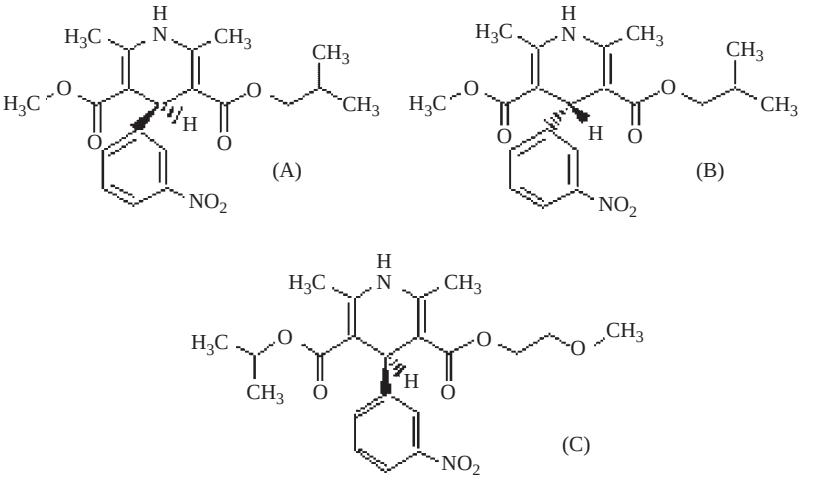

FIGURE 1. Chemical structures of *m*-nisoldipine enantiomers and nimodipine. (A) S-(+)-*m*-nisoldipine, (B) R-(-)-m-nisoldipine, and (C) nimodipine.

active molecules, environmental, and agrochemicals (Aboul-Enein & Wainer, 1997). Generally speaking, while one of the enantiomers of a molecule shows an active biological behavior such as therapeutic activity, the other one may be inactive or even cause serious side effects. These enantiomers may also have different kinetics, metabolism, excretion, and stability behavior in their environment (Ali & Aboul-Enein, 2004).

In this study, first an ES-OVM chiral column was used to prove that no racemization occurred in rat tissues after a single enantiomer administration. Then on a reverse-phase C_{18} column (5 μm, 250 × 4.6 mm i.d.), a simple and sensitive HPLC method was established for the determination and quantification of R-(–)- and S-(+)-*m*-nisoldipine in rat tissue samples. The method was applied to study the tissue distribution of R-(–)- and S-(+)-*m*-nisoldipine for the first time after a single oral dose administration. To understand the tissue distribution process of R-(–)- and S-(+)-*m*-nisoldipine, it is essential to investigate their amounts in different tissues. The study of tissues distribution between enantiomers may reflect whether the stereoselectivity, therapeutical effect, and excretion pathways of *m*-nisoldipine enantiomers are same. Meanwhile, the study of tissues distribution of each enantiomer of *m*-nisoldipine after single enantiomer administration to rats would give answers to the question whether the racemization occurs in vivo or not, and furthermore, it is helpful to solve whether it is worth developing the enantiomerically pure final dosage form.

EXPERIMENTAL

Materials and Methods

Animals

Male Sprague–Dawley rats (Experimental Animals Center of Hebei Province, Shijiazhuang, P.R. China), weighing 250 ± 10 g, were kept in environmentally controlled breeding room for 5 days before the experiment and fed with standard laboratory food and water ad libitum, and all rats were dosed following an overnight fast (except for water).

Drugs

m-Nisoldipine enantiomers were supplied by School of Pharmacy, Hebei Medical University (Shijiazhuang, China). Nimodipine (Figure 1), used as internal standard (I.S.), was obtained from Chinese Drug and Biological Products Quality Control Institute (Beijing, China). *m*-Nisoldipine enantiomers were suspended in 0.5% CMC-Na (sodium carboxymethylcellulose) and the suspension (2.5 mg/mL) was orally administrated to rats at a dose of 20 mg/kg, respectively.

Chromatographic Systems

The high-performance liquid chromatography (HPLC) systems consisted of Waters 1525 pump, Waters UV 2487 dual λ absorbance detector, and a Waters Empower Project workstation. A Diamonsil C_{18} column (5 μm, 250 × 4.6 mm i.d.) was used. The separation was carried out with the mobile phase consisting of acetonitrile (HPLC grade; Tedia Company, Inc., Fairfield, USA)–water (62:38, vol/vol) at a flow rate of 1.0 mL/min. Chromatograms were monitored at 237 nm, and the temperature of column was kept at 30°C.

Sample Preparation

After oral administration of 20 mg/kg suspension of R-(–)- and S-(+)-*m*-nisoldipine (2.5 mg/mL), heart, liver, lung, spleen, kidney, brain, stomach, and small intestine samples were quickly taken out at 10, 30, 90, and 150 min. The time points were determined according to the concentration–time curves of *m*-nisoldipine (Wang, Zhang, Wang, Yuan, & Li, 2007; Wang et al., 2006). Tissue samples were rapidly put into normal saline solution to remove the blood or content, blotted on filter paper, and then were weighed for wet weight

and homogenized in saline solution (0.3 g/mL). The obtained tissue homogenates were added with 50 μL of 5 mol/L NaOH and 10 μL of 20 μg/mL internal standard (nimodipine). After vortex-mixing for 10 s, the homogenates were extracted with 3 mL of *n*-hexane-ether (1:1, vol/vol) and vortexed for 1 min and centrifuged for 10 min at 1,000 × *g*. The bottom aqueous layer was discarded and the upper organic layer (2 mL) was transferred into a clean glass tube, evaporated to dryness under nitrogen, reconstituted in 40 μL of methanol, and vortexed for 2 min. After centrifuging at 3,000 × *g* for 10 min, 20 μL of the reconstituted material was injected into the HPLC system for determination of the concentration of R-(–)- and S-(+)-*m*-nisoldipine.

Preparation of Standard and Quality Control Samples

Stock solution of each enantiomer was prepared in methanol. Standard solutions with a series of concentration were obtained by further dilution of the stock solution with methanol. All the solutions were stored at −20°C and were brought to room temperature before use.

A series of adequate quantity of standards of each enantiomer for the calibration curve were prepared in blank rat tissues homogenates, which were spiked with R-(–)-, S-(+)-*m*-nisoldipine, and nimodipine, the mixture was then treated following sample extraction procedure described above. Quality control samples were prepared separately for each pure, single enantiomer at three concentrations (low, medium, and high), respectively. The processed procedure was the same as for the standard calibration samples.

Statistical Analysis

Results were presented as $M \pm SD$. Two-sample t test was used for comparison of the two group means after R-(–)- and S-(+)-*m*-nisoldipine administration at the same time point. The criterion for statistical significance was set at $\alpha = 0.05$. Quantifications of R-(–)- and S-(+)-*m*-nisoldipine in tissues were obtained from the peak areas of R-(–)- and S-(+)-*m*-nisoldipine. The observed mass concentration was calculated by the regression equation. The column diagrams of R-(–)- and S-(+)-*m*-nisoldipine in tissues at different time after a single enantiomer administration were drawn according to the data.

RESULTS

Validation of the Enantioselective Assay Method for R-(–)- and S-(+)-*m*-Nisoldipine in Rat Tissues

Validation of the enantioselective assay method for R-(–)- and S-(+)-*m*-nisoldipine in rat tissues was performed according to Ch.P threshold (Zheng, Jiang, Shao, Li, & Yuan, 2005). The parameters tested in the validation procedure were limit of detection (LOD) and lower limit of quantitation (LLOQ), linearity and range, specificity, precision and accuracy, recovery and stability. Each parameter was tested for R-(–1)- and S-(+)-*m*-nisoldipine.

Limit of Detection and Lower Limit of Quantitation

The LOD for R-(–)- and S-(+)-*m*-nisoldipine were determined at a signal-to-noise ratio of 3:1 and were all found to be 0.2 ng. The LLOQ for R-(–)- and S-(+)-*m*-nisoldipine was defined as the lowest concentration at which both precision and accuracy were less than or equal to 20% and were found to be 0.5 ng in tissues ($n = 6$). The limits were sufficient for studies of tissue distribution following a single oral administration of R-(–)- and S-(+)-*m*-nisoldipine.

Specificity

The degree of interference by endogenous substances was assessed by inspection of chromatograms derived from processed blank and rat tissue samples. Figure 2 presented typical chromatograms of blank tissues, blank tissue spiked with R-(–)- and S-(+)-*m*-nisoldipine, and rat tissue samples after a single oral administration of R-(–)- and S-(+)-*m*-nisoldipine. It indicated that a good separation was obtained under the described condition, and R-(–)-and S-(+)-*m*-nisoldipine were equally eluted at 16.0 min approximately, respectively, the internal standard was eluted at 10.5 min approximately. No interfering peaks from endogenous substances were found at the retention times of R-(–)-, S-(+)-*m*-nisoldipine, or the internal standard.

Linearity and Range for R-(–)- and S-(+)-*m*-Nisoldipine

The standard curves of the peak area (Y) to the concentration (C) were constructed using $1/\chi^2$ weighted linear least-squares regression model. The standard curves, correlation coefficients, and linear ranges of *m*-nisoldipine enantiomers in each tissue are listed in Table 1.

Precision and Accuracy for R-(–)- and S-(+)-*m*-Nisoldipine

Accuracy, intraday, and interday precisions for R-(–)- and S-(+)-*m*-nisoldipine were evaluated from the results of quality control (QC) samples. Six replicates of QC samples for the individual enantiomer at three concentration levels were determined on three consecutive days. The intraday and interday precisions of the method were expressed as relative standard deviation (RSD) of the measurements. The accuracy of the method was determined by calculating the percentage deviation observed in the analysis of QC samples and expressed as the relative error (RE). All numbers of accuracy, intraday, and interday precisions were below the 15% Ch.P threshold (Zheng et al., 2005). It was suggested that the method was accurate and reproducible for the determination of *m*-nisoldipine enantiomers in rat tissues.

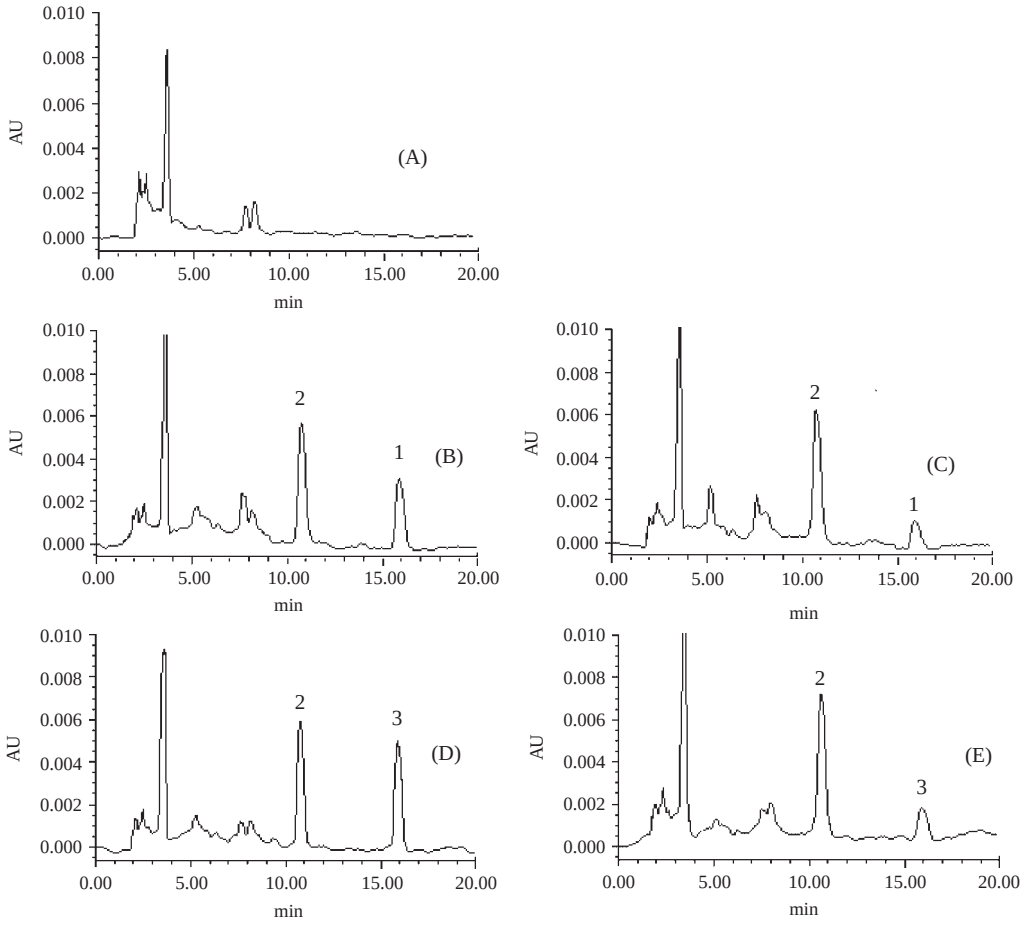

FIGURE 2. Chromatograms of *m*-nisoldipine enantiomers in rat liver. (A) Blank tissue, (B) blank tissue spiked with R-(-)-*m*-nisoldipine, (C) tissue sample after R-(-)-*m*-nisoldipine administration, (D) blank tissue spiked with S-(+)-*m*-nisoldipine, and (E) tissue sample after S-(+)-*m*-nisoldipine administration. 1, R-(-)-*m*-nisoldipine; 2, nimodipine (internal standard); and 3, S-(+)-*m*-nisoldipine.

Recovery and Stability

The recoveries of *m*-nisoldipine enantiomers were tested at three QC sample levels by comparing the peak areas from extracted tissue samples with those found by direct injection of standard solutions at the same concentration. The results showed that the mean recoveries of R-(–)-*m*-nisoldipine were 104.5, 96.2, and 105.5% (n = 6) and RSD were 10.6, 8.2, and 5.5%; and the mean recoveries of S-(+)-*m*-nisoldipine were 112.5, 98.2, and 101.3% (n = 6) and RSD were 13.4, 4.8, and 6.6%, respectively, at the three concentrations of quality control levels.

The stability of *m*-nisoldipine enantiomers was investigated at low, medium, and high concentrations under a variety of storage and processed conditions. The analyte was found to be stable (RE within ± 15%) after three cycles of freeze (–20°C)-thaw (room temperature) in rat tissues. The analyte was also shown to be stable in the reconstituted solution for 24 h at room temperature (RE within ± 15%) and in rat tissues at room temperature for at least 4 h (R.E. within ± 15%). No signs of degradation were found under the freeze condition (–20°C) for 15 days. Nevertheless, most of the 1,4-DHP derivatives were sensitive to light as a result of photo-degradation, and the solution of *m*-nisoldipine enantiomers is not stable when they are under lighting condition for a long time. To prevent oxidation of the DHP ring and transmutation of the nitro group in nitrobenzene ring, sample preparation and instrumental analyses were performed under feeble yellow light (Chen, Bao, Ma & Yuan, 2002).

Application of Analytical Method in Tissue Distribution for R-(–)- and S-(+)-*m*-Nisoldipine After a Single Enantiomer Administration

Concentrations of *m*-nisoldipine enantiomers in eight tissues of rats such as heart, liver, lung, spleen, kidney, brain, stomach, and small intestine were determined by HPLC, respectively, according to the procedure described above. The results are listed in Table 2. The distribution of *m*-nisoldipine enantiomers in various tissues after a single enantiomer oral administration of 20 mg/kg to rats is shown in Figures 3–5.

TABLE 1
Standard Curves, Correlation Coefficients and Linear Ranges of *m*-Nisoldipine Enantiomers in Tissue Samples

Tissues	*m*-Nisoldipine	Standard Curves	Correlation	Linear Ranges, ng/g
Heart	R-(–)	$Y = 0.0053C + 0.1135$	0.9946	3–1,500
	S-(+)	$Y = 0.0051C + 0.2114$	0.9983	30–4,200
Liver	R-(–)	$Y = 0.0053C + 0.115$	0.9953	7–1,400
	S-(+)	$Y = 0.0066C + 0.0452$	0.9984	3–720
Spleen	R-(–)	$Y = 0.0053C + 0.1325$	0.9887	6–1,800
	S-(+)	$Y = 0.0084C + 0.0991$	0.9926	5–1,000
Kidney	R-(–)	$Y = 0.0078C + 0.0882$	0.9964	3–180
	S-(+)	$Y = 0.0056C + 0.027$	0.9891	15–2,500
Lung	R-(–)-	$Y = 0.0053C + 0.1727$	0.9982	10–1,400
	S-(+)	$Y = 0.0053C + 0.0778$	0.9925	30–3,600
Stomach	R-(–)	$Y = 0.0065C - 0.8938$	0.9902	100–11,000
	S-(+)	$Y = 0.0064C - 0.9284$	0.9861	150–12,000
Small intestine	R-(–)	$Y = 0.0052C + 0.1718$	0.9897	160–4,200
	S-(+)	$Y = 0.0059C - 0.1155$	0.9915	30–4,800
Brain	R-(–)	$Y = 0.0077C + 0.0792$	0.9973	3–360
	S-(+)	$Y = 0.0063C + 0.0395$	0.9987	3–1,500

TABLE 2
Distribution of m-Nisoldipine Enantiomers in Various Tissues After a Single Oral Administration of 20 mg/kg *m*-nisoldipine Enantiomers to Rats ($M \pm SD$, $n = 5$ μg/g)

	10 min		30 min		90 min		150 min	
Tissues	R-(–)-	S-(+)-	R-(–)-	S-(+)-	R-(–)	S-(+)-	R-(–)	S-(+)-
Stomach	1.74 ± 0.67	1.15 ± 0.54	1.82 ± 0.84	1.14 ± 0.97	No data	1.91 ± 0.81	1.51 ± 1.03	0.90 ± 0.64
Intestine	1.54 ± 0.87	1.69 ± 0.79	1.44 ± 0.85	2.05 ± 0.79	0.49 ± 0.33	0.72 ± 0.71	1.40 ± 0.58	1.48 ± 0.91
Lung	0.23 ± 0.18	0.46 ± 0.26	0.48 ± 0.34	1.34 ± 0.85	0.04 ± 0.05	0.10 ± 0.10	0.21 ± 0.20	0.73 ± 0.68
Heart	0.31 ± 0.34	0.64 ± 0.51	0.77 ± 0.67	1.41 ± 0.69	0.16 ± 0.17	0.81 ± 0.52	0.16 ± 0.15	0.18 ± 0.11
Kidney	0.04 ± 0.02	0.17 ± 0.08	0.06 ± 0.04	0.75 ± 0.88	0.06 ± 0.05	0.52 ± 0.64	0.05 ± 0.03	0.04 ± 0.03
Liver	0.13 ± 0.21	0.11 ± 0.11	0.35 ± 0.50	0.18 ± 0.14	0.03 ± 0.03	0.02 ± 0.02	0.07 ± 0.07	0.07 ± 0.11
Brain	0.03 ± 0.02	0.11 ± 0.08	0.06 ± 0.06	0.30 ± 0.27	0.06 ± 0.05	0.14 ± 0.20	0.02 ± 0.01	0.12 ± 0.12
Spleen	0.2 ± 0.15	0.15 ± 0.18	0.57 ± 0.55	0.30 ± 0.18	0.07 ± 0.06	0.10 ± 0.03	0.40 ± 0.49	0.05 ± 0.05

Intestine is from small intestine.

DISCUSSION

For biological samples, the sample handling step is usually necessary before a HPLC analysis of drugs to remove proteins and to increase the selectivity and sensitivity of the method. In our study, protein precipitation first was chosen as the method of pretreating tissue samples. However, it is difficult to achieve good extraction recovery from tissue samples which was confirmed in our study, the extraction recovery is below 50%; furthermore, interfering peaks from endogenous substances were also found at the retention times of R-(–)- or S-(+)-*m*-nisoldipine. Therefore, liquid–liquid extraction method was chosen as a sensitive and effective pretreated method. Different extraction solvents such as ether, acetoacetate, toulene, *n*-hexane, and cyclohexane were compared. It was showed that interfering peaks were detected in pretreated samples with acetoacetate, cyclohexane, and toulene. There were no interfering peaks in pretreated samples with ether or *n*-hexane, but it was hard to control the quantum of ether. So *n*-hexane-ether (1:1, vol/vol) was selected as the best extraction solvent, and the extraction recovery was above 70%.

First, *m*-nisoldipine enantiomers were determined on an ES-OVM chiral column (5 μm, 150 × 4.6 mm) after a single administration of *m*-nisoldipine enantiomers. The mobile phase consisted of methanol–acetonitrile(5 mmol/L)–ammonium acetate (15:15:70, vol/vol/vol) with a flow rate of 0.8 mL/min and the detection wavelength was at 237 nm. After a single

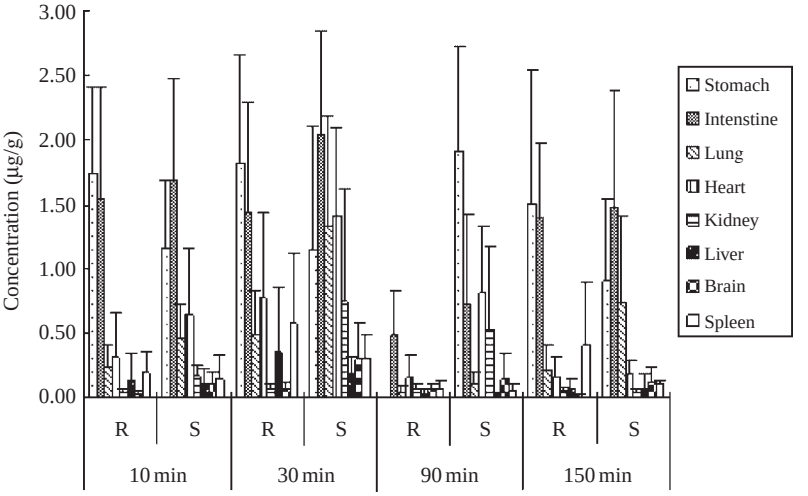

FIGURE 3. Distribution of *m*-nisoldipine enantiomers in various tissues after a single enantiomer oral administration of 20 mg/kg to rats ($n = 5$).

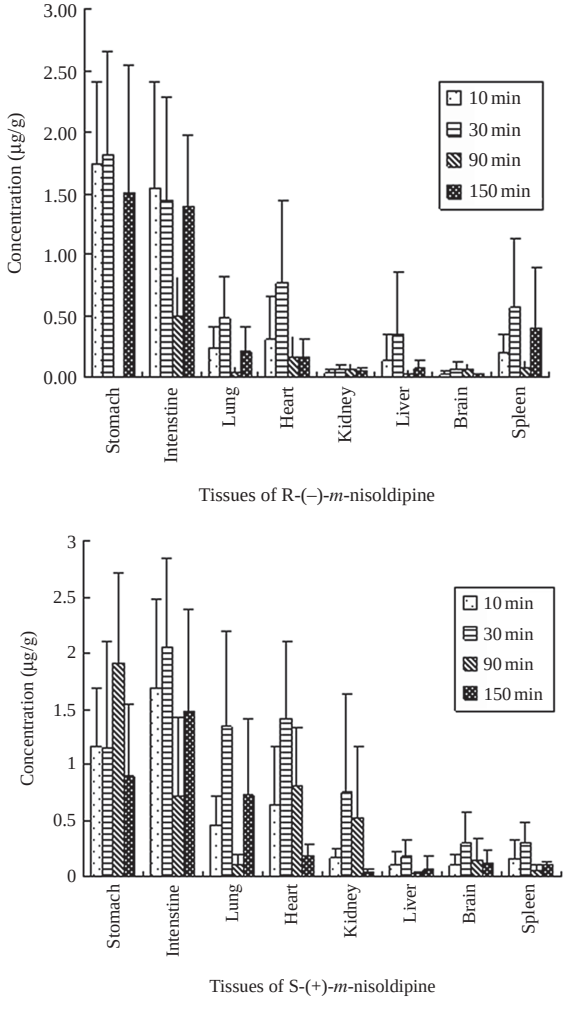

FIGURE 4. Distribution of *m*-nisoldipine enantiomers in various tissues at the same time points after a single enantiomer oral administration of 20 mg/kg to rats ($n = 5$).

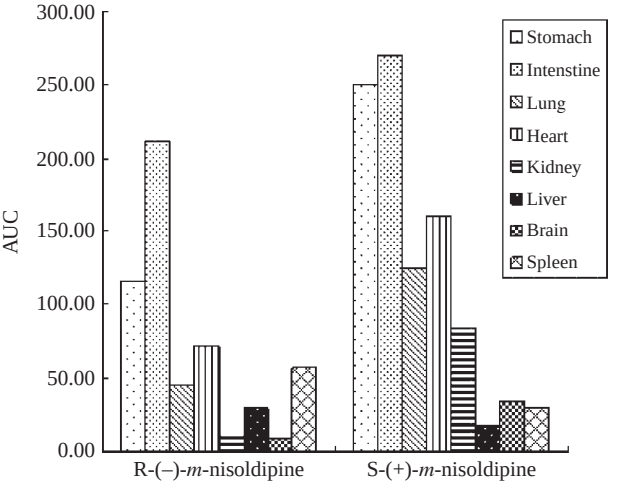

FIGURE 5. Area under the curve (AUC) of *m*-nisoldipine enantiomers in different tissues after oral administration of 20 mg/kg *m*-nisoldipine enantiomers to rats ($n = 5$).

enantiomer oral administration, there was no the peak of the other enantiomer in the chromatograms of R-(–)- or S-(+)-*m*-nisoldipine sample by using the ES-OVM column. The distinction of R-(–)- and S-(+)-*m*-nisoldipine in different tissues was compared, and the results showed that no racemization occurred in rat tissues after a single enantiomer oral administration. The chromatograms of R-(–)- and S-(+)-*m*-nisoldipine were shown in Figure 6. Therefore, we choose the Diamonsil C_{18} column (5 μm, 250 × 4.6 mm i.d.) to determine the concentrations of R-(–)- and S-(+)-*m*-nisoldipine in different tissues.

As shown in Table 2 and Figure 3, after a single enantiomer oral administration of 20 mg/kg R-(–)- and S-(+)-*m*-nisoldipine, R-(–)-*m*-nisoldipine was mainly observed in stomach, intestine, heart, lung at 10 min; stomach, intestine, heart, spleen at 30 min; intestine, lung, heart, spleen at 90 min; and stomach, intestine,

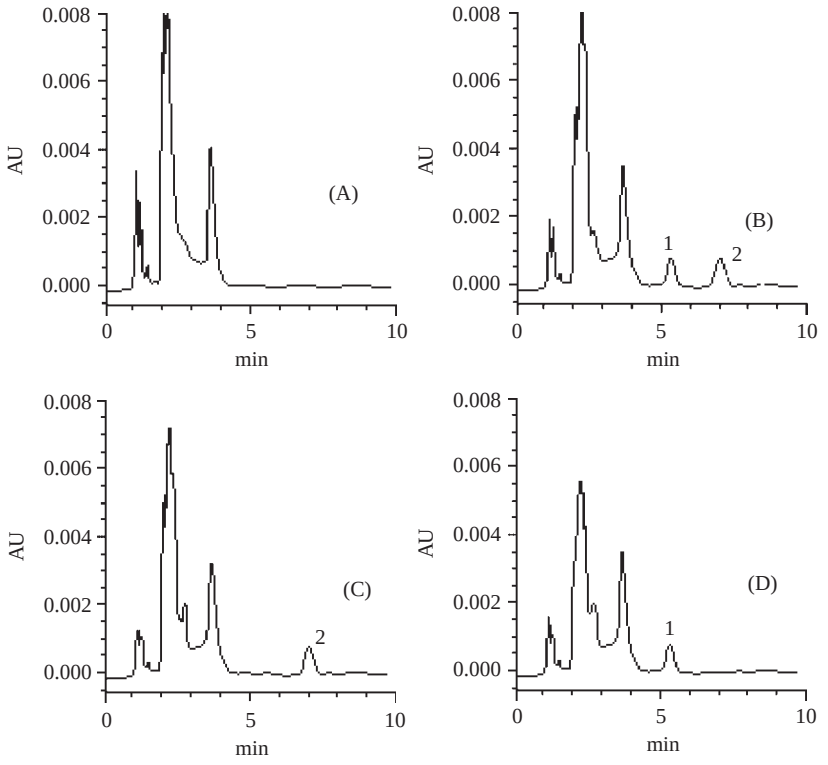

FIGURE 6. Chromatograms of *m*-nisoldipine enantiomers in rat liver sample on ES-OVM column. (A) Blank tissue, (B) blank tissue spiked with R-(–)- and S-(+)-*m*-nisoldipine, (C) tissues sample after R-(-)-*m*-nisoldipine administration, and (D) tissue sample after S-(+)-*m*-nisoldipine administration. 1, S-(+)-*m*-nisoldipine and 2, R-(-)-*m*-nisoldipine.

spleen, heart at 150 min while the quantity of R-(-)-*m*-nisoldipine was relatively low in liver, kidney, and brain. S-(+)-*m*-nisoldipine was mainly observed in stomach, intestine, heart, lung at 10 min; intestine, heart, lung, stomach at 30 min; stomach, heart, lung, intestine at 90 min; and intestine, stomach, heart, brain at 150 min while S-(+)-*m*-nisoldipine was relatively low in brain, spleen, and liver. By the two-sample *t* test, there were no significant differences between the two enantiomers in each tissue ($p > .05$, $n = 5$).

From Table 2, Figures 3, and 5, the highest amounts of *m*-nisoldipine enantiomers was observed in stomach and intestine, on one hand, which might be attributed to the way of oral administration, on the other hand, which also explained that R-(–)- and S-(+)-*m*-nisoldipine had special affinity to small intestine. There were all kinds of digestive enzymes and enzymes produced by enteric microbial flora in rat digestive tract, so R-(–)- and S-(+)-*m*-nisoldipine might be converted to its metabolites by these enzymes after ingestion. It was yet found that there was a small quantity of the prototype of R-(–)- and S-(+)-*m*-nisoldipine in brain which indicated that they can cross the blood–brain barrier to arrive at the brain tissue. The distribution in lung and brain also implies that the lipophilicity of the drug is powerful. *m*-Nisoldipine enantiomers easily dissolve in chloroform and acetone, sparingly dissolve in methanol and alcohol, and scarcely dissolve in water, which just show the powerful lipophilicity of the drug. In addition, the small intestine, stomach, lung, and heart contain a large quantity of R-(–)- and S-(+)-*m*-nisoldipine compared to other tissues. These tissues may have been associated with R-(–)- and S-(+)-*m*-nisoldipine toxicity. For this reason, the efficacy or toxic effects of *m*-nisoldipine enantiomers in vivo should be further explored.

From Figure 4, the quantity of *m*-nisoldipine enantiomers in rat tissues was basically high at 30 min and then on the whole declined gradually, which might mainly be attributed to the oral administration and endogenous material of different tissues. Simultaneously, the results indicated that the distribution of *m*-nisoldipine enantiomers in different tissues showed dynamic changes. It was also found that the concentrations of R-(–)-and S-(+)-*m*-nisoldipine were high at 30 and 150 min than that at 10 and 90 min in small intestine, lung, liver, and spleen. Information on R-(–)- and S-(+)-*m*-nisoldipine tissue distribution is not only helpful in understanding various pharmacological responses observed after R-(–)- and S-(+)-*m*-nisoldipine administration and of their clinical significance but also for the evidence for later pharmacodynamics.

Therefore, it is indispensable to further investigate the toxicology, pharmacodynamics, active and passive transport, and biotransformation of R-(–)- and S-(+)-*m*-nisoldipine in vivo by using some other stereoselective methods. We will

continually reveal the chiral pharmacodynamics characteristics of *m*-nisoldipine enantiomers. If the pharmacodynamics is same, it is not worth developing the single enantiomer formulation.

CONCLUSION

In summary, a rapid and selective high-performance liquid chromatographic method with an ES-OVM column was first presented to determine *m*-nisoldipine enantiomers that no racemization occurred in tissue samples. Afterwards, we developed a rapid and sensitive method with a C_{18} column for determination of R-(–)- and S-(+)-*m*-nisoldipine in tissue samples. The method was simple and showed high precision and good repeatability and it could be successfully utilized to study the tissues distribution of *m*-nisoldipine enantiomers in rats. This study would make it possible in vivo to carry out pharmacokinetics and pharmacodynamic studies of R-(–)- and S-(+)-*m*-nisoldipine in humans.

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