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Interaction study of acetylcholinestrase inhibitors on pharmacokinetics of memantine in rat plasma by HPLC-fluorescence method

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ABSTRACT: The present study aims to investigate the possibility of interaction of donepezil (DP) and galantamine (GAL) as acetylcholinestrase inhibitors, on memantine (MT) hydrochloride in rat plasma by HPLC-fluorescence detection. The separation of MT was achieved within 12 min without interference of DP and GAL on the chromatogram. MT levels in rat plasma with a single administration of MT (2.5 mg/kg, i.p.) and those with a co-administration of DP (5.0 mg/kg, i.p.) and GAL (3 mg/kg, i.p.) were monitored. MT concentrations determined in rat plasma ranged from 10.0 to 245.6 ng/mL. Significant difference was observed in the behavior of MT with a co-administration of DP, while no significant difference was observed with a co-administration of GAL.

Keywords: memantine; acetylcholinestrase inhibitors; HPLC-fluorescence detection; pharmacokinetics

Introduction

Alzheimer's disease (AD) is the most common form of dementia (Amar and Wilcock, 1996), with about 20–30 million sufferers worldwide. In 2006, this incurable, degenerative and terminal disease is predicted to affect 1 in 85 people globally by 2050 (Brookmeyer *et al*., 2007). Four medications are currently approved by regulatory agencies such as the US Food and Drug Administration and the European Medicines Agency to treat the cognitive manifestations of AD. Three of them are acetylcholinesterase (AChE) inhibitors and the other is memantine (MT) (Orgogozo *et al*., 2002). AChE inhibitors are indicated for the mild to moderately severe stages of AD. Donepezil (DP) a reversible AChE inhibitor, is indicated for severe disease in theUSA and some other markets worldwide. Galantamine (GAL) is also a reversible AChE inhibitor. In addition to inhibition of AChE, GAL interacts with nicotinic acetylcholine receptors to potentiate the action of agonists at these receptors (Scott and Goa, 2000).

MT is a low-affinity voltage-dependent uncompetitive antagonist for glutamatergic *N*-methyl-D-aspartate (NMDA) receptors (Rogawski and Wenk, 2003). MT binds to the NMDA receptor with a higher affinity than Mg^{2+} ions. Therefore, MT is able to inhibit the prolonged influx of Ca^{2+} ions which form the basis of neuronal excitotoxicity (Robinson and Keating, 2006).

Recently, some interest has emerged in using a combination therapy for the treatment of AD. The proposal for such a combined treatment was originally put forward for DP and MT (Rogawski, 2000). Thus, it is necessary to study the effect of these drugs on the pharmacokinetics of MT.

In our previous report, a simple and sensitive high-performance liquid chromatography method with fluorescence (HPLC-FL) detection for determination of MT in plasma was developed (Hassan *et al*., 2012). This method was successfully applied to determine MT in rat plasma samples and to study the pharmacokinetic interaction between MT and methazolamide (a carbonic anhydrase inhibitor) after a single administration of MT.

The aim of this study is to investigate the possibility of pharmacokinetic interaction of MT with DP or GAL as a AChE inhibitors in rat preliminarily by HPLC-FL detection. In this study the methodology is not original and the method has been described before (Hassan *et al*., 2012). The chemical structures of examined compounds in this study are shown in Figure 1. MT concentrations with co-administration of DP or GAL were monitored in rat plasma. Then, pharmacokinetic parameters of MT were calculated from the results and compared with those after a single intraperitoneal (i.p.) administration of MT alone.

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Abbreviations used: AChE, acetylcholinesterase; AD, Alzheimer's disease; DP, donepezil; FLX, fluoxetine; GAL, galantamine; MT, memantine; NMDA, *N*-methyl-D-aspartate.

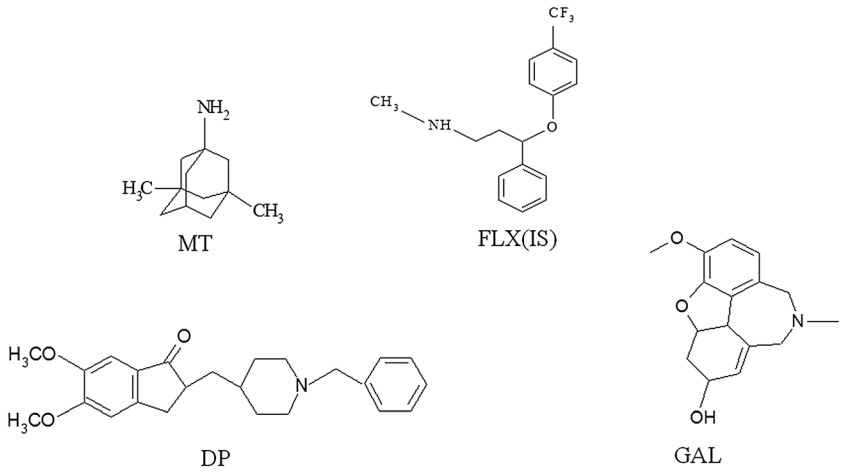

Figure 1. Chemical structures of memantine (MT), donepezil (DP) and galantamine (GAL). IS: fluoxetine hydrochloride.

Experimental

Reagents and standards

MT·HCl, fluoxetine (FLX) hydrochloride as an internal standard (IS), DP and GAL were obtained from Sigma Chemical Co. (St Louis, MO, USA). The labeling reagent, 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride was synthesized as reported previously (Nakashima *et al.*, 1995). Sodium carbonate, sodium hydrogen carbonate, acetonitrile and methanol were obtained from Wako Pure Chemical Co. (Osaka, Japan). Water was passed through a pure line WL21P (Yamato Science, Tokyo, Japan) and other chemicals were of extra pure grade. Nitrogen gas of 99.999% purity was provided by Daiichisanso (Nagasaki, Japan).

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.

Chromatographic apparatus and conditions

A detailed description of the HPLC method can be found in our previous report (Hassan *et al.*, 2012). Briefly, the HPLC system consisted of a Jasco 880-PU, liquid chromatographic pump (Jasco, Tokyo), a 7725 injector with a 20 μL sample loop (Rheodyne, CA, USA), a Wakopack Handy-ODS column (150 × 4.6 mm i.d.; 5 μm, Wako Pure Chemical), an RF-10A XL spectrofluorometric detector set at 330 (λ_{ex}) and 440 (λ_{em}) nm (Shimadzu, Kyoto, Japan), a CTO-10AS$_{VP}$ column oven set at 30°C (Shimadzu) and an R-111 recorder (Shimadzu). MT and IS were isocratically separated with acetonitrile-water with a ratio of 70:30 (v/v, %). The mobile phase was degassed and pumped into the column at a flow rate of 1.3 mL/min.

Animal treatment

Male Wistar rats were used in the experiments (250–290 g; Kyudo Co. Ltd, Saga, Japan). The rats were housed in the conditions of constant temperature (24 ± 1°C) and provided with standard laboratory food (Oriental Yeast, Tokyo, Japan) 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.). After operating on rats and inserting the cannula into the femoral artery, animals were stabilized for 1 h and then blood samples (250 μL) were collected into EDTA tubes following a single administration of MT (2.5 mg/kg, i.p.) with and without DP (5.0 mg/kg, i.p.) and with and without GAL (3.0 mg/kg, i.p.) at 0, 5, 10, 15, 30, 60, 120, 180, 240, 360 and 480 min. Blood samples were centrifuged at 2000g for 10 min at 20°C, the obtained plasma samples were kept at −30°C.

Pharmacokinetics and statistical analysis 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); also, 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

HPLC determination of MT in rat plasma with co-administration of DP and GAL

The MT peak was clear from interference of plasma components when DP or GAL were co-administrated according to our previously reported method (Hassan *et al.*, 2012). As a result, HPLC analysis of MT could be completed within 12 min with a good recovery. The retention times for IS and MT were 9 and 11 min, respectively. Figure 2 represents typical chromatograms obtained from rat plasma (a) before and (b) 120 min after administration of MT (2.5 mg/kg, i.p.) with DP (5.0 mg/kg, i.p.). MT and IS were well separated from DP and the endogenous components of plasma. The peak of MT corresponds to 146.5 ng/mL. The concentrations of MT were calculated from the working curve prepared by rat plasma samples spiked with known concentrations of MT standard. The working range was from 10 to 400 ng/mL plasma with a good correlation coefficient ($r = 0.999$). The regression equation of MT was $y = 0.013x + 0.050$, where y represents the peak height ratio of MT to IS and x is MT concentration in ng/mL plasma. The limits of detection and quantitation for the proposed method were 2.0 ng/mL and 6.6 at signal-to-noise ratios of 3 and 10, respectively.

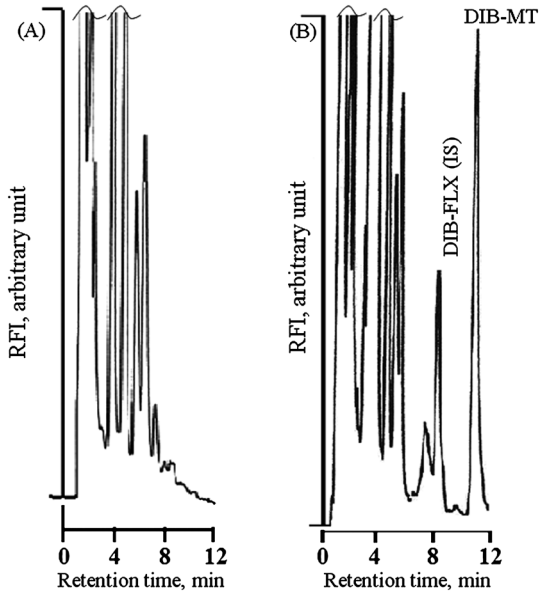

Figure 2. Chromatograms of MT in rat plasma: (A) pre-administration and (B) after 120 min of MT (2.5 mg/kg, i.p.) co-administered with DP (5.0 mg/kg, i.p.) to rats.

Pharmacokinetic study of MT co-administered with DP

The time courses of MT concentration with and without DP are presented in Figure 3. 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 1; 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, AUC_{0-480} of 2.3 ± 0.2 × 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. Among the pharmacokinetic parameters of MT co-administered with DP, C_{max}, AUC_{0-480} and MRT_{0-480} significantly increased to 245.6 ± 17.9 ng/mL ($p < 0.05$), 4.8 ± 0.4 × 10^4 ($p < 0.01$) and 158.3 ± 4.2 ($p < 0.05$), respectively, while the *CL* of MT decreased by half to 52.3 ± 3.0 ($p < 0.01$).

Many pharmacokinetic studies of DP with other medicines, such as cimetidine, ketoconazole, theophilline and digoxin, have been reported. Among them, the combination of DP and ketoconazole resulted only in a statistically significant increase in plasma concentration of DP (Tiseo *et al*., 1998a–d). For treatment of Alzheimer's disease, a pharmacokinetic interaction study between DP and risperidone was carried out, because risperidone has been used to reduce both psychosis and behavioral symptoms in patients with dementia (Zhao *et al*., 2003). Although DP and selegiline do not independently exert potential efficacy, their coadministration significantly improved the

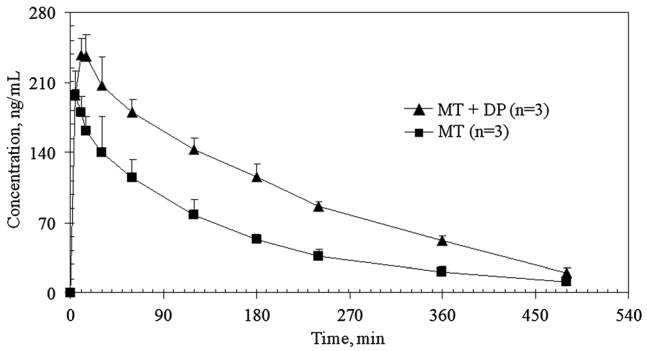

Figure 3. Concentration–time profile of MT in rat plasma after a single administration of MT with and without DP. Data are expressed as mean ± SD ($n = 3$).

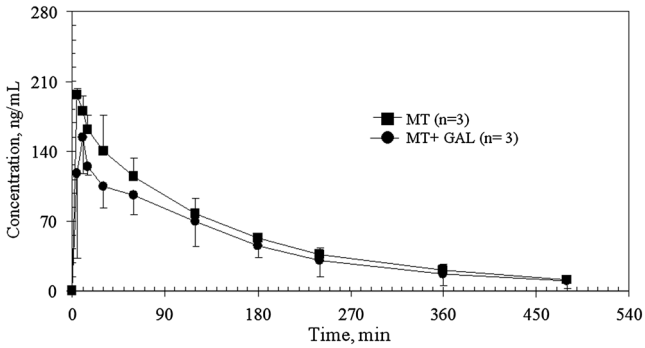

Figure 4. Concentration–time profile of MT in rat plasma after a single administration of MT with and without GAL. Data are expressed as mean ± SD ($n = 3$).

Table 1. Pharmacokinetic parameters of memantine (MT; 2.5 mg/kg) with and without donepezil (DP; 5.0 mg/kg) in plasma following a single intraperitoneal (i.p.) administration to rats

Parameter	MT	MT + DP	*p*-Value
T_{max} (min)	5.0 ± 0.0	10.0 ± 5.0	0.158
C_{max} (ng/mL)	197.0 ± 20.6	245.6 ± 17.9	0.031*
$T_{1/2}$ (min)	142.0 ± 13.1	122.0 ± 20.5	0.230
$AUC_{0-480} \times 10^4$ (ng min/mL)	2.3 ± 0.2	4.8 ± 0.4	0.005**
MRT_{0-480} (min)	137.6 ± 7.2	158.3 ± 4.2	0.013*
CL (mL/min/kg)	110.6 ± 10.1	52.3 ± 3.0	0.004**

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

Table 2. Pharmacokinetic parameters of MT (2.5 mg/kg) with and without galantamine (GAL; 3.0 mg/kg) in plasma following a single intraperitoneal (i.p.) administration to rats

Parameter	MT	MT + GAL	*p*-Value*
T_{max} (min)	5.0 ± 0.0	10.0 ± 5.0	0.150
C_{max} (ng/mL)	197.0 ± 20.6	169.3 ± 45.0	0.388
$T_{1/2}$ (min)	142.0 ± 13.1	204.6 ± 60.9	0.156
$AUC_{0-480} \times 10^4$ (ng min/mL)	2.3 ± 0.2	3.2 ± 0.7	0.353
MRT_{0-480} (min)	137.6 ± 7.2	187.6 ± 21.0	0.276
CL (mL/min/kg)	110.6 ± 10.1	82.0 ± 20.0	0.386

Data are expressed as mean ± SD ($n = 3$).
* Student's t-test: $p < 0.05$, no significantly different from MT alone.

scopolamine and *p*-chlorophenylalanine-induced memory deficits in rats (Takahata *et al.*, 2005). Furthermore, *N*-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide monohydrate (FK960), an anti-dementia drug, has been demonstrated to have potential cognitive-improving actions through enhancement of somatostatin release. Concurrent administration of FK960 and DP significantly improved memory impairment in animals (Tokita *et al.*, 2002). Therefore, the drug–drug interaction of DP and other drugs that may be co-administrated in clinical practice seems to be significant.

Pharmacokinetic study of MT with co-administration of GAL

The time courses of MT concentration after a single i.p. administration of MT with and without GAL are presented in Figure 4 and the pharmacokinetic parameters are summarized in Table 2. The concentration ranges of MT with and without GAL were 10.0–169.3 and 10.0–197.0 ng/mL, respectively. Although GAL is metabolized with the same enzymes that are responsible for metabolizing DP (CYP2D6 and 3A4), the pharmacokinetic parameters of MT after co-administration of MT with GAL, were not significantly changed. The dose of 5.0 for DP and 3.0 mg/kg for GAL was higher than that in clinical use, and only one dose study was performed in this study.

In our previous work (Hassan *et al.*, 2012), the influence of methazolamide on the excretion of MT was expected owing to the effect of methazolamide on urine pH, and the significant changes in the pharmacokinetic parameters of MT such as AUC_{0-480}, *CL*, MRT_{0-480} were acceptable. In case of the effect of AChE inhibitors on the pharmacokinetics of MT, it was reported that MT major metabolites excreted in urine are hydroxylated and *N*-oxidized derivates (typical reactions catalyzed by cytochrome P450, CYP450; Stanislav *et al.*, 2004).

Therefore, CYP450 may have a role in the pharmacokinetics of MT. DP and GAL are metabolized with CYP450 (2D6 and 3A4) and have no effects on urine pH. Therefore, pharmacokinetic interaction of MT with AChE inhibitors was expected owing to the effect of CYP450. However, it was found that there were significant changes in the pharmacokinetic parameters of MT when co-administered with DP and no significant changes when MT co-administered with GAL.

Therefore, the possible reason for the significant change in the pharmacokinetic parameters of MT in case of the co-administration of DP and no significant change in case of the co-administration of GAL may be the effect of DP metabolites on the urine pH. Therefore, alterations of urine pH towards alkaline conditions with DP metabolites may lead to an accumulation of MT in blood. More detailed studies should be performed to clarify the reason for the significant change in the pharmacokinetics of MT after co-administration of DP and whether this change has clinical relevance or not.

Conclusion

The pharmacokinetic interaction of MT with DP and GAL was studied by HPLC-FL detection. Significant differences in almost all pharmacokinetic parameters of MT were found between rats administered MT alone and with co-administration of DP (except for MT vs GAL). The proposed method is considered to be a powerful tool for pharmacokinetic interaction studies of MT with other drugs.

References

Amar K and Wilcock G. Fortnightly review: vascular dementia. *British Medical Journal* 1996; **312**: 227–231.

Brookmeyer R, Johnson E, Ziegler-Graham K and Arrighi HM. Forecasting the global burden of Alzheimer's disease. *Alzheimer's & Dementia* 2007; **3**: 186–191.

Hassan MG, Emara KM, Mohamed HA, Abdel-Wadood, HM, Ikeda R, Wada M, Kuroda N and Nakashima K. Determination of memantine in rat plasma by HPLC-fluorescence method and its application to study of the pharmacokinetic interaction between memantine and methazolamide. *Biomedical Chromatography* 2012; **26**: 214–219.

Nakashima K, Yamasaki H, Kuroda N and Akiyama S. Evaluation of lophine derivatives as chemiluminogens by a flow-injection method. *Analytica Chimica Acta* 1995; **303**, 103–107.

Orgogozo JM, Rigaud, AS, Stoffler A, Mobius HJ and Forette F. Efficacy and safety of memantine in patients with mild to moderate vascular dementia: a randomized, placebo-controlled trial (MMM 300). *Stroke* 2002; **33**: 1834–1839.

Robinson DM and Keating GM. Memantine: a review of its use in Alzheimer's disease. *Drugs* 2006; **66**: 1515–1534.

Rogawski MA. Low affinity channel blocking (uncompetitive) NMDA receptor antagonists as therapeutic agents – toward an understanding of their favorable tolerability. *Amino Acids* 2000; **19**: 133–149.

Rogawski MA and Wenk GL. The neuropharmacological basis for the use of memantine in the treatment of Alzheimer's disease. *CNS Drug Reviews* 2003; **9**: 275–308.

Scott, L. J. and Goa, K. L. Galantamine: a review of its use in Alzheimer's disease. *Drugs* 2000; **60**: 1095–1122.

Stanislav M, Lucie M, Eva A, Pavel A, Jaroslav C, Leos F and Jirina M. Inhibitory effects of memantine on human cytochrome P450 activities: prediction of in vivo drug interactions. *European Journal of Clinical Pharmacology* 2004; **60**: 583–589.

Takahata K, Minami A, Kusumoto H, Shimazu S and Yoneda F. Effect of selegiline alone or with donepezil on memory impairment in rats. *European Journal of Pharmacology* 2005; **518**: 140–144.

Tiseo PJ, Perdomo CA and Friedhoff LT. Concurrent administration of donepezil HCl and cimetidine: assessment of pharmacokinetic changes following single and multiple doses. *British Journal of Clinical Pharmacology* 1998a; **46**: 25–29.

Tiseo PJ, Perdomo CA and Friedhoff LT. Concurrent administration of donepezil HCl and ketoconazole: assessment of pharmacokinetic changes following single and multiple doses. *British Journal of Clinical Pharmacology* 1998b; **46**: 30–34.

Tiseo PJ, Perdomo CA and Friedhoff LT. Concurrent administration of donepezil HCl and theophylline: assessment of pharmacokinetic changes following multiple-dose administration in healthy volunteers. *British Journal of Clinical Pharmacology* 1998c; **46**: 35–39.

Tiseo PJ, Perdomo CA and Friedhoff LT. Concurrent administration of donepezil HCl and digoxin: assessment of pharmacokinetic changes. *British Journal of Clinical Pharmacology* 1998d; **46**: 40–44.

Tokita K, Yamazaki S, Yamazaki M, Matsuoka N and Mutoh S. Combination of a novel antidementia drug FK960 with donepezil synergistically improves memory deficits in rats. *Pharmacology Biochemistry and Behavior* 2002; **73**: 511–519.

Zhao Q, Xie C, Pesco-Koplowitz L, Jia X and Parier JL. Pharmacokinetic and safety assessments of concurrent administration of risperidone and donepezil. *Journal of Clinical Pharmacology* 2003; **43**: 180–186.

