Chronic tobacco smoking and gender as variables affecting amantadine disposition in healthy subjects

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Amantadine HCl (3 mg kg⁻¹) was administered orally to 20 young healthy adults. Its apparent volume of distribution (V_z/F) was higher in smokers than nonsmokers, 6.05 ± 0.86 vs 4.87 ± 0.85 l kg⁻¹; (mean $\pm$ s.d., 10/group, $P < 0.011$), and no gender-associated effect was observed. Renal clearance did not vary with time-interval, but urinary recovery at 48 h was higher in men than in women ($60.2 \pm 7.5\%$ vs $47.0 \pm 15.0\%$, $P < 0.032$). Males had higher renal clearances than females when normalised for body mass index (BMI, 0.492 ± 0.284 vs 0.248 ± 0.137 l⁻¹ BMI h⁻¹, (10/group, $P < 0.032$)). On combining data from a previous study, the weight normalised renal clearance was also higher in men than in women, 0.160 ± 0.075 vs 0.102 ± 0.053 l kg⁻¹ h⁻¹ (19/group, $P < 0.01$). Chronic tobacco smoking did not alter the plasma or renal amantadine clearance. We conclude that gender and tobacco smoking are independent variables effecting amantadine disposition.

Keywords amantadine gender renal clearance smoking
apparent volume of distribution

Introduction

Amantadine, an aliphatic tricyclic primary amine ($pK_a = 10.1$), is excreted predominantly unchanged into the urine and undergoes limited metabolism in man [1]. Nicotine, the major pharmacologically active component of tobacco smoke, is a weak organic cation ($pK_a = 7.9$) [2]. At concentrations equivalent to those in the plasma of chronic smokers, nicotine, and its major metabolite, cotinine, increase amantadine accumulation in rat isolated renal proximal tubules [3]. The transport affinity for amantadine in rat renal distal tubules is lower in females than males [4]. In man, quinine and quinidine inhibit the renal clearance of amantadine only in males [5]. We assessed the influence of cigarette smoking on amantadine pharmacokinetics in humans as an extension of our *in vitro* studies in rats [3]. We tested the hypothesis that there is a gender-associated difference in amantadine renal clearance in humans by analyzing data from the present study combined with similar data from a previous study in a separate group of healthy men and women [5].

Methods

Study design and drug analysis

A non-randomised parallel-group study was completed in 20 healthy male and female volunteers, balanced for gender and smoking status (five subjects/group). The protocol was approved by the Faculty Committee on the Use of Human Subjects in Research at the University of Manitoba. All participants gave written informed consent. No medication was given for 2 weeks prior to and during the study. All subjects abstained from alcohol- and caffeine-containing beverages during the study. Tobacco users had smoked ≥ 20 cigarettes per day for the preceding 6 months. They were permitted to smoke freely throughout the experimental period and tobacco consumption was monitored. Each smoker was advised of the health risks associated with this habit, and was offered access to a smoking cessation program.

Following an overnight fast, subjects received 3 mg kg⁻¹ amantadine HCl syrup (Symmetrel®, Du Pont Merck Pharma, Mississauga, Ontario) orally

with 200 ml water. Further ingestion of water and food was avoided for the subsequent 2 and 4 h, respectively. Blood (initial 2 ml discarded followed by 10 ml sample) was collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h from a 20 gauge intravenous catheter. Collections at 24, 32 and 48 h were taken by venepuncture. Blood was mixed immediately with sodium oxalate and placed on ice. Plasma was separated by centrifugation (1000 g, 25 min, 4° C) and stored at -70° C until assay. Urine was collected from 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–32 and 32–48 h after drug administration. Urine volumes and pH were measured. Aliquots were stored at -70° C until assay.

Amantadine HCl concentrations in plasma and urine were assayed in duplicate by gas chromatography [6]. The lower assay limit in urine was 50 ng ml⁻¹ and standard curves ranged from 130 to 1000 ng ml⁻¹ ($r^2 > 0.99$, coefficient of variation = 5.5%). The lower assay limit in plasma was 10 ng ml⁻¹ and standard curves ranged from 50 to 800 ng ml⁻¹ ($r^2 > 0.99$, coefficient of variation = 7.0%).

Pharmacokinetic parameters and statistical analysis

Terminal plasma half-life ($t_{1/2z}$) and disposition rate constant (λ_z) were determined by least squares regression of the final linear phase of the log plasma amantadine concentration decay. Values of AUC were estimated by the linear trapezoidal method with extrapolation to infinity using C_t/λ_z . Plasma clearance (CL) was calculated as amantadine HCl dose/AUC. Renal clearance (CL_R) was determined as

urinary recovery of amantadine HCl/AUC in the corresponding time period. All clearance values were normalised for body weight, surface area and body mass index (BMI). Apparent volume of distribution (V_z/F) was determined as CL/λ_z .

Statistical comparisons were made using an ANOVA model in conjunction with Tukey's Honestly Significant Difference *post-hoc* test [7]. Most comparisons were made with a two-way ANOVA model accounting for patient gender, smoking status, and gender by smoking-status interaction. To improve the statistical power of the gender comparisons, the 0–8 h amantadine CL_R values ($n = 20$) were combined with corresponding data from other healthy subjects ($n = 18$) who participated in a separate study [5]. All data are reported as mean $\pm$ s.d. or median and (range). Mean differences and 95% confidence intervals of differences (CI) of selected parameters were calculated [8].

Results

Demographic characteristics of the subjects are shown in Table 1. Mean body weights and heights were higher in males than females ($P < 0.001$ and $P < 0.012$, respectively). Body surface area was higher in males than females ($P < 0.004$), but mean BMI values were comparable amongst the groups. Amantadine dosage adjusted for body weight or surface area was similar amongst the groups, whereas when adjusted for BMI it was greater in male than female subjects ($P < 0.005$).

Table 1 Demographics of the subjects of the study and pharmacokinetic parameters of amantadine (mean $\pm$ s.d.)

	Male		Female	
	Smoker	Abstainer	Smoker	Abstainer
Number of subjects	5	5	5	5
Age (years)	33.8 $\pm$ 3.7	26.8 $\pm$ 6.6	34.2 $\pm$ 10.0	27.6 $\pm$ 7.2
Weight (kg) ^a	73.5 $\pm$ 14.2	75.5 $\pm$ 11.8	56.8 $\pm$ 11.4	62.5 $\pm$ 8.7
Height (m) ^a	1.78 $\pm$ 0.06	1.79 $\pm$ 0.05	1.64 $\pm$ 0.09	1.66 $\pm$ 0.07
Body surface area (m ²) ^a	1.90 $\pm$ 0.20	1.94 $\pm$ 0.16	1.61 $\pm$ 0.20	1.70 $\pm$ 0.14
Body mass index	23.1 $\pm$ 3.3	23.5 $\pm$ 2.6	21.0 $\pm$ 2.6	22.5 $\pm$ 2.0
0–48 h urinary recovery ^c	0.613 $\pm$ 0.059	0.592 $\pm$ 0.094	0.465 $\pm$ 0.190	0.475 $\pm$ 0.120
V_z/F (l kg ⁻¹) ^b	5.85 $\pm$ 0.90	4.99 $\pm$ 0.59	6.23 $\pm$ 0.96	4.78 $\pm$ 1.11
V_z/F (l m ⁻²) ^b	223.5 $\pm$ 35.7	194.1 $\pm$ 32.1	217.2 $\pm$ 22.5	174.7 $\pm$ 34.7
V_z/F (l ⁻¹ BMI) ^b	18.37 $\pm$ 2.21	15.99 $\pm$ 2.29	16.59 $\pm$ 1.79	13.09 $\pm$ 2.20
CL (l kg ⁻¹ h ⁻¹)	0.243 $\pm$ 0.086	0.246 $\pm$ 0.043	0.263 $\pm$ 0.064	0.206 $\pm$ 0.055
CL (l m ⁻² h ⁻¹)	9.09 $\pm$ 2.32	9.48 $\pm$ 1.29	9.34 $\pm$ 1.86	7.47 $\pm$ 1.71
CL (l ⁻¹ BMI h ⁻¹)	0.756 $\pm$ 0.232	0.784 $\pm$ 0.115	0.708 $\pm$ 0.112	0.565 $\pm$ 0.150
CL _{R(0-8h)} (l kg ⁻¹ h ⁻¹) ^d	0.150 $\pm$ 0.096	0.162 $\pm$ 0.101	0.114 $\pm$ 0.071	0.072 $\pm$ 0.034
CL _{R(0-8h)} (l m ⁻² h ⁻¹) ^d	5.70 $\pm$ 3.59	6.25 $\pm$ 3.79	4.00 $\pm$ 2.55	2.70 $\pm$ 1.45
CL _{R(0-8h)} (l ⁻¹ BMI h ⁻¹) ^c	0.472 $\pm$ 0.296	0.513 $\pm$ 0.305	0.295 $\pm$ 0.163	0.201 $\pm$ 0.100
CL _R (l kg ⁻¹ h ⁻¹)	0.170 $\pm$ 0.059	0.163 $\pm$ 0.039	0.153 $\pm$ 0.085	0.101 $\pm$ 0.043
CL _R (l m ⁻² h ⁻¹) ^d	6.35 $\pm$ 1.71	6.34 $\pm$ 1.62	5.35 $\pm$ 3.09	3.66 $\pm$ 1.48
CL _R (l ⁻¹ BMI h ⁻¹) ^c	0.528 $\pm$ 0.162	0.521 $\pm$ 0.121	0.395 $\pm$ 0.190	0.276 $\pm$ 0.117

^a $P < 0.05$ males vs females; ^b $P < 0.05$ tobacco smokers vs abstainers; ^c $P < 0.05$ males vs females;

^d $0.05 < P < 0.10$ males vs females.

Figure 1 Renal clearance of amantadine as a function of time. Dashed lines indicate the 95% confidence interval ($F = 3.06$; $0.05 < P < 0.1$; $r^2 = 0.033$). ♦ smoking males, ◇ non-smoking males, ● smoking females, ○ non-smoking females.

Mean pharmacokinetic parameters are shown in Table 1. Values of $t_{1/2}$, and CL were unrelated to gender or smoking status. However, V_z/F , normalised for weight (mean difference $\pm$ 95% CI, 1.20 ± 0.80 l kg^{-1}) and height (mean difference $\pm$ 95% CI, 35.9 ± 29.0 l m^{-2} , 2.94 ± 2.23 l BMI^{-1}), was greater in chronic smokers than non-smokers ($P < 0.011$). Plasma amantadine concentrations from 2 to 24 h were lower in smokers, but this difference was not reflected in AUC data (mean difference $\pm$ 95% CI, 1.50 ± 3.27 mg l $^{-1}$ h). C_{max} and t_{max} values were similar in smokers and non-smokers (565 ± 125 vs 620 ± 93 ng ml $^{-1}$ and 1.75 (1.5–6) h vs 2.5 (1–8) h, respectively).

Renal clearance was similar throughout the 0–6, 6–12, 12–24, 24–32, and 32–48 h collection periods ($P > 0.087$ for weight-, $P > 0.115$ for surface area-, and $P > 0.116$ for BMI-normalisation) (Figure 1). Urinary drug recovery at 48 h (mean difference $\pm$ 95% CI, 0.132 ± 0.111) and CL_R/CL ratios (mean difference $\pm$ 95% CI, 0.173 ± 0.148) were higher in male than female subjects irrespective of their smoking status ($P < 0.032$ in both cases). Both 0–8 h and total CL_R values normalised for BMI showed gender-related differences ($n = 20$; $P \leq 0.032$ and $P \leq 0.013$ respectively). When the present weight-corrected 0–8 h CL_R data were combined with corresponding data from a previous study [5], data for both genders were consistent between the studies. The mean CL_R in male subjects (0.160 ± 0.075 l kg^{-1} h $^{-1}$) was approximately 1.5-fold greater than in females (0.102 ± 0.053 l kg^{-1} h $^{-1}$) (mean difference $\pm$ 95% CI, 0.058 ± 0.057 ; $P < 0.01$; $n = 38$).

Discussion

The pharmacokinetic parameters of amantadine normalised for body weight were consistent with previous reports [1, 5]. Also, we have shown that the

renal clearance of amantadine is time invariant. In contrast to our previous findings with rat renal tubules that nicotine and cotinine increase the renal tubular uptake of amantadine [4], we showed no effect of chronic smoking on the renal clearance of amantadine in man. There may be differences in CL and CL_R between female smokers and abstainers. A statistical power calculation with $\alpha = 0.05$ and $\beta = 0.20$ indicated a minimum sample size of 10 female subjects/group to demonstrate a difference.

Conversely, the higher V_z/F in smokers than non-smokers was independent of gender. It is unlikely that such a difference is explained by variation in absorption, as 0–48 h urinary amantadine recoveries were similar in the two groups. Substantial amantadine sequestration has been demonstrated in the kidney, where a tissue to blood ratio of 29 was found 1 h after a 1.6 mg kg^{-1} dose to mice [9]. The differences in V_z/F may be attributed to a nicotine/cotinine-induced increase in renal amantadine uptake.

The finding of a higher renal clearance of amantadine in male than female subjects confirmed a previous observation [5]. The direction and magnitude were similar when renal clearance was normalised by three measures, indicating that variation in subject surface area and adiposity do not contribute to the gender effect. In the study of Gaudry *et al.* [5] a power calculation with $\alpha = 0.05$ and $\beta = 0.20$, indicated that 28 subjects would be required to demonstrate a gender difference in the renal clearance of amantadine. Combination of relevant data from both studies ($n = 38$) satisfied the sample size requirement for body weight-normalised CL_R data [5]. Together, these data provide evidence in support of the hypothesis that gender differences in renal organic cation handling observed in the rat apply also to humans.

In conclusion, our results support a pharmacokinetic explanation for a reported higher occurrence of adverse events associated with therapeutic amantadine doses in female compared with male subjects [10], since a lower renal clearance of amantadine would predict a greater body burden at steady-state.

The authors thank Heather Peters and Jackie Golembioski for nursing assistance, and James Sherwin for assaying amantadine concentrations. This work was supported by grants from the Medical Research Council of Canada (MA-11664) and Apotex, Inc.

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(Received 4 May 1994,
accepted 22 September 1994)