8-Hydroxylation and glucuronidation of mirtazapine in Japanese psychiatric patients: Significance of the glucuronidation pathway of 8-hydroxy-mirtazapine

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Running title: 8-Hydroxylation and glucuronidation of mirtazapine

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Abstract (233words)

Introduction

To investigate the metabolism of mirtazapine (MIR) in Japanese psychiatric patients, we determined the plasma levels of MIR, *N*-desmethylmirtazapine (DMIR), 8-hydroxy-mirtazapine (8-OH-MIR), mirtazapine glucuronide (MIR-G), and 8-hydroxy-mirtazapine glucuronide (8-OH-MIR-G).

Methods

Seventy-nine Japanese psychiatric patients were treated with MIR for 1–8 weeks to achieve a steady-state concentration. Plasma levels of MIR, DMIR, and 8-OH-MIR were determined using high-performance liquid chromatography. Plasma concentrations of MIR-G and 8-OH-MIR-G were determined by total MIR and total 8-OH-MIR (i.e., concentrations after hydrolysis) minus unconjugated MIR and unconjugated 8-OH-MIR, respectively. Polymerase chain reaction was used to determine CYP2D6 genotypes.

Results

Plasma levels of 8-OH-MIR were lower than those of MIR and DMIR (median 1.42 nmol/L vs. 92.71 nmol/L and 44.96 nmol/L, respectively). The plasma levels (median) of MIR-G and 8-OH-MIR-G were 75.00 nmol/L and 111.60 nmol/L, giving MIR-G/MIR and 8-OH-MIR-G/8-OH-MIR ratios of 0.92 and 59.50, respectively. Multiple regression analysis revealed that smoking was correlated with the plasma MIR concentration (dose- and body weight-corrected; p=0.040) and that age (years) was significantly correlated with the plasma DMIR concentration (dose- and body weight-corrected; p=0.018). The steady-state plasma concentrations of MIR and its metabolites were unaffected by the number of *CYP2D6*5* and *CYP2D6*10* alleles.

Discussion

The plasma concentration of 8-OH-MIR was as low as 1.42 nmol/L, whereas 8-OH-MIR-G had an approximate 59.50-times higher concentration than 8-OH-MIR, suggesting a significant role for hydroxylation of MIR and its glucuronidation in the Japanese population.

Keywords

Mirtazapine・Hydroxylation・Glucuronidation・Pharmacokinetics・Pharmacogenetics

Introduction

Mirtazapine (MIR) has been approved by the Japanese Ministry of Health and Welfare for the treatment of depressive disorders or a depressive state and its therapeutic reference range is suggested at 30–80 ng/mL (113.1–301.6 nmol/L) [1]. The pharmacological profile of MIR is different from that of conventional antidepressants such as tricyclic antidepressants. For this reason, it has been classified as a noradrenergic and specific serotonergic antidepressant (NaSSA) that increases noradrenaline and serotonin release via blockade of adrenergic α_2-autoreceptors and α_2-heteroreceptors, respectively [2,3]. MIR also antagonizes both serotonin 2 ($5HT_2$) and serotonin 3 ($5HT_3$) receptors [4], which results in a net increase in serotonin 1 ($5HT_1$)-mediated neurotransmission [5]. Studies on the pharmacological role of metabolites are available only for DMIR; *In-vitro*, in comparison to MIR, seems to be a ten-fold stronger ligand for $5HT_1$ receptors, however, ten-fold less potent as an α_2-antagonist [6], which suggests contribution of DMIR to clinical effect.

MIR is available as a racemic mixture of S-(+)-MIR and R-(-)-MIR, and MIR is enantioselectively metabolized by the cytochrome P450 enzymes CYP1A2, CYP2D6, CYP3A4 [7], and potentially CYP3A5 [8]. The S-(+)-MIR enantiomer is primarily metabolized by CYP2D6 via 8-hydroxylation (Figure 1), contributing to approximately 40% of racemic MIR metabolism [9]. Some studies have shown that chronic smoking induces CYP1A2 activity, allowing it to increase the 8-hydroxylation of S-(+)-MIR [10]. The R-(-)-MIR enantiomer is directly conjugated to MIR-*N*-glucuronide, which represents about 25% of racemic MIR metabolism. Other metabolic pathways include N^2-oxidation and *N*-desmethylation, accounting for 10% and 25% of racemic MIR metabolism, respectively [9]. The metabolism of MIR to *N*-desmethylmirtazapine (DMIR) is primarily handled by CYP3A4 [11]. MIR-*N*-glucuronide and 8-OH-MIR glucuronide account for

most of the urinary metabolites of MIR, with only 4% excreted as unmetabolized MIR. The main metabolic pathway for R-(-)-8-OH-MIR is hydroxyl glucuronidation, whereas it is *N*-ammonium glucuronidation for R-(-)-MIR [9].

Here, we examined the plasma levels of MIR and its metabolites and investigated the factors influencing the pharmacokinetics of MIR and its metabolites. Previously, Watanabe et al. investigated the effect of CYP2D6 polymorphisms, particularly *CYP2D6*10* alleles, on the steady-state plasma concentrations of racemic MIR and its metabolite DMIR in 75 Japanese psychiatric patients [12]. No significant differences in the plasma concentrations of MIR and DMIR were noted among different *CYP2D6* genotypes. Multiple regression analysis also revealed that neither sex nor the number of *CYP2D6*10* alleles was a significant factor affecting the plasma concentration of MIR or DMIR. However, there was a significant effect of age on the plasma level of DMIR.

Our preliminary investigation of the plasma concentrations of 8-OH-MIR in 57 Japanese psychiatric patients revealed that the 8-OH-MIR/MIR ratio was 0.03±0.03 (0.01–0.13), with a plasma MIR level of 29.5±21.9 (1.6–112.4) ng/mL, meaning that the steady-state plasma concentrations of 8-OH-MIR were as low as 1 ng/mL (Pierce et al, in preparation).

We speculated that the glucuronidation pathway would play an important role after forming the hydroxylated metabolite of MIR (i.e., 8-OH-MIR). There is only one report on the glucuronidation of MIR in the literature—a study performed in the cat [13]—and there have been no human *in vivo* studies. Accordingly, in this study, we determined the plasma levels of MIR, DMIR, 8-OH-MIR, MIR glucuronide (MIR-G), and

8-OH-MIR glucuronide (8-OH-MIR-G) to investigate the role played by glucuronidation in the metabolism of MIR.

Methods

Patients

In total, 82 Japanese psychiatric patients were examined in this study. Of the 82 patients, 77 were from our earlier study [14]. However, we newly determined the plasma concentrations of racemic compounds in the 82 participants instead of summing the plasma enantiomer concentration data from Hayashi et al. [14]. Three patients whose plasma levels of both MIR and its metabolites DMIR and 8-OH-MIR were below the lower limit of detection were excluded from the analysis because of non-adherence, and so 79 patients with valid data were included in the analysis. Of these patients, 36 (23 outpatients and 13 inpatients) were treated at Dokkyo Medical University Hospital and 43 (36 outpatients and 7 inpatients) were treated at Hirosaki University Hospital. MIR (mirtazapine HCl; Reflex®; Meiji Seika Pharma Co., Ltd., Tokyo, Japan) was administered at dosages of 7.5–45 mg/day (25.4±13.1 mg/day) or 0.12–1.10 mg/kg body weight (BW) (0.46±0.26 mg/kg BW). All patients were instructed to take mirtazapine once daily before bedtime. To promote adherence, family members were instructed to ensure that outpatients took the drug and nursing staff confirmed that inpatients took the drug. To achieve steady-state plasma levels, the same daily dose was maintained for 1–8 weeks. Patients receiving neuroleptics or barbiturates as subordinate prescriptions were excluded. Patients were not permitted to take additional antidepressants, including tricyclic antidepressants or selective serotonin reuptake inhibitors (e.g., paroxetine, fluvoxamine), or other medications with potential for

CYP isozyme inhibition or induction. Benzodiazepines were permitted at typical doses for sleep disturbance or anxiety. Of the total 79 patients, 16 (20.3%) were smokers and 63 (79.7%) were nonsmokers. Patients were excluded if they had a poor general medical condition or a major abnormality in laboratory findings. Psychiatric diagnoses were major depressive disorders in 78 patients and bipolar disorder, panic disorder, dysthymic disorder, and social anxiety disorder in 1 patient each, based on DSM-IV-TR criteria.

This study was approved by the Ethics Committee of Dokkyo Medical University Hospital and the Ethics Committee of Hirosaki University Hospital. Written informed consent for participation was obtained from the patients or their families prior to the study.

Blood sampling

MIR was administered to patients for 1–8 weeks to achieve a steady-state concentration. About 10 to 15 h after the final dose, venous blood (7 mL) was sampled using Venoject tubes containing EDTA-Na (Terumo Japan, Tokyo, Japan) and the plasma was separated via centrifugation at 3000 × *g* for 10 min. The separated plasma and cell fractions were stored at –80°C until analysis.

Determination of plasma concentrations of unconjugated MIR and its metabolites unconjugated DMIR and unconjugated 8-OH-MIR

Plasma levels of racemic MIR, DMIR, and 8-OH-MIR were measured by high-performance liquid chromatography (HPLC) using the method of Paus et al. [15], with minor modifications. Assay standards for

MIR, DMIR, and 8-OH-MIR were provided by N.V. Organon (Oss, Netherlands). The HPLC apparatus consisted of an LC-10A pump system, AS-8020 automated sample processor, RF20A fluorescence spectrophotometer (Shimadzu Corporation, Kyoto, Japan), and a 250 × 4.6-mm STR-ODS-II column with 5-μm particle size (Shinwa Chemical Industries, Kyoto, Japan). The HPLC flow rate was 0.6 mL/min and the mobile phase was methanol-ethanol-0.02 M ammonium acetate with volume ratios of 18:9:73 (solution A) and 25:12:63 (solution B).

Next, 100 μL methanol, 100 μL cisapride (10.9 μg/mL, internal standard), 0.5 mL sodium hydroxide buffer (pH=10), 0.5 mL blank serum, 0.5 mL Milli-Q water, and 3.5 mL *n*-heptane-chloroform (70:30 by volume) were added to 0.5 mL of heparinized plasma. Extraction was performed by shaking for 10 min and centrifugation for 8 min at 3000 rpm. The solution was stored at –80°C for 10 min. The organic phase was then transferred into a separate tube and dried for 45 min by vacuum centrifugation at 45°C. Next, 200 μL of solution A was added to the tube and mixed, and 100 μL of the solution was injected into the HPLC system. Linearity was shown over the range of 2.5-100 ng/mL for MIR and DMIR (MIR=9.25-370nmol/L, DMIR=10.0-400 nmol/L) and 1-20 ng/mL for 8-OH-MIR (3.57-71.4nmol/L). The lower limit of detection was determined to be 1.0 ng/mL (MIR = 3.7 nmol/L, DMIR = 4.0 nmol/L and 8-OH-MIR = 3.57 nmol/L). The intra-assay coefficients of variation (CVs) of MIR at 5.0, 50.0 and 100 ng/mL (18.5, 185 and 370 nmol/L) were 3.7%, 3.0% and 2.8%, respectively. The intra-assay CVs of DMIR at 5.0, 50.0 and 100 ng/mL (20.0, 200, and 400 nmol/L) were 3.5, 3.0 and 2.7%, respectively. The intra-assay CVs 8-OH-MIR at 3.0, 18.0 and 36.0 ng/mL (10.7, 64.2, and 128.5 nmol/L) were 2.9, 2.4 and 2.1%, respectively. The inter-assay CVs of MIR

at 5.0, 50.0 and 100 ng/mL (18.5, 185 and 370 nmol/L) were 8.8%, 7.4% and 6.4%, respectively. The inter-assay CVs of DMIR at 5.0, 50.0 and 100 ng/mL (20.0, 200, and 400 nmol/L) were 8.7%, 7.3% and 6.2%, respectively. The inter-assay CVs of 8-OH-MIR at 3.0, 18.0 and 36.0 ng/mL (10.7, 64.2, and 128.5 nmol/L) were 7.0%, 7.5%, and 6.5%, respectively.

Deconjugation and determination of the plasma concentrations of MIR-G and 8-OH-MIR-G

Plasma levels of glucuronidated MIR and 8-OH-MIR were determined using HPLC with the same setup and mobile phase solutions described above, with some minor modifications. Briefly, 0.5 mL of plasma was mixed with 100 μL methanol, 100 μL cisapride (internal standard, 30 μg/mL), 200 μL Milli-Q water, 1 mL 0.2 M ammonium acetate (pH=5.0), 0.3 mL β-glucuronidase, and 0.05 mL 4% sodium azide and incubated for 15 h at 37°C (the optimal hydrolytic condition was determined in the preliminary experiments using pooled plasma from patients, and the plasma levels of MIR and 8-OH-MIR reached a plateau after 12 h incubation). Then, 1.0 mL sodium hydroxide buffer (pH=10.0) and 3.5 mL *n*-heptane-chloroform (70:30) was added and shaken for 10 min, followed by centrifugation at 3000 rpm for 5 min. The organic layer was then removed and dried at 45°C for 45 min. Finally, 200 μL of solution A was added and mixed, and 100 μL of the solution was injected into the HPLC system.

Plasma concentrations of MIR-G and 8-OH-MIR-G were determined by total MIR and total 8-OH-MIR (i.e., concentrations after hydrolysis) minus unconjugated MIR and unconjugated 8-OH-MIR, respectively.

Calculation of the glucuronidation ratio

The glucuronidation ratios were defined as MIR-G/MIR and 8-OH-MIR-G/8-OH-MIR and were calculated in each patient from the plasma concentrations of MIR-G, MIR, 8-OH-MIR-G, and 8-OH-MIR.

CYP2D6 genotyping

To determine genotype with respect to CYP2D6, DNA was first isolated from the cell fraction via a QIAamp Blood Kit (QIAGEN Inc., Valencia, CA). The *CYP2D6*2* and *CYP2D6*10* alleles were then determined using the mutation-specific polymerase chain reaction (PCR) amplification method reported by Johansson et al. [16]. The *CYP2D6*5* allele was identified using the long PCR analysis method reported by Steen et al. [17].

Data availability

Requests for data availability made to the corresponding author will be discussed by the Ethics Committee of Dokkyo Medical University Hospital and the Ethics Committee of Hirosaki University Hospital.

Statistical analysis

The Kolmogorov-Smirnov test revealed that the data did not follow a normal distribution. The Spearman rank correlation test was conducted to determine the correlation between the dosage of MIR (corrected for

BW) and the plasma levels of MIR, 8-OH-MIR, DMIR, MIR-G, and 8-OH-MIR-G. Multiple regression analysis (stepwise method) was performed to analyze the relationship between subject-independent variables (sex, age, smoking status, and the number of *CYP2D6*5* and *CYP2D6*10* alleles) and subject-dependent variables such as the plasma concentrations of MIR, DMIR, 8-OH-MIR, MIR-G, and 8-OH-MIR-G (nmol/L/mg/kg; all dose- and BW-corrected). The Mann-Whitney test was used to confirm the difference in the plasma concentrations of MIR between smoking group and non-smoking group. All statistical tests were two-tailed, and p values <0.05 were considered significant. Statistical analyses were conducted using IBM SPSS statistics software version 25 (Japan IBM, Tokyo, Japan).

Results

Participants

Three patients had MIR, DMIR, and 8-OH-MIR concentration below the lower limit of detection and were excluded from the analysis because of non-adherence. Thus, data from 79 patients were analyzed in this study. Their demographic characteristics are shown in Table 1.

Plasma levels of MIR and its metabolites

Plasma concentrations of MIR, DMIR, 8-OH-MIR, MIR-G, and 8-OH-MIR-G are shown in Table 2. The relationships between the daily dose of MIR corrected for weight and the plasma concentrations of MIR, DMIR, and 8-OH-MIR are shown in Figure 2. Positive significant correlations were found between the daily

dose of MIR corrected for BW and the plasma concentrations of MIR ($p<0.0001$), DMIR ($p<0.0001$), and 8-OH-MIR ($p<0.0001$).

The plasma level of 8-OH-MIR (bottom panel, Figure 2) was low compared with that of MIR (top panel, Figure 2) and DMIR (middle panel, Figure 2). Note that the difference in the upper limit of the ordinate, that is, the upper limit of the ordinate for the plasma levels of MIR and DMIR, is 500 nmol/L but is 50 nmol/L for 8-OH-MIR.

As shown in Figure 3, the daily dose of MIR corrected for BW had significant positive correlations with the plasma concentrations of MIR-G ($p=0.007$) and 8-OH-MIR-G ($p=0.001$).

The plasma levels (median, range) of MIR-G and 8-OH-MIR-G were 75.00 (4.52–722.82) nmol/L and 111.60 (3.20–1234.41) nmol/L, respectively. The MIR-G/MIR and 8-OH-MIR-G/8-OH-MIR ratios were 0.92 (0.07–7.00) and 59.50 (4.50–287.17), respectively.

CYP2D6 genotypes

Twenty-three patients had no mutated allele (*CYP2D6*1/CYP2D6*1* or *CYP2D6*1/CYP2D6**2); 40 patients (50.6%) had 1 mutated allele (*CYP2D6*1/CYP2D6*10* or *CYP2D6*2/CYP2D6*10*, including 3 patients with *CYP2D6*1/CYP2D6*5*); and 16 patients (20.3%) had 2 mutated alleles (12 patients with *CYP2D6*10/CYP2D6*10* and 4 patients with *CYP2D6*10/CYP2D6*5*).

Multiple regression analysis

Multiple regression analysis (stepwise method) revealed that smoking was a significant factor correlated with the plasma concentration of MIR (dose- and BW-corrected) (p=0.040).The following equation describes the final model (Table 3): Plasma concentration of MIR (dose- and BW-corrected) = 291.17 – 92.71 × smoking (smoker=1, smoker=0) (R=0.23, p=0.040, R^2=0.054). The smoking group had trend for lower plasma concentration of MIR (dose- and BW-corrected) than the non-smoking group (198.5±98.5 nmol/L/mg/kg vs. 291.2±169.8 nmol/L/mg/kg ; Figure 4).

Multiple regression analysis also revealed that smoking (p=0.080) tended to decrease the plasma concentration of 8-OH-MIR (dose- and BW-corrected; Table 3). The smoking group tended to have a lower plasma concentration of 8-OH-MIR (dose- and BW-corrected) than the non-smoking group (3.70±2.38 nmol/L/mg/kg vs 8.75±10.45 nmol/L/mg/kg, p=0.080). In addition, age (years) was a significant factor correlated with the plasma concentration of DMIR (dose- and BW-corrected) (p=0.018; Table 3). The final model was described by the following equation: Plasma concentration of DMIR (dose- and BW-corrected) = 33.45 + 2.15 × age (R=0.27, p=0.018, R^2=0.071) (see Figure 5).

Multiple regression analysis also determined that there were no significant factors correlated with the plasma concentration of MIR-G or 8-OH-MIR-G, as shown in Table 3. Further analysis also revealed no significant difference in the MIR-G/MIR or 8-OH-MIR-G/8-OH-MIR ratio (Table 3).

Discussion

In this study, the plasma level of 8-OH-MIR was much lower than that of MIR and DMIR. Additionally,

smoking habit and age were found to significantly influence the plasma levels of MIR and DMIR, respectively. Direct comparison suggested that trend for lower plasma concentrations of MIR in smoking group than those of non-smoking group, however, the difference did not reach significance level ($p=0.056$, Mann-Whitney test, Figure 4). Age or sex has been reported to have a significant effect on the plasma level of MIR. Timmer et al. found that the MIR concentration was approximately 2–3 times higher in elderly patients (age, 65–74 years) than in younger adult patients (age, 25–48 years) [18]. Shams et al. determined that patients older than 60 years of age (age, 72.2±7.1 years) had higher dose-corrected concentrations of MIR and DMIR than younger patients (age, 43.3±10.6 years) [19]. The significance of therapeutic drug monitoring for the relatively well-tolerated MIR is still a matter of debate [20],[21]. The frequency of the poor metabolizer CYP2D6 is extremely low in Asian populations such as the Japanese, which means that hydroxylation pathways of MIR to 8-OH-MIR are relatively common in Japanese people. Additionally, as shown in the present work, the capacity for the glucuronidation of 8-OH MIR to 8-OH-MIR-G is relatively high, which is a factor underlying the good tolerance of MIR. However, age was found to significantly influence the plasma levels of DMIR and clinicians should be cautious when MIR is administered to the elderly. Conflicting findings have been reported for the role of sex in MIR metabolism. Timmer et al. found that the MIR area under the curve (AUC) was about half in adult men (age=25–48 years) compared with adult women (age=25–43 years) [18]. Sirot et al. also noted significantly lower mean S-(+)-MIR, R-(-)-MIR, and R-(-)-DMIR concentrations in male patients than in female patients [10]. According to Borobia et al., the dose- and BW-adjusted AUC of MIR was higher in men than in women but dose- and BW-adjusted peak concentrations were lower in men than in

women after a single 30-mg oral dose of MIR [22]. In addition, Shams et al. found that dose-corrected concentrations of MIR and DMIR were significantly higher in women than in men [19].

Some studies indicated that smoking significantly affects the plasma concentration of MIR. In a comparison of smokers and nonsmokers, Lind et al. reported that smokers had significantly lower concentrations of S-(+)-MIR (23 vs. 39 nmol/L, respectively; p=0.026) and R-(-)-DMIR (39 vs. 51 nmol/L, respectively; p=0.036) and a significantly lower S-(+)-MIR/R-(-)-MIR ratio (0.28 vs. 0.37, respectively; p=0.014) [23]. According to Sirot et al. [10], the plasma levels of S-(+)-MIR, R-(-)-MIR, and S-(+)-DMIR, as well as the S-(+)-MIR/R-(-)-MIR ratio, were significantly higher in nonsmokers than in smokers, which was attributed to the induction of CYP1A2 in smokers and the putative contribution of CYP1A2 to the metabolism of MIR. In a previous study, we investigated the effects of various factors on the metabolism of MIR and reported that smokers (n=15) had a significantly lower dose- and BW-corrected S-(+)-MIR level than nonsmokers (n=55; 15.1±17.8 vs. 23.9±17.8 ng/mL/mg/kg [=56.9±67.1 nmol/L/mg/kg vs. 90.1±67.1 nmol/L/mg/kg, respectively], Kruskal-Wallis test, p=0.034) [14].

In the present study, the plasma level of 8-OH-MIR was extremely low (1.42 nmol/L), whereas that of 8-OH-MIR-G was quite high (111.60 nmol/L; Table 2). The ratios of the plasma levels of glucuronidated compounds to unconjugated compounds, that is, the glucuronidation ratio, were calculated to be 0.92 (0.07–7.00) and 59.50 (4.50–287.17) for MIR-G/MIR and 8-OH-MIR-G/8-OH-MIR, respectively, which indicates the importance of the glucuronidation pathway of 8-OH-MIR. Shimoda et al. investigated the significance of the glucuronidation pathways of the tricyclic antidepressant clomipramine and determined the plasma

concentrations of 8-hydroxyclomipramine (HC), 8-hydroxy-*N*-desmethylclomipramine (HDC), and the glucuronide conjugates HC glucuronide (HCG) and HDC glucuronide (HDCG) in 108 Japanese adults [24]. The data revealed that the glucuronidation ratio [(HC+HDC)/(HCG+HDCG)] was 0.56±0.45, which means that an approximate 1.8-fold higher plasma concentration of glucuronidated metabolites was found compared with unconjugated metabolite in the patients treated with clomipramine.

Someya et al. determined the plasma concentrations of haloperidol (HAL), reduced haloperidol (RHAL), haloperidol glucuronide (HALG), and reduced haloperidol glucuronide (RHALG) [25]. Although they reported that the plasma HALG concentration was significantly higher than that of HAL, RHAL, or RHALG, the glucuronidation ratio [(HALG+RHALG)/(HAL+RHAL)] was 3.0±1.9. In addition, when HALG/HAL and RHALG/RHAL were recalculated from the raw data shown in their report, the HALG/HAL and RHALG/RHAL ratios were 3.15±1.64 and 3.16±4.78, respectively.

Although no human studies have investigated the glucuronidation pathway of MIR, Quimby et al. reported the results from a single-dose pharmacokinetic study of MIR in young healthy cats [13]. The AUCs of 8-OH-MIR in serum were 4.16 ng/mL·h and 2.63 ng/mL·h (median) in the high-dose (3.75 mg) and low-dose (1.88 mg) groups, respectively. In addition, the AUCs of 8-OH-MIR-G in serum were 11.63 ng/mL·h and 14.32 ng/mL·h (median) in the high-dose (3.75 mg) and low-dose (1.88 mg) groups, respectively. Thus, the levels of 8-OH-MIR-G were approximately 2.8- and 5.4-fold higher than those of 8-OH-MIR in the serum of young healthy cats.

In the present study, the 8-OH-MIR-G/8-OH-MIR ratio was 59.50, which was much higher than the

metabolic ratio of glucuronidation in previous reports [13,24,25]. As far as we know, the data on the half-lives of MIR-G and 8-OH-MIR-G are not available, however, MIR increases its hydrophilicity via hydroxylation and glucuronic acid conjugation and is then mainly excreted in the urine. Delbressine et al. reported that 14%–34% of the administered dose of MIR was excreted in the urine as 8-OH-MIR-G in 5 healthy participants (3 men, 2 women) after 20 mg/day of MIR for 5 days [9].

The finding that the plasma concentration of 8-OH-MIR-G was much higher than that of MIR, despite possibly higher clearance from plasma into the urine due to the hydrophilic nature of glucuronide compared with the unconjugated form, strongly suggests that glucuronidation of the MIR is the major metabolic pathway in humans.

Conclusion

The MIR plasma concentration was much lower in smokers than in nonsmokers. Additionally, age was a significant factor affecting the DMIR plasma concentration. The CYP2D6 genotype does not show a significant effect on the plasma concentration of MIR or its metabolites (i.e., DMIR, 8-OH-MIR, MIR-G, and 8-OH-MIR-G). The plasma concentration of 8-OH-MIR was as low as 1.42 nmol/L while 8-OH-MIR-G showed an approximate 59.5-times higher plasma concentration than 8-OH-MIR, which suggests the significance of the metabolic pathways of hydroxylation of MIR and its glucuronidation in the Japanese population.

Funding

This study was funded by JSPS KAKENHI Grants #25461739 and #17K10280 and Seki Minato Memorial Awards to Kazutaka Shimoda and by JSPS KAKENHI Grants #15K19239, #15K19710, and #15H04754 to Norio Yasui-Furukori. The funding sources played no role in the study design; collection, analysis, or interpretation of data; writing of the report; or decision to submit the paper for publication.

Conflicts of interest

Kazutaka Shimoda has received research support from Novartis Pharma K.K., Dainippon Sumitomo Pharma Co., Astellas Pharma Inc., Meiji Seika Pharma Co., Ltd., Eisai Co., Ltd., Pfizer Inc., Otsuka Pharmaceutical Co., Ltd., Daiichi Sankyo Co., and Takeda Pharmaceutical Co., Ltd., and honoraria from Eisai Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Takeda Pharmaceutical Co., Ltd., Meiji Seika Pharma Co., Ltd., Janssen Pharmaceutical K.K., Shionogi & Co., Ltd., Dainippon Sumitomo Pharma Co., Daiichi Sankyo Co., and Pfizer Inc. Norio Yasui-Furukori has received grant/research support or honoraria from and has been a speaker for Dainippon Sumitomo Pharma, Mochida Pharmaceutical, MSD, and Otsuka Pharmaceutical. All other authors declare no biomedical or financial interests or potential conflicts of interest directly relevant to the content of this study.

Authorship

All authors fulfill the criteria of authorship based on their substantial contribution to the conception and

design, analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; or final approval of the version to be published. No one who fulfills these criteria has been excluded as an author.

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Figure legends

Figure 1: Structural formulae of MIR and its metabolites. The metabolic pathways of MIR are also shown[10,11].

Figure 2: Relationship between the plasma concentrations of MIR (topmost panel), DMIR (middle panel), and 8-OH-MIR (lowest panel) and the daily dose of MIR (all corrected for BW).

Figure 3: Relationships between the plasma concentrations of MIR-G (upper panel) and 8-OH-MIR-G (lower panel) and the daily dose of MIR (all corrected for BW).

Figure 4: Trend for lower plasma concentrations of MIR (dose- and BW-corrected) in smoking group than in non-smoking group (180.7 (34.9-436.1) nmol/L/mg/kg vs. 241.2 (26.1-780.3) nmol/L/mg/kg (Mann-Whitney test ($p=0.056$)).

Figure 5: Relationship between participant age and the steady-state plasma concentrations of DMIR ($R=0.27$, $p=0.018$, $R^2=0.071$).

Table legends

Table 1: Demographic characteristics of the 79 participants (mean±SD, range). BW, body weight; MIR, mirtazapine.

Table 2: Plasma concentrations of mirtazapine, *N*-desmethylmirtazapine, 8-OH-mirtazapine, mirtazapine glucuronide, and 8-OH-mirtazapine glucuronide. To convert nmol/L to ng/ml, refer to the following formulae: Mirtazapine (ng/mL) = Mirtazapine (nmol/L) × 0.27; *N*-desmethylmirtazapine (ng/mL) = *N*-desmethylmirtazapine (nmol/L) × 0.25; 8-OH-mirtazapine (ng/mL) = 8-OH-mirtazapine (nmol/L) × 0.28; Mirtazapine glucuronide (ng/mL) = Mirtazapine glucuronide (nmol/L) × 0.44; and 8-OH-mirtazapine glucuronide (ng/mL) = 8-OH-mirtazapine glucuronide (nmol/L) × 0.46.

Table 3: Results of stepwise multiple regression analysis (p value) for the corrected blood concentrations of MIR, 8-OH-MIR, DMIR, MIR-G and 8-OH-MIR-G. *Indicates statistical significance. DMIR, N-desmethylmirtazapine; MIR, mirtazapine; MIR-G, mirtazapine glucuronide; 8-OH-MIR, 8-hydroxy-mirtazapine; 8-OH-MIR-G, 8-hydroxy-mirtazapine glucuronide.