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Determination of memantine in rat plasma by HPLC-fluorescence method and its application to study of the pharmacokinetic interaction between memantine and methazolamide

Mohamed G. Hassan[a,b], Kamla M. Emara[c], Horria A. Mohamed[c], Hanaa M. Abdel-Wadood[c], Rie Ikeda[b], Mitsuhiro Wada[b], Naotaka Kuroda[b] and Kenichiro Nakashima[b]*

ABSTRACT: A sensitive high-performance liquid chromatographic method with fluorescence detection was developed to determine memantine (MT) in rat plasma. The method consists of pre-column labeling of MT with 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride (DIB-Cl) and a clean-up step with solid-phase extraction. A good separation of DIB-MT was achieved within 12 min on an octadecylsilica (ODS) column (150 × 4.6 mm i.d.; 5 µm) with a mobile phase of acetonitrile–water (70:30, v/v). The calibration curve prepared with fluoxetine as an internal standard showed good linearity in the range of 10–400 ng/mL ($r = 0.999$). The limits of detection and quantitation at signal-to-noise ratios of 3 and 10 were 2.0 and 6.6 ng/mL, respectively. The method was shown to be reliable with precisions of <5% for intra-day and <9% for inter-day as relative standard deviation. The fluorescence property and reaction yield of authentic DIB-MT were also examined. The proposed method was successfully applied to study the pharmacokinetic interaction between MT and methazolamide. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: memantine; HPLC-FL detection; 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride (DIB-Cl); rat plasma; pharmacokinetics

Introduction

Memantine (1-amino-3,5-dimethyladamantane, MT), a tricyclic amine, is chemically related to the antiviral amantadine and its *α*-methyl derivative rimantadine (Suckow *et al.*, 1999). Amantadine and rimantadine are used for prophylaxis and treatment of influenza, while MT is used for the treatment of Alzheimer's disease (Shuangjin *et al.*, 2007).

Recently, MT has been demonstrated to be useful in dementia syndrome. Although MT is a noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist, and has been clinically used for many years in Europe, it has few side effects, even among the geriatric patients, who are typical candidates for this drug (Narola *et al.*, 2010).

MT is a weak base with a pK_a of 10.27 and is predominantly excreted into urine in its unchanged form through the kidney. The plasma concentration of MT depends on urine pH; alkaline urine at pH 8 results in a reduced renal excretion and renal clearance of MT (Freudenthaler *et al.*, 1998). Carbonic anhydrase inhibitors (CAI) that alkalinize the urine, such as methazolamide (MZA), would be expected to reduce the renal elimination of MT. In general, for preclinical evaluation of pharmacokinetic drug interaction using small animals, sample size is restricted. Therefore, a sensitive determination method which requires a small sample size for an assay is preferable for the purpose.

MT is a primary amine with aliphatic tricyclic moiety having neither ultraviolet (UV) absorption nor fluorescence (FL) properties, and not even electroactive groups (Yeh *et al.*, 2010). Some techniques have been used to determine MT in a variety of matrices. These methods include high-performance liquid chromatography (HPLC)–UV detection methods (Narola *et al.*, 2010; Shuangjin *et al.*, 2007), HPLC-FL detection methods with labeling (Duh *et al.*, 2005; Higashi *et al.*, 2006; Suckow *et al.*, 1999; Xie *et al.*, 2011; Zarghi *et al.*, 2010), liquid chromatography–mass spectrometry (LC-MS; Almeida *et al.*, 2007; Dubey *et al.*, 2009; Koeberle *et al.*, 2003; Liu *et al.*, 2008, 2009), gas chromatography–mass spectrometry (GC-MS; Leis *et al.*, 2002) and micellar electrokinetic chromatography with laser-induced FL detection (Yeh *et al.*, 2010).

Among these, HPLC-UV is simple, but less sensitive for pharmacokinetic study of MT. Thus, a large sample size is needed for determination of MT in biological samples. The LC- or GC-MS

* Correspondence to: Kenichiro Nakashima, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan. E-mail: naka-ken@nagasaki-u.ac.jp

[a] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Assiut 71524, Egypt

[b] Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

[c] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt

Abbreviations used: CAI, carbonic anhydrase inhibitors; DIB-Cl, 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride; FLX, fluoxetine; MT, memantine; MZA, methazolamide; NMDA, *N*-methyl-D-aspartate; RFI, relative fluorescence intensity.

(or -MS/MS) methods require higher sensitivity, expensive instrument and/or tedious procedures. In addition, sample preparation in most of the methods includes tedious traditional liquid–liquid extraction, in which large amounts of toxic and flammable organic solvents are used. 4-(4,5-Diphenyl-1*H*-imidazol-2-yl)benzoyl chloride (DIB-Cl) was originally synthesized in our laboratory as a fluorescence labeling reagent for primary and secondary amines and phenols (Nakashima *et al.*, 1995). It has been proved to be a useful labeling reagent for the HPLC analysis of compounds such as amphetamine, methamphetamine, morphine and naphthols (Nakamura *et al* 2006; Ohyama *et al.*, 2009; Tomita *et al.*, 2007; Wada *et al.*, 2008).

The merits of DIB-Cl labeling for HPLC analysis include high selectivity, mild labeling conditions and high fluorescence quantum yield. MT possesses a primary amine group for labeling with DIB-Cl, as shown in Figure 1.

In this study, a simple and sensitive HPLC-FL method for the determination of MT in rat plasma was developed using DIB-Cl as a fluorescent label. Moreover, the proposed method was applied to the pharmacokinetic interaction study between MT and MZA as a CAI after a single intraperitoneal (i.p.) administration of MT with/without MZA to rat.

Experimental

Chemicals

MT·HCl, fluoxetine (FLX) hydrochloride as an internal standard (IS) and MZA were obtained from Sigma Chemical Co. (MO, USA). The labeling reagent, DIB-Cl, was synthesized in our laboratory as reported previously (Nakashima *et al.*, 1995). Sodium carbonate, sodium hydrogen carbonate, acetonitrile and methanol were obtained from Wako Pure Chemical (Osaka, Japan). Water was passed through a pure line WL21P (Yamato Science, Tokyo) and other chemicals were of extra pure grade. Stock solutions of MT and FLX (1 mg/mL) were prepared in methanol. These solutions were diluted appropriately with methanol to prepare the working solutions.

Apparatus

The HPLC system consisted of a Jasco 880-PU, liquid chromatographic pump (Jasco, Tokyo, Japan), a Wakopack Handy-ODS column (150 × 4.6 mm i.d.; 5 μm, Wako Pure Chemical), an RF-10A_{XL} fluorometeric detector (Simadzu, Kyoto, Japan) set at 330 nm (λ_{ex}) and 440 nm (λ_{em}), an R-111 laboratory recorder (Shimadzu), a CTO-10AS_{VP} column oven set at 30°C (Shimadzu) and a 7725 injector with a 20 μL sample loop (Rheodyne, CA, USA). DIB-MT was separated with a mixture of acetonitrile–water (70:30, v/v) as the mobile phase at a flow rate of 1.3 mL/min.

Animal treatment

Male Wistar rats were used in the experiments (250–290 g; Kyudo Experimenal Animals, Saga, Japan). They were housed in conditions of constant temperature (24 ± 1°C) and provided with standard laboratory food (Oriental Yeast, Tokyo) and water *ad libitum*. All animal procedures and care in this experiment were permitted by the Nagasaki University Animal Care and Use Committee.

The rats were anesthetized with ethyl carbamate (1.5 g/kg, i.p.). Blood samples were collected into the tubes containing EDTA, following a single administration of MT (2.5 mg/kg, i.p.) with/without MZA (5.0 mg/kg, i.p.) at 0, 5, 10, 15, 30, 60, 120, 180, 240, 360 and 480 min. Blood samples were centrifuged at 2000*g* for 10 min at 20°C and the resultant plasma samples were kept at −30°C prior to use.

Labeling with DIB-Cl

Ten-microliter aliquots of IS (50 ng/mL) were transferred into a mini amber reaction vial and evaporated under N_2 gas. To the residue, 25 μL of rat plasma, 50 μL of 1.5 mM DIB-Cl suspension in acetonitrile and 50 μL of carbonate buffer (0.2 M, pH 8.75) were added. The contents of the vial were vortex-mixed for 30 s, heated for 35 min at 45°C and cooled in ice-water. The reaction was stopped by addition of 10 μL of 25% aqueous ammonia solution and the sample was applied for clean-up with solid phase extraction (SPE).

Clean-up with SPE

A Bond Elut® cartridge packed with C_{18} resin (100 mg/mL; Varian, CA, USA) was used for clean-up. After the cartridge was conditioned with each of water and acetonitrile (1 mL × 2), the reaction mixture was applied and washed in triplicate with 1 mL of a mixture of acetonitrile–water (40:60, v/v). The cartridge was dried by suction for 2 min and the retained DIB-MT was eluted with 500 μL of methanol. The eluate was dried under N_2 gas, reconstituted with 135 μL of mobile phase and 20 μL of the resultant was injected into the HPLC system.

Preparation of authentic DIB-MT

Authentic DIB-MT was prepared as follows: to the suspension of DIB-Cl (15.0 mg, 41.8 μmol) in 7.5 mL of acetonitrile, MT (9.0 mg, 41.8 μmol) and

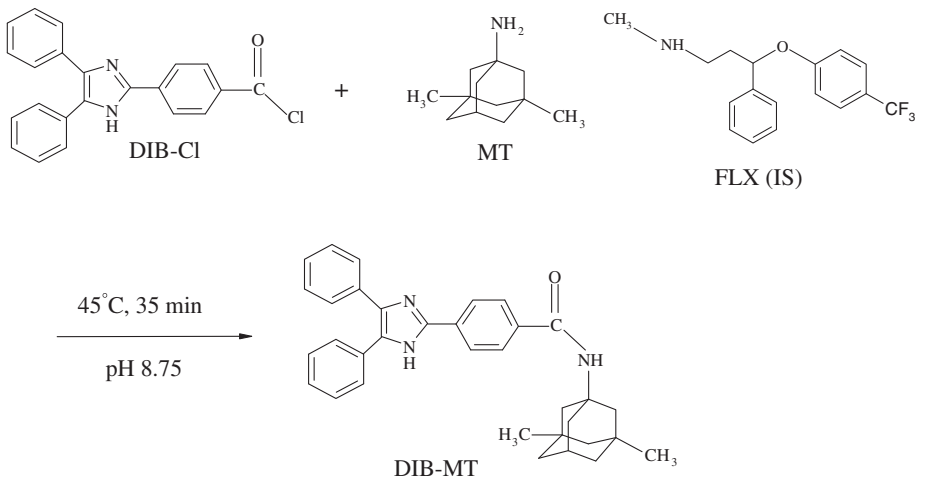

Figure 1. Labeling reaction of MT with DIB-Cl.

20.0 μL of triethylamine were added and mixed. The mixture obtained was heated at 45°C for 3 h, filtered, washed with acetonitrile and dried up. The resulted crude cotton white crystals were dissolved in 10 mL of ethanol and purified by the column chromatography (1.5 × 10 cm column packed with 5 g of Wako gel C-200, particle size 75–150 μm and activated with ethyl acetate, Wako Pure Chemical). A 40 mL aliquot of ethyl acetate was used as the eluting solvent, and the eluate was dried up with a centrifugal evaporator to yield white crystals. Yield: 6.7 mg, 32%. FAB-MS (*m/z*): 502.36 $[M+H]^+$ (JMS-MS 700 fast atom bombardment mass spectrometer, Jeol, Tokyo).

Fluorescence quantum yield of DIB-MT

The fluorescence quantum yield (ϕ_F) of DIB-MT was calculated in several solvents (methanol, acetonitrile, the mobile phase, ethyl acetate and chloroform) using quinine sulfate as the standard sample. To measure the UV and FL spectral data, 1.3×10^{-5} and 2.0×10^{-8} M of both DIB-MT and quinine sulfate were used, respectively. The ϕ_F of DIB-MT in each solvent was calculated by the following equation:

$$\phi_{system} = \phi_{ref}(A_{ref}/A_{system})(F_{system}/F_{ref})$$

where ϕ is the fluorescence quantum yield, *A* is the absorbance at the excitation wavelength and *F* is the area under the emission curve (Muino and Callis, 2009). The reaction yield of MT with DIB-Cl was calculated as peak height of MT labeled with DIB-Cl/peak height of authentic DIB-MT.

Method validation

The validation of the proposed method was performed using rat plasma spiked with known concentrations of standard MT. The calibration curve was prepared by the internal standard method using the peak height ratio of MT to IS. The limit of detection (LOD) and the limit of quantitation (LOQ) of MT were calculated as the peak height at signal-to-noise (S/N) ratios of 3 and 10, respectively.

The precisions of intra- and inter-day assays were assessed by six measurements of the plasma samples spiked with 20, 100 and 200 ng/mL of MT, and were evaluated as the relative standard deviation (RSD). The accuracy of the method was calculated as found concentration/nominal concentration. The recovery was calculated as peak height ratio of MT to IS in plasma/peak height ratio of standard MT to standard IS.

Pharmacokinetic of MT in rat plasma

The concentrations of MT in rat plasma were calculated from the corresponding calibration curve. The peak concentration (C_{max}) and concentration peak time (T_{max}) were obtained directly from the original data. The elimination half-life ($T_{1/2}$) was calculated using the equation 0.693/*k* (*k* = rate constant). Clearance (*Cl*) was calculated as the dose/area under the curve for concentration vs time (AUC_{0-480}). Other parameters such as AUC_{0-480}, and the mean residence time (MRT_{0-480}) were calculated by moment analysis, and all the data are presented as the mean ± standard deviation (SD, $n = 3$). Statistical analysis was assessed by Student's *t*-test with $p < 0.05$ being considered significant.

Results and discussions

Chromatographic separation

The separation of DIB-MT in rat plasma was performed by an ODS column with a mobile phase of acetonitrile–water (70:30, v/v). The retention times of IS and MT were 9 and 11 min, respectively. Figure 2 shows typical chromatograms obtained from (A) drug-free plasma and (B) that spiked with FLX 50 ng/mL and MT 100 ng/mL. DIB-MT could be satisfactorily separated from the interfering peaks.

Labeling conditions

In this study, FLX (50 ng/mL) and MT (100 ng/mL) were used to study parameters affecting the relative fluorescence intensity (RFI). The effect of carbonate buffer pH was examined in the range of 8.25–9.25. A maximum RFI was obtained at pH 8.75, and thus pH 8.75 was selected for the following experiments. The carbonate buffer concentration was also examined in the range of 0.10–0.25 M. A maximum and constant RFI was achieved at a concentration of 0.15 M. Therefore, 0.20 M was used for the subsequent experiments.

As DIB-Cl is insoluble in acetonitrile at concentrations higher than 1 mM, we used different concentrations of DIB-Cl suspensions (1.00–1.75 mM). A maximum and constant RFI could be obtained with 1.25 mM DIB-Cl. Therefore, 1.50 mM of DIB-Cl was selected for the subsequent experiments.

The peak heights of MT obtained at different temperatures (45, 60 and 80°C) and reaction times (10, 20, 25, 30, 35, 45 and 60 min) were evaluated; there was no significant effect of the different heating temperatures on the RFI, while the RFI increased with increasing heating time up to 30 min and after that achieved a steady-state. Therefore, heating at 45°C for 35 min was selected for the subsequent experiments.

The stability of the labels in the reaction mixture was also traced in the dark. No significant decrease in the RFI was observed with elapse of time after labeling, and the labels were confirmed to be stable for 24 h (95–100%).

SPE conditions

SPE treatment was applied to remove the huge blank peak that appeared in the chromatogram as a broad band and caused a decrease in the sensitivity of the method. First, SPE conditions were optimized for the determination of MT. Acetonitrile contents from 30 to 60% in the washing solution were examined. A maximum and constant RFI could be obtained by increasing the acetonitrile content of the washing solution to 50%, while increasing in the content more than this ratio caused a decrease in the RFI; 40% aqueous acetonitrile solution was chosen for the subsequent experiments. Elution of DIB-MT was examined with

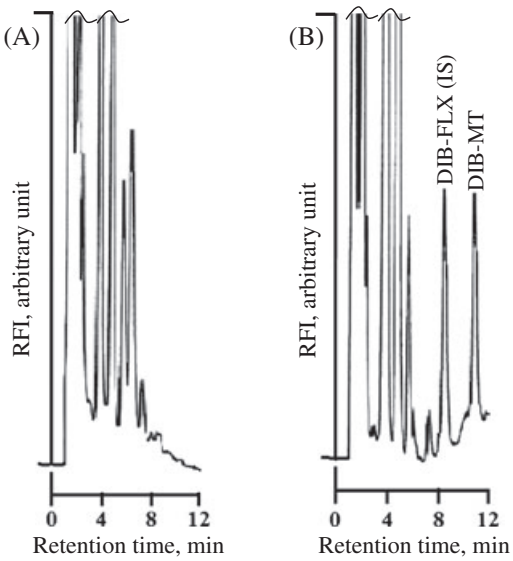

Figure 2. Chromatograms of (A) plasma and (B) spiked plasma with FLX 50 ng/mL and MT 100 ng/mL.

methanol and acetonitrile; there was no significant difference between the solvents, and thus methanol was selected.

Identification of authentic DIB-MT

The synthesized DIB-MT was identified with FAB-MS and UV and FL spectra. The molecular ion obtained at 502.36 is in agreement with that calculated for the mono-labeled DIB-MT. UV and FL spectra of the authentic DIB-MT were well corresponded to this of the DIB-MT peak on the chromatogram. The reaction yield of MT with DIB-Cl was estimated to be 90%.

ϕ_F of DIB-MT

UV and FL spectra and ϕ_F of DIB-MT were measured in several solvents – methanol, acetonitrile, the mobile phase, ethyl acetate and chloroform – and quinine sulfate was used as the standard compound for ϕ_F estimation. Unfortunately, no available information to compare with our data has been reported. DIB-MT shows intense RFI in organic solvents such as acetonitrile and methanol, which are frequently used as the mobile phase in reversed-phase chromatography. Owing to this desirable property, DIB-MT gave relatively intense RFI in the mobile phase. The ϕ_F values of DIB-MT in the different solvents were found to be 0.48 for acetonitrile, 0.40 for mobile phase, 0.35 for methanol, 0.30 for ethyl acetate and 0.24 for chloroform. The difference in ϕ_F values of DIB-MT may be due to the difference in solvent polarity; the ϕ_F values increased in proportion to the solvent polarity. The results are summarized in Table 1.

Method validation

Under the optimal reaction conditions, the calibration curve for rat plasma spiked with MT was prepared by plotting the peak height ratio of MT to IS vs the concentration in ng/mL. The obtained calibration curve was linear in the range of 10–400 ng/mL with a good correlation coefficient ($r = 0.999$). LOD and LOQ at S/N ratios of 3 and 10 were 2.0 and 6.6 ng/mL (15.4 and 50.8 fmol on column),

Table 1. UV and FL spectral data and FL quantum yield of DIB-MT in several solvents

Solvent	λ_{max} (nm)[a]	ε[b]	λ_{ex} (nm)	λ_{em} (nm)	RFI[c]	ϕ_F[d]
Methanol	323	11628	305	437	100	0.35
Acetonitrile	325	12015	308	422	195	0.48
Mobile phase	323	11860	326	430	103	0.40
Ethyl acetate	327	11163	319	402	79	0.30
Chloroform	318	10465	316	415	63	0.24

[a]Concentration used, 1.3×10^{-5} M of DIB-MT.
[b]ε, Molar absorptivity (L/mol/cm).
[c]Concentration used, 2.0×10^{-8} M of DIB-MT.
[d]ϕ_F. FL quantum yield.
[e]Mobile phase, acetonitrile–water (70:30, v/v).

Table 2. Linearity range, LOD and LOQ of MT in spiked rat plasma

Concentration (ng/mL)	r[a]	Regression equation[b]	LOD,[c] ng/mL (fmol on column)	LOQ,[d] ng/mL (fmol on column)
10–400	0.999	$y = 0.013x + 0.050$	2.0 (15.4)	6.6 (50.8)

[a]Correlation coefficient.
[b]y = peak height ratio of MT to IS; x = sample concentration, ng/mL.
[c]Limit of detection at S/N = 3.
[d]Limit of quantitation at S/N = 10.

Table 3. Method precision, accuracy and recovery of rat plasma spiked with MT

Spiked concentration (ng/mL)	Recovery (%),[a] mean ± SD	Accuracy (%),[b] mean ± SD	Precision (%) (RSD)[c]	
			Intra-day	Inter-day
20	86 ± 1.7	100.9 ± 3.8	3.8	7.7
100	90 ± 1.0	100.8 ± 3.9	3.9	8.7
200	89 ± 1.2	99.5 ± 2.3	2.3	6.6

[a]$n = 3$.
[b]Accuracy (%) = (found concentration/nominal concentration) × 100, $n = 6$.
[c]$n = 6$.

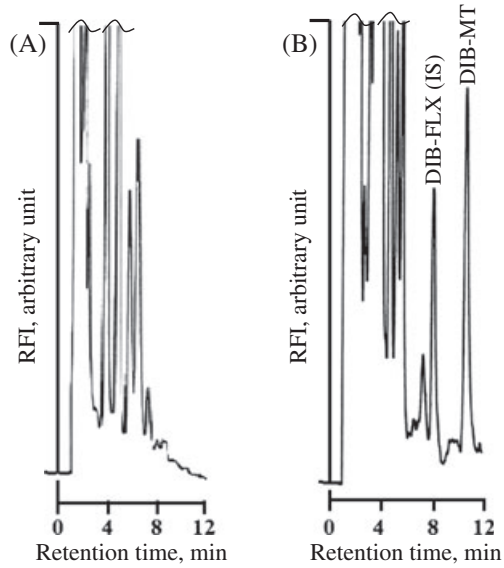

Figure 3. Chromatograms obtained from rat plasma: (A) pre-administration and (B) after 120 min of MT administration to rat.

respectively. The proposed method is sensitive enough for drug monitoring and also other purposes such as pharmacokinetic studies by using a small amount of plasma sample (25 µL). The parameters of the calibration curve for the proposed method are summarized in Table 2.

The proposed method is considered to be more sensitive than other HPLC-FL methods such as 4-fluoro-7-nitro-2,1,3-benzoxadiazole (Higashi *et al.*, 2006), (2-naphthoxy)acetyl chloride (Duh *et al.*, 2005) and 9-fluorenylmethyl chloroformate (Xie *et al.*, 2011). The proposed method is also comparable to the *o*-phthaldialdehyde method with an LOQ of 2 ng/mL using 430 µL of human plasma (Zarghi *et al.*, 2010) and the dansyl chloride method with LOQ of 3 ng/mL using 1 mL of human plasma (Suckow *et al.*, 1999). On the other hand, LC-MS methods showed lower LOQs – 0.1 ng/mL (Almeida *et al.*, 2007), 0.2 ng/mL (Liu *et al.*, 2008), 0.2 ng/mL (Dubey *et al.*, 2009) and 1 ng/mL (Liu *et al.*, 2009) – as did GC-MS, with a reported LOQ of 0.1 ng/mL (Leis *et al.*, 2002). Plasma samples in the range of 50–5000 µL were required for these methods.

Intra- and inter-day precisions of the proposed method were evaluated by analyzing six plasma samples spiked with known concentrations of MT (20, 100 and 200 ng/mL). The intra-day assay precisions (RSDs) were 3.8% (20 ng/mL), 3.9% (100 ng/mL) and 2.3% (200 ng/mL). Inter-day assay precisions (RSDs) were 7.7% (20 ng/mL), 8.7% (100 ng/mL) and 6.6% (200 ng/mL). The assay accuracies were also found to be 100.9 ± 3.8% (20 ng/mL), 100.8 ± 3.9% (100 ng/mL) and 99.5 ± 2.3% (200 ng/mL). The recoveries obtained were 86 ± 1.7% (20 ng/mL), 90 ± 1.0% (100 ng/mL) and 89 ± 1.2% (200 ng/mL). The precision, accuracy and recovery of the method are summarized in Table 3. As a result, a reliable and precise method could be developed using an internal standard method and SPE extraction.

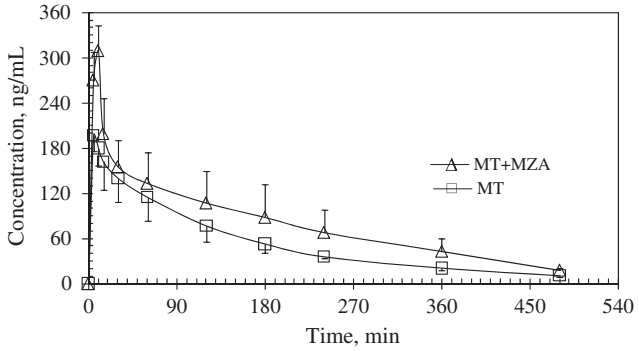

Figure 4. Concentration–time profile of MT in rat plasma after a single administration of MT with/without MZA. Data are expressed as mean ± SD ($n = 3$).

Pharmacokinetics of MT in plasma

To evaluate the applicability of the proposed method to a pharmacokinetic study, MT in rat plasma was monitored after a single administration of MT (2.5 mg/kg, i.p.) with/without MZA (5.0 mg/kg, i.p.), which is an effective CAI and has been used for the treatment of glaucoma.

Figure 3 shows typical chromatograms for rat plasma (A) pre-administration and (B) after 120 min of MT administration. The peak of MT corresponded to 93.8 ng/mL. No interference on the chromatogram of MT after co-administration with MZA was found. Figure 4 shows the time course of MT after a single administration of MT (2.5 mg/kg, i.p.) with/without MZA (5.0 mg/kg, i.p.). The concentration of MT immediately increased after i.p. administration and was determined by the proposed method for at least 360 min. The pharmacokinetic parameters of MT in rat plasma are summarized in Table 4; T_{max} of 5.0 ± 0.0 min, C_{max} of 197.0 ± 20.6 ng/mL, $T_{1/2}$ of 142.0 ± 13.1 min,

Table 4. Pharmacokinetic parameters of MT (2.5 mg/kg) with/without MZA (5.0 mg/kg) in plasma following a single i.p. administration to rats

Parameter	MT	MT + MZA	*p*-Value
T_{max} (min)	5.0 ± 0.0	8.3 ± 2.8	0.178
C_{max} (ng/mL)	197.0 ± 20.6	309.0 ± 29.3	0.006[a]
$T_{1/2}$ (min)	142.0 ± 13.1	152.2 ± 50.7	0.753
$AUC_{0-480} \times 10^4$ (ng·min/mL)	2.3 ± 0.2	4.3 ± 0.9	0.042[b]
MRT_{0-480} (min)	137.6 ± 7.2	157.3 ± 7.2	0.029[b]
Cl (mL/min/kg)	110.6 ± 10.1	58.8 ± 6.8	0.001[a]

Data are expressed as mean ± SD ($n = 3$).
[a]Student's *t*-test: $p < 0.01$, significantly different from MT alone.
[b]Student's *t*-test: $p < 0.05$, significantly different from MT alone.

AUC_{0-480} of $2.3 \pm 0.2 \times 10^4$ ng·min/mL, MRT_{0-480} of 137.6 ± 7.2 min and *Cl* of 110.6 ± 10.1 mL/min/kg were obtained.

Little information is available for comparison with our results are available. Xie *et al.* (2011) and Liu *et al.* (2009) administered MT to rats via intravenous (i.v.) and/or oral (p.o.) routes. The pharmacokinetic parameters after i.p. administration using an HPLC-UV method were reported; however, a larger dose (50 mg/kg) was needed (Shuangjin *et al.*, 2007).

Among the pharmacokinetic parameters of MT co-administered with MZA, C_{max}, AUC_{0-480} and MRT_{0-480} significantly increased to 309.0 ± 29.3 ng/mL ($p < 0.01$), $4.3 \pm 0.9 \times 10^4$ ng·min/mL ($p < 0.05$) and 157.3 ± 7.2 min. ($p < 0.05$), respectively, while the *Cl* of MT decreased by half to 58.8 ± 6.8 mL/min/kg ($p < 0.01$). As MZA is a noncompetitive CAI, an increase in urine volume occurs with a decrease in renal bicarbonate reabsorption, which causes a change in urine to an alkaline pH. Therefore, alterations of urine pH towards the alkaline condition with MZA may lead to an accumulation of MT in blood.

As the influence of MZA on the excretion of MT was expected owing to the effect of MZA on urine pH, the significant changes of pharmacokinetic parameters of MT such as AUC_{0-480}, *Cl*, MRT_{0-480} are acceptable. The potential increase of plasma level of MT after co-administration with agents using the same renal cationic transport system, such as cimethidine, ranithidine, procainamide and quinidine, has been pointed out (Jones, 2010). MT has little interaction with CYP450 isoenzymes (Periclou *et al.*, 2004).

This paper is the first report of pharmacokinetic interaction study of MT with MZA. However, further study is needed to clarify the interaction mechanism.

Conclusion

A sensitive HPLC method with FL detection for measuring MT in rat plasma was developed. The method is based on labeling of MT with DIB-Cl to yield the fluorescent DIB-MT. The labeling at 45°C for 35 min is simple and mild. Using this method, a well-defined peak of the DIB-MT was obtained by the separation on a C_{18} column without any interfering peaks. The FL property of DIB-MT with authentic sample was also clarified. As an application experiment, a pharmacokinetic interaction study of MT with MZA was carried out and showed some interaction effects. The proposed method is considered to be a powerful tool for pharmacokinetic interaction studies of MT with other drugs.

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