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Pharmacokinetics and pharmacodynamics of a novel Acetylcholinesterase Inhibitor, DMNG-3

Xin-Guo Zhang*, Fei Kou, Guo-Di Ma, Peng Tang, and Zhong-Duo Yang

*School of Life Science and Engineering, Lanzhou University of Technology, Key Laboratory of screening for and Deep processing in new Tibetan medicine of Gansu Province, Gansu, P.R. China, *Email: biodrug@163.com*

DMNG-3 (3β-Methyl-[2-(4-nitrophenoxy)ethyl]-amino]con-5-enine), is a new and the potentially most potent acetylcholinesterase inhibitor recently obtained from conessine by N-demethylation and nucleophilic substitution reaction. In the present study, a step-down passive avoidance test was used to investigate whether DMNG-3 could modulate impairment of learning and memory induced by scopolamine, and a high performance liquid chromatography (HPLC) method for the determination of DMNG-3 in biological samples was applied to study its pharmacokinetics and tissues distribution. Separation was achieved on C18 column using a mobile phase consisting methanol-water (70:30, v/v) at a flow rate of 1.0 ml/min. The intra- and inter-day precisions were good and the RSD was all lower than 1.30%. The mean absolute recovery of DMNG-3 in plasma ranged from 88.55 to 96.45%. Our results showed oral administration of DMNG-3 (10, 25, 50 mg/kg/day) can significantly improve the latency and number of errors and had a positive effect of improvement of learning and memory in mice in passive avoidance tests. The elimination half-life (T1/2) was 14.07±1.29, 15.87±1.03 h, and the total clearance (CL) values were 0.70±0.11, 0.78±0.13 L/h/kg, respectively. The pharmacokinetic studies showed that DMNG-3 has a slowly clearance and large distribution volume in experimental animals, and its disposition is linear over the range of doses tested. The liver, small intestine, stomach, and large intestine were the major distribution tissues of DMNG-3 in mice. It was found that DMNG-3 could be detected in brain, suggesting that DMNG-3 can cross the blood-brain barrier. The present study shows that DMNG-3 can be possible developed as a new drug for the treatment of Alzheimer's disease in the future.

Key words: DMNG-3, step-down passive avoidance test, HPLC, pharmacokinetics, tissue distribution

INTRODUCTION

Alzheimer's disease (AD) is a common form of dementia, characterized by the degeneration of basal forebrain cholinergic neurons innervating the cortex amygdale and hippocampus, with difficulty in maintaining and sustaining attention, and with profound cognitive impairment, such as loss of memory and learning ability (Michael et al. 2003, Lanz et al. 2004, Fodale et al. 2006). Four different forms of AD are reported: with delirium, with delusions, with depressed mood and uncomplicated, each indicating the predominant feature of clinical presentation. The course of the disease is fluctuant, and cognitive impairment, personality change, psychotic symptoms, incontinence, gait and motor disturbance, seizures and myoclonus can occur. These cognitive deficits cause significant impairment in social and occupational function.

AD is a progressive neurodegenerative disorder which is characterized by memory loss and other cognitive impairments. There is not a clear theory on disease due to its complex etiology. AD includes several diverse hallmarks such as β-amyloid deposits, τ-protein aggregation, oxidative stress, or low levels of acetylcholine (ACh) (Morain et al. 2000). The cholinergic hypothesis of AD suggests that low levels of ACh in specific regions of the brain result in learning and memory dysfunction. This hypothesis indicates that increase of ACh levels by inhibition of acetylcholinesterase (AChE) is beneficial for the treatment of AD. Today, AChE inhibitors are first-line medications for the treatment of AD, and are associated with mild improvements in cognitive function, behavior, and activities of daily living; However, the most adverse effects of AChE inhibitors, such as nausea, vomiting, diarrhea, dizziness, confusion, and cardiac arrhythmia, are still inevitable (Wilkinson et al. 2004, Winslow et al. 2011). Recently, 11 new AChE inhibitors were obtained from conessine by N-demethylation and nucleophilic substitution reaction. The most potent potential inhibitor was DMNG-3 (3β-Methyl-[2-(4-nitrophenoxy)ethyl]-amino]con-5-enine, Fig. 1) with a 4-nitrophenoxyethyl at 3-N position of isoconessimine, which showed an IC_{50} value of 0.11 μmol/L, being close to those of huperzine A (IC_{50}, 0.07 μmol/L) (Jin et al. 2013).

Correspondence should be addressed to X. Zhang
Email: biodrug@163.com

Fig. 1. Chemical structures of DMNG-3 (3β-Methyl-[2-(4-nitrophenoxy) ethyl]-amino]con-5-enine).

Although DMNG-3 showed a higher inhibition of AChE activity, little is known regarding their biological activity in vivo and pharmacokinetic studies. Poor pharmacokinetics is one of the reasons for the withdrawal of drug candidates from clinical trials (Kuuranne et al. 2008). Thereby, the objective of this study was to develop a step-down passive avoidance test to investigate whether DMNG-3 could modulate impairment of learning and memory induced by scopolamine. In addition, a sensitive and precise analytical method for determining DMNG-3 in biological samples was applied and further to understand its pharmacokinetic characteristics and tissues distribution in rats after oral administration. Our results suggested that DMNG-3 can be possible developed as a new drug for the treatment of Alzheimer's disease in the future.

METHOD

Chemical reagents

The baicalein was purchased from the company of Biotechnology company of ShangHai YuanYe, with over 98% purity. DMNG-3 was offered by School of Life Science and engineering of Lanzhou university of technology, with over 98% purity. The chromatographic methanol purchased from the company of DaMao chemical reagent factory in TianJin. Deionized water was purchased from the company of WaHaHa in HangZhou. Huperzine A was purchased from the Wanbang pharmaceutical company in ZheJiang. The Scopolamine hydrobromide was purchased from the first pharmaceutical company in ChengDu. Other reagents were all of analytical grade.

Step-down passive avoidance test

ICR mice, weighing 20±2 g, were obtained from the Lanzhou university animal center. According to the requirement of the National Act on the Use of Experimental Animal (People's Republic of China); the protocols of animal experiments were approved by the Animals Ethics Committee of Lanzhou

University of Technology. For the pharmacodynamics study, 60 ICR mice were randomly assigned to six groups: 1 – normal group; 2 – scopolamine-treated for control group; 3 – scopolamine with 2 mg/kg Huperzine A for positive control group; 4–6 – scopolamine with 10, 25, 50 mg/kg DMNG-3 for Drug treatment groups. The positive control group (2 mg/kg/day Huperzine A) and three DMNG-3 treatment groups (10, 25 and 50 mg/kg/day) were orally administered indicated doses for 15 days in a row once daily prior to testing in the apparatus. The normal groups and control groups were orally administrated 0.2 ml saline per 20 g for 15 days. Scopolamine (3 mg/kg, ip) was given to all scopolamine--treated groups 60 minutes before testing.

The inhibitory avoidance apparatus was a 50×25×25-cm xyloid box whose floor consisted of brass wires (1 mm in diameter) spaced 1 cm apart. A 6 cm wide, 2.5 cm high platform was placed on the floor of the box against the left wall. Animals were placed on the platform and their latency to step down on the grid with all four paws was measured. At the beginning of training trial, mice were placed in the box to adapt for 3 min. In the training session, mice were exposed to a 5 min learning course, during which they received an electric foot shock (36 V, AC) if the animals stepped down from the platform. After 24 h, in the test session, 24 h after the training test was performed, and mice were placed on the platform. The latency to step down onto the grid for the first time and the number of errors when subjected to shocks with 5 min (the shocks were maintained for 5 min) were measured as learning performances (Roesler et al. 2000, Wu et al. 2012).

Chromatographic equipments and conditions

The HPLC system consists of a PU-2086 pump, a 2487 UV/VIS detector set at 300 nm, and a Mx-2080-31. The analyzes were separated on a Kromasil ODS C18 reversed-phase column (4.6×250 mm, 5 μm) at the room temperature with the mobile phase of methanol-water (70:30 v/v) at a flow rate of 1.0 ml/min.

Plasma sample preparation

The IS solution (50 μl, 5 μg/ml in methanol) was added to 100 μl of plasma samples or tissue homogenate samples, then the mixture was precipitated with 500 μl methanol. After vortexing for 5 min, the denatured protein precipitate was separated by centrifugation at 12000 rpm for 10 min at room temperature (Adessi et al. 2003, Li et al. 2012). The supernatant was evaporated to dryness in a water-bath at 37°C and then dissolved in 100 μl mobile phase. A 20-μl volume of this sample solution was injected onto HPLC for analysis. Simple one-step protein precipitation with

methanol was used to minimize baseline distortion and optimize extraction recovery (Hasim et al. 2011).

Preparation of calibration standards, quality control samples and low limit of quantitation

The configuration of the DMNG-3 standard solution: DMNG-3 (10 mg) and internal standard (IS) (10 mg) were prepared by accurately weighing the required amounts into separate volumetric flasks and dissolving in methanol. The methanol stock solution of DMNG-3 was serially diluted to get the standard working solutions with the desired concentrations: 0.08, 0.1, 0.2, 0.5, 1.0, 10.0 μg/ml for plasma and 0.08, 0.1, 0.2, 0.5, 1.0, 10.0 μg/ml for tissue homogenates and IS was diluted to 10 μg/ml with methanol. All solutions were stored at 4°C (Li et al. 2012).

Quality control (QC) standards were prepared by spiking blank plasma with appropriate volumes of DMNG-3 standard solutions to make low, medium and high drug concentrations of 0.08, 0.20, 1.00 μg/ml, respectively. Preparation and extraction of the plasma samples and the spiked samples were carried out as the above-mentioned extraction method. A standard curve was constructed by plotting the observed peak area ratios of DMNG-3 and IS against the respective standard concentration using the least-squares method.

The low limit of quantification (LLOQ) in plasma was defined as ten times the signal-to-noise ratio (S/N) and the lowest concentration on the calibration curve for which an acceptable accuracy (RE) within ±20% and a precision (RSD) below 20% can be obtained.

Accuracy and precision

For the determination of intra-day and inter-day accuracy and precision of the proposed method, the QC samples at low, medium and high levels (0.08, 0.20, and 1.00 μg/ml) were repeatedly injected into the HPLC column. In order to determine intra-day accuracy and precision, the assays were carried out on the same samples at five duplicates on the same day. This process was repeated on three consecutive days in order to determine the inter-day accuracy and precision. The precision was expressed as the relative standard deviation (RSD), and intra- and inter-day assay accuracies were described as percentages of theoretical concentration (Lin et al. 2010, Kwon et al. 2004).

Extraction recovery

The recovery of the DMNG-3 was calculated by the comparison between peak areas of the calibration samples analyzed by the normal procedure and those obtained after adding the same amounts of reference substances to blank plasma after extraction. Experiments were performed at the three QC concentration levels (0.08, 0.20, 1.00 μg/ml) in triplicate (Lin et al. 2010).

Stability study of plasma sample

The stabilities of the analyte in plasma were assessed by analyzing triplicate QC samples. These QC samples were stored at –20°C for 2 weeks, then all samples were thawed and analyzed along with the freshly prepared set of QC samples for storage stability study (Lin et al. 2010, Zhuang et al. 2011, Wang et al. 2011). Moreover, the QC samples were determined after three freeze-thaw cycles and the concentration were compared to their nominal concentration for freeze-thaw stability testing study.

Pharmacokinetic study

The healthy sprague-dawley (SD) rats (220±20 g) were provided from the Experimental Animal Center of Lanzhou University (GanSu, China). According to the requirement of the National Act on the Use of Experimental Animal (People's Republic of China); the protocols of animal experiments were approved by the Animals Ethics Committee of Lanzhou University of Technology. For the pharmacokinetic study, 12 SD rats were randomly assigned to 2 groups and each group contained 6 rats, respectively. According to the results of step-down passive avoidance test, the SD rats were orally administered DMNG-3 DMSO solution which was suspended by 0.5% CMC-Na (DMSO<10%) at the dose of 20 mg/kg, 40 mg/kg. 250 μl blood samples were collected in heparin containing tubes from the retro-orbital plexus of rats at times 0, 0.5, 1, 3, 5, 8, 12, 16 24, 36, 48 and 72 h after oral administration, and immediately centrifuged at 5000 RPM for 10 min (Hasim et al. 2011, Zhuang et al. 2011, Lin et al. 2009). The plasma samples were frozen at –20°C until analysis.

Tissue distribution study

30 ICR mice were randomly assigned to 3 groups, respectively. Each group contained 10 mice, DMSO solution of DMNG-3 was suspended by 0.5% CMC-Na (DMSO<10%) was administered orally at a dose of 25 mg/kg to each group. The different tissue including the heart, liver, lung, spleen, kidney, brain, stomach, small intestine, large intestine and fat samples were collected at 6, 9, and 12 h, respectively (Hasim et al. 2011, Zhang et al. 2003). These time points were selected on the basis of pharmacological

and pharmacokinetic studies in rats. Tissue samples were individually homogenized with normal saline at the rate of 1:1 (weight/volume) using an homogenizer. The obtained tissue homogenates were centrifuged at approximately 12,000 rpm for 10 min and supernatants were stored at –20°C until analysis (Wang et al. 2012, Guo et al. 2013, Hao et al. 2005).

Data analysis

Pharmacodynamic data were analyzed by one-way analysis of variance followed by Duncan's multiple range test when the T-test was significant, and which using the SPSS 17.0 software. Statistically significant differences between control and experimental groups (P<0.05) are indicated.

Pharmacokinetic parameters of DMNG-3 were calculated by non-compartmental and compartmental methods using the DAS 3.0 software (Medical College of Wannan, People's Republic of China). The maximum plasma concentrations (Cmax) and the time to reach the maximum concentrations (Tmax) were obtained directly from the observed data. The elimination rate constant (kel) was calculated on the slope of terminal portion of the log-transformed plasma concentration versus time curve by linear regression. The elimination half-life ($T_{1/2}$) was calculated using 0.693/kel. The area under curve from 0 hour to t (AUC_{0-t}) in rats was calculated via the trapezoidal rule. The area under the plasma concentration-time curve to time infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-\infty}=AUC_{0-t}+Ct/Kel$, with 'Ct' defined as the last measured plasma concentration at time t (Wang et al. 2011).

RESULTS AND DISCUSSION

Effects of DMNG-3 on acquisition of memory in step-down passive avoidance test

AD is one of the most frequent causes of dementia, representing 50–60% of all dementia cases and affecting 10–20% of people older than 65 years. Although many intellectual functions are impaired (attention, orientation, language, judgment), the most prominent symptom of AD is represented by a progressive memory loss (Winslow et al. 2011). In general, memory processes are divided into three stages: learning acquisition, memory retention, and reappearance (Lu 2001). The step-down type passive avoidance is used to measure the three stages of memory process depending on drug-treated period. Chemical agents such as scopolamine, sodium nitrite, and 45% ethanol can cause memory impairment in mice (Luo et al. 2003). In this present paper, as can be seen from the Figure 2, in the control

group, the latencies were significantly shortened and the number of errors markedly increased compared with the normal group in the step-down test (p<0.01). After mice were orally administered DMNG-3 (10, 25 and 50 mg/kg/day) for 15 days in a row, the influence of DMNG-3 on AD model mice is obviously higher than control group and the latencies were significantly increased compared with the control rats (p<0.05, p<0.01). In addition, the errors number of mice administered DMNG-3 (10, 25 and 50 mg/kg/day) for 15 days in a row were significantly decreased compared with the control group (p<0.05, p<0.01).

Huperzine A, derived from the Chinese herb *Huperzia serrata* as a potent, reversible, selective inhibitor of AChE, which has a mechanism of action similar to donepezil, rivastigmine and galantamine. A large number of preclinical studies and clinical trials had shown the effect of Huperzine A in treating AD (Yang et al. 2013). In our study, in the high dose group, the latencies were no significantly shortened and the number of errors markedly increased compared with Huperzine A-the positive drug group in the step-down test (p>0.05).

AD is a neurodegenerative disorder characterized by a progressive loss of memory and cognition. It has long been recognized that the cholinergic system is critically involved in the control of cognition, and AD can be reversed by preventing the breakdown of Ach in the synaptic cleft with AChE inhibitors (Yan and Feng 2004, Wu et al. 2012). Our results demonstrated that DMNG-3 can significantly improve the learning performances in scopolamine-induced learning and memory impairments in mice with increasing latency and a decreasing number of errors in the step-down test in a dose-dependent manner, which indicated that DMNG-3 might be useful for the treatment of AD.
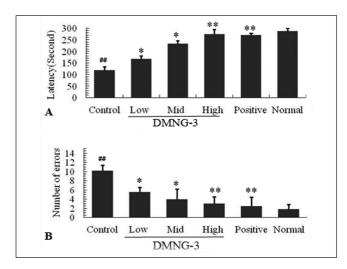

Fig. 2. The effect of DMNG-3 on scopolamine-induced learning and memory impairment in the Step-down test. *P<0.05, **P<0.01 compared with the control group, and ##P<0.01 compared with the normal group.

Method validation

Specificity

A wavelength of 300 nm was chosen according to maximum absorption spectrum of DMNG-3 to achieve a high selectivity. Representative chromatograms of blank plasma sample, plasma spiked with 100 μg/ml of DMNG-3, plasma sample at 6 h after administration of DMNG-3 at a dose of 20 mg/kg are presented in (Fig. 3). The mobile-phase composition played a vital role in obtaining appropriate retention time and higher mass spectrum sensitivity. The mobile phase consisting of A methanol and B H_2O (70:30, v/v)was approved to be optimal for this study. Several possible internal standards were tested including Acetic acid-alpha naphthyl esters, baicalein, baicalin, alantolactone. Acetic acid-alpha naphthyl esters and alantolactone were firstly considered as IS, but DMNG-3 had peaks that could not be separated with them. The extraction efficiency and chromatographic behavior of baicalein (5-, 6-, 7-trihydroxyflavone) were similar to those of DMNG-3, so it was tested in the same conditions as DMNG-3, and good peak shape in a proper time was achieved. Retention times of DMNG-3 and IS were approximately 15 and 18 min, respectively. There was no interfering peak from endogenous substances in the blank plasma and tissue.

Linearity of Calibration Curves and Limit of Determination

The standard curve was prepared for DMNG-3 in the range of 0.08–10.00 μg/ml, which covered the levels following the administration of a single dose of 20 mg/kg DMNG-3. The standard curve was described by equations
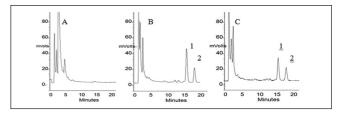

Fig. 3. Representative HPLC Chromatograms of (A) blank rat plasma sample; (B) blank rat plasma spiked with DMNG-3 and the IS; (C) rat plasma sample obtained after administration DMNG-3 (1: DMNG-3, 2: IS).

are listed in Table 1, where y is the DMNG-3-to-IS peak-area ratio, x the concentration, and the correlation coefficient and the linear ranges for DMNG-3 correlation coefficients and linear ranges of DMNG-3 in each tissue are also listed in Table 1. The LOQ in plasma was defined as the lowest concentration that can be determined with relative error and relative standard deviation (RSD) <10%, LODs for Plasma and different tissues are also shown in Table 1.

Recovery

The recoveries of DMNG-3 from the rat plasma were 88.55±0.99%, 89.49±1.36%, and 96.45±0.63% for low (0.08 μg/ml), medium (0.20 μg/ml), and high (1.00 μg/ml) concentrations, respectively. The results are summarized in Table 2, and the mean recovery in most tissues at three different concentrations were all above 83.19%.

Precision and Accuracy

The intra-and inter-day accuracies were estimated and the studied concentrations (0.08 μg/ml, 0.20 μg/ml,

Table 1. Regression equation, linear ranges and detection limits of the method

sample	standard curve	R^2	linear range (μg/ml)	LOD (μg/ml)
plasma	y=2.5336x−0.0318	0.9999	0.08–10.00	0.08
heart	y=2.347x−0.0053	0.9969	0.08–10.00	0.08
liver	y=2.603x−0.0411	0.9995	0.08–10.00	0.08
spleen	y=2.4456x−0.0104	0.9993	0.08–10.00	0.08
lung	y=2.5963x−0.0368	0.9996	0.08–10.00	0.08
kidney	y=2.3254x+0.012	0.9976	0.08–10.00	0.08
large intestine	y=2.6256x−0.0618	0.9998	0.08–10.00	0.08
small intestine	y=2.5951x−0.0456	0.9995	0.08–10.00	0.08
stomach	y=2.3489x+0.0108	0.9987	0.08–10.00	0.08
fat	y=2.3415x+0.0189	0.9987	0.08–10.00	0.08
brain	y=2.346x+0.0268	0.9973	0.08–10.00	0.08

Table 2. Recovery of DMNG-3 in plasma of rats (n=5)

spiked concentration (µg/ml)	recovery (Mean±SD, %)	RSD (%)
0.08	88.55±0.99	1.12
0.20	89.49±1.36	1.52
1.00	96.45±0.63	0.67

1.00 µg/ml) were lower than 10%, and the accuracy was within ±1.30%, These values were within the acceptable range (±15%) indicating that the present method had a good accuracy and precision. The result of accuracy and precision were presented in Table 3.

Table 3. Precision and accuracy data for DMNG-3 in plasma of rats (n=3 days, 5 times per day)

concentration µg/ml	intra-day		inter-day	
	RSD (%)	accuracy (%)	RSD (%)	accuracy (%)
0.08	0.72	92.63	0.75	95.14
0.20	1.03	93.47	1.30	92.29
1.00	1.03	97.16	0.94	91.35

Stability

The stability of DMNG-3 in samples was investigated under a variety of storage and process conditions. For storage stability, samples were prepared and stored at –20°C for 2 weeks. On the 15th day, all samples were thawed and analyzed along with the freshly prepared set of the quality control (QC) samples. Analysis of these samples were equal to the freshly prepared samples (RSD<15%). The results showed that all the samples were stable during these tests, and there were no stability-related problems during the routine analysis of samples.

Application to pharmacokinetics study

In order to ensure enough plasma containing DMNG-3 to analysis in the current pharmacokinetic study, pharmacokinetic data was obtained from rats but mice; Furthermore, the conversion of drug doses between species were used, and dose is adjusted and rounded from 25.50 mg/kg to 20.40 mg/kg, respectively. The method described above was successfully applied to a pharmacokinetics study in rat after oral administration of 20.40 mg/kg DMNG-3. The mean plasma concentration-time profiles of DMNG-3 are shown in (Fig. 4). Based on these results, the PK parameters were calculated and summarized in Table 4 (Li et al. 2012, Hasim et al. 2011, Lin et al. 2009).

Table 4. Main pharmacokinetic parameters of DMNG-3 after oral administration with two kinds dose of 40 mg/kg and 20 mg/kg to SD rats (X±SD, n=6)

parameter	unit	40 mg/kg		20 mg/kg	
		mean	SD	mean	SD
AUC (0–t)	µg/L.h	54.61	2.03	30.86	1.31
AUC (0–∞)	µg/L.h	57.09	1.97	32.01	1.72
MRT (0–t)	h	22.31	1.45	23.35	1.36
MRT (0–∞)	h	25.46	1.69	25.82	2.06
Kel	1/h	0.04	0.01	0.05	0.01
$T_{1/2}$	h	15.87	1.03	14.07	1.29
Tmax	h	16.13	0.23	12.59	0.17
V	L/kg	16.05	1.46	15.85	1.02
CL	L/h/kg	0.70	0.11	0.78	0.13
Cmax	µg/ml	2.06	0.21	0.97	0.11

After oral administration, DMNG-3 was absorbed slowly from rat gastrointestinal tract; the drug was detected in plasma from the first blood sampling time (30 min) and slowly reached Tmax values of 12.59±0.17, 16.13±0.23 h for 20 and 40 mg/kg, respectively. DMNG-3 showed slowly systemic clearance (CL 0.70±0.11, 0.78±0.13 L/h/kg) and big volume of distribution (Vd 16.05±0.14, 15.85±0.10 L/kg). The volume of distribution was almost 20 times total body water at 0.668 L/kg in rats (Davies et al. 1993), suggesting that DMNG-3 may be more distributed into extravascular systems. Moreover, the elimination half-life ($T_{1/2}$) was 14.07±1.29, 15.87±1.03 h. The corresponding value for $AUC_{0–t}$ and $AUC_{0–∞}$ was 54.61±2.03, 30.86±1.31, 57.09±1.97, and 32.01±1.72 µg/L.h, indicating that DMNG-3 was orally available.

Application to tissues distribution study

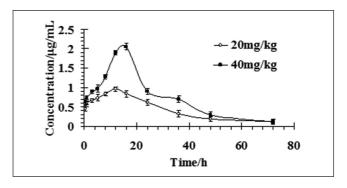Because the maximum concentration was at 12 h at the dose of 25 mg/kg, we chose 6, 9, and 12 h after

Fig. 4. Mean plasma DMNG-3 concentration versus time profiles after oral administration with a single dose of 40 mg/kg, 20 mg/kg in SD rats (n=6).

oral administration as the detected time points. In this experiment (n=10), we found that DMNG-3 could be detected in brain, suggesting that DMNG-3 can cross the blood-brain barrier and coincided with the influences of DMNG-3 on step-down activities in AD model mice. At 3 h after administration, DMNG-3 could be detected in most of the inspected tissues as is shown in (Fig. 5). This suggested that DMNG-3 is mainly absorbed in large intestine and stomach and digested slowly. The content of DMNG-3 in liver and small intestine showed similar dynamic change: maximum concentration was at 12 h, which might mainly be attributed to the oral administration (Yan et al. 2005), but it showed that DMNG-3 has retention in most tissues, which made it necessary to further investigate whether it accumulations were toxic (Li et al. 2012, Zhang et al. 2003, Hao et al. 2005).
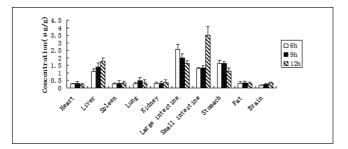

Fig. 5. Concentration of DMNG-3 in tissues after oral administration at the dose of 25 mg/kg to rats (n=10).

CONCLUSIONS

For the first time, a simple and reliable HPLC method to determine DMNG-3 levels in rat plasma and different tissues was established and validated. The method has been also successfully applied to the pharmacokinetics studies and tissues distribution. In conclusion, the results of our study provide the first evidence that oral administration of DMNG-3 can ameliorate impaired learning and memory processes. Therefore, DMNG-3 may be a potent potential AChE inhibitor that can be used for the treatment of AD in the future. However, considerable research is still necessary to fully understand the potential utility of DMNG-3 in the treatment of cognitive dysfunction of AD.

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