

CYP3A and P-Glycoprotein Activity Induction With St. John's Wort in Healthy Volunteers From 6 Ethnic Populations

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It has been reported that St. John's Wort (SJW) may have inducing effects on the cytochrome P450 enzyme system, specifically the CYP3A4 isoform (CYP3A4)^{1,2} and the intestinal P-glycoprotein (Pgp) efflux membrane transporter.^{1,3} This may have profound implications on patients taking medications that are substrates for Pgp and/or CYP3A4. Ethnic difference is an important factor to determine drug metabolism and response. Varying results have been reported in studies evaluating ethnic differences in the pharmacokinetic disposition of Pgp/CYP3A4 substrates and suggest that these differences may be attributed to ethnically associated differences in intestinal Pgp and CYP3A4 activity.⁴⁻⁸

Fexofenadine and midazolam have been widely used as probes for intestinal and hepatic Pgp transporter and CYP3A enzyme activities in human interaction studies.^{6,9-11} In vivo studies examining SJW induction on CYP3A4 and Pgp substrates' pharmacokinetic (PK) parameters and its relationship among various ethnic groups have not been examined extensively.

The purpose of the current study was to investigate whether any differences in the inducibility of Pgp and CYP3A by SJW are present among 6 ethnic groups in healthy volunteers using fexofenadine and midazolam as probes.

METHODS

Subjects and Treatment

Thirty subjects, who signed written informed consent, completed the trial. They were Caucasians, African Americans, Hispanics, Chinese, Indians, and Malays (5 per group). All subjects met the inclusion and exclusion criteria written in the study protocol, which was approved by the Bronson Center for Clinical and Community Research Institutional Review Board (Kalamazoo, Mich) and the Ethics Committee of Singapore General Hospital (Singapore). Every subject provided a family history of ethnic heritage going back 2 generations and of all family members. Subjects who received any known enzyme-altering drugs or herbal preparations within 30 days prior to the first dosing were excluded from the study.

Each subject received a single dose of 60 mg of fexofenadine, 5 mg of midazolam syrup, and 2 mg of midazolam intravenous infusion (6 hours after the oral dose of midazolam) on study days 1 and 11. All subjects took 300 mg of SJW 3 times a day, starting on day 2 through day 11. Plasma and urine samples were taken for PK analyses of fexofenadine and midazolam. One blood

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sample was taken for the genotyping of CYP3A4, CYP3A5, Pgp, and Pregnane X Receptor (PXR) analyses.

Sample Analysis

Fexofenadine and midazolam concentrations in plasma and urine samples were analyzed using validated high-performance liquid chromatographic (HPLC)/tandem mass spectrometric (LC-MS-MS) methods at Cedra Corporation (Austin, Tex). Genomic DNA samples were analyzed using a combination of TagMan-based assays and ABI PRISM-based sequencing at EPIDAUROS Biotechnologie AG (Bernried, Germany).

Pharmacokinetic Analysis

Pharmacokinetic parameters of midazolam and fexofenadine for day 1 (D1) and day 11 (D11) were calculated by the standard method for noncompartmental analysis using a nonlinear regression program, Kinetica (Innaphase, Pa). The blood-to-plasma concentration ratio of midazolam is 0.8¹²; therefore, blood clearance of midazolam was equal to 1.25 • systemic clearance. It was assumed that systemic blood clearance of midazolam was equivalent to hepatic blood clearance, and hepatic availability (F_H) was expressed as

$$F_H = 1 - 1.25 \cdot CL_{IV}/Q_H,$$

where Q_H is the hepatic blood flow, taking the value of 1.35 L/min. Intestinal availability (F_c) was calculated as the ratio of oral bioavailability (F) and F_H (F/F_H).

Statistical Analysis

Statistical analysis of PK parameters was performed by 1-way analysis of variance (ANOVA). Point estimates and 95% confidence intervals (95% CIs) were calculated for the ratio of PK parameters for day 11 to day 1. The Bonferroni/Dunn test was used to perform unpaired comparisons between ethnic groups for PK parameters and ratios of parameters at an overall significance level of 5% (with the individual unpaired significance level being less than .0033) for independent groups. Within each ethnic group and for combined data, a 1-sample t test was used to evaluate the mean ratio, and the hypothesized mean was set equal to 1.

RESULTS

Subjects

Twenty-two male and 8 female subjects completed the study. The average age of subjects was 30 ± 8 years (range, 19-51 years), and the mean body mass index was 25 ± 3 kg/m² (range, 20-30 kg/m²). The mean body weight of subjects was 70 ± 9 kg (range, 53-91 kg), and there were no statistically significant differences for the demographics among the 6 ethnic groups.

Fexofenadine

The plasma concentrations of fexofenadine on day 11 decreased in all groups compared to day 1. Caucasians had the lowest plasma concentrations of fexofenadine on both day 1 and day 11 at all time points among the 6 ethnic groups (data not shown). After SJW treatment, mean oral clearance (CL_{oral}) and apparent volume of distribution at steady state ($V_{ss,oral}$) of fexofenadine increased in all groups, about 2 times higher than those of day 1 based on all subjects (Table I). Significant increases of fexofenadine's CL_{oral} on day 11 were observed for Caucasians and Hispanics, whereas nonsignificant but substantial changes were observed for the other ethnic groups (Table I). SJW exhibited no significant effect on terminal half-lives ($t_{1/2}$) (5.2 hours, day 1 vs 5.3 hours, day 11; $P = .3580$) and renal clearance (CL_R) of fexofenadine (4.0 L/h, day 1 vs 4.2 L/h, day 11; $P = .1816$) based on all subjects. Among the groups, CL_{oral} means were comparable for day 1 and day 11, as well as CL_{oral} and $V_{ss,oral}$ ratios of day 11 to day 1 (Table I).

Midazolam

Midazolam plasma concentrations were lower on day 11 compared to those on day 1 in all subjects. Caucasians showed the lowest plasma concentrations among the populations for both day 1 and day 11 (data not shown). After SJW treatment, significant increases of midazolam systemic clearance (CL_{IV}) and CL_{oral} were observed in all groups (Table I). Caucasians had the highest mean CL_{IV} on day 1 and day 11 compared to other populations; it was significantly higher than that of Hispanics, Chinese, and Indians on day 1 and that of Chinese on day 11 (Table I). However, CL_{IV} ratios of day

Table I Mean \pm SD Pharmacokinetic Parameter Estimates and Point Estimates (95% Confidence Interval) for the Parameter Ratios of Fexofenadine (Fex) and Midazolam (Mid) After Oral and Intravenous Dose With (D11) and Without (D1) Coadministration of St. John's Wort

Parameter	Treatment	Ethnicity						
		African American	Caucasian	Hispanic	Chinese	Indian	Malay	All
CL _{renal,fex} ^a , L/h	D1	91 \pm 47	108 \pm 47	74 \pm 26	75 \pm 27	62 \pm 17	55 \pm 12	77 \pm 34
	D11	158 \pm 92	195 \pm 46	141 \pm 58	112 \pm 54	86 \pm 14	98 \pm 49	132 \pm 64
	D11/D1	1.86 (0.81, 2.91)	1.97 (1.31, 2.63)*	2.02 (1.08, 2.96)*	1.48 (0.97, 2.00)	1.54 (0.69, 2.40)	1.78 (0.85, 2.72)	1.78 (1.53, 2.00)*
V _{ss,renal,fex} ^a , L	D1	589 \pm 312	851 \pm 481	543 \pm 233	581 \pm 243	467 \pm 183	314 \pm 81	556 \pm 313
	D11	1035 \pm 788	1390 \pm 260	930 \pm 418	796 \pm 412	525 \pm 137	528 \pm 293	867 \pm 500
	D11/D1	1.80 (0.80, 2.81)	2.00 (0.97, 3.03)	1.88 (0.93, 2.84)	1.37 (0.77, 1.97)	1.31 (0.53, 2.12)	1.63 (0.76, 2.48)	1.67 (1.41, 1.93)*
CL _{renal,mid}} ^a , L/h	D1	17.9 \pm 5.3	23.1 \pm 3.3	14.3 \pm 3.7	14.0 \pm 1.5	12 \pm 3.4	16.7 \pm 3.6	16.4 \pm 4.9
	D11	24.6 \pm 4.9	31.3 \pm 4.3	25.4 \pm 4.9	19.6 \pm 3.0	23 \pm 5.5	23.3 \pm 4.2	24.5 \pm 5.5
	D11/D1	1.40 (1.18, 1.62)*	1.36 (1.25, 1.48)*	1.83 (1.37, 2.28)*	1.41 (1.04, 1.78)*	1.94 (1.43, 2.45)*	1.41 (1.19, 1.63)*	1.56 (1.43, 1.69)*
V _{ss,renal,mid}} ^a , L	D1	75.2 \pm 25.9	69.2 \pm 22.0	62.2 \pm 13.1	40.7 \pm 5.8	76.4 \pm 23.4	49.1 \pm 8.8	62.1 \pm 21.3
	D11	58.1 \pm 16.5	73.1 \pm 18.3	83.5 \pm 35.3	41.0 \pm 9.0	94.0 \pm 49.2	54.4 \pm 13.7	67.3 \pm 31.0
	D11/D1	0.80 (0.60, 1.01)	1.08 (0.98, 1.18)	1.32 (0.78, 1.87)	1.00 (0.86, 1.14)	1.33 (0.32, 2.34)	1.12 (0.81, 1.43)	1.11 (0.96, 1.26)
CL _{renal,mid}} ^a , L/h	D1	108 \pm 20	132 \pm 49	74 \pm 25	69 \pm 9	61 \pm 12	82 \pm 14	88 \pm 34
	D11	278 \pm 112	370 \pm 120	246 \pm 86	173 \pm 66	192 \pm 79	230 \pm 91	248 \pm 108
	D11/D1	2.59 (1.32, 3.86)*	2.91 (1.75, 4.07)*	3.4 (2.57, 4.24)*	2.53 (1.44, 3.63)*	3.21 (1.29, 3.43)*	2.74 (1.86, 3.62)*	2.9 (2.55, 3.25)*
F _{renal} ^a	D1	0.17 \pm 0.02	0.19 \pm 0.06	0.20 \pm 0.02	0.21 \pm 0.04	0.21 \pm 0.03	0.21 \pm 0.03	0.20 \pm 0.04
	D11	0.09 \pm 0.02	0.08 \pm 0.03	0.11 \pm 0.02	0.12 \pm 0.04	0.14 \pm 0.04	0.12 \pm 0.04	0.11 \pm 0.04
	D11/D1	0.52 (0.30, 0.75)*	0.46 (0.31, 0.61)*	0.54 (0.43, 0.65)*	0.57 (0.37, 0.77)*	0.66 (0.39, 0.94)*	0.55 (0.22, 0.88)*	0.55 (0.48, 0.62)*
F _{renal,mid}}	D1	0.59 \pm 0.08	0.62 \pm 0.05	0.76 \pm 0.06	0.77 \pm 0.02	0.80 \pm 0.06	0.72 \pm 0.06	0.73 \pm 0.18
	D11	0.25 \pm 0.07	0.48 \pm 0.07	0.58 \pm 0.08	0.67 \pm 0.05	0.62 \pm 0.09	0.61 \pm 0.07	0.59 \pm 0.09
	D11/D1	0.84 (0.77, 0.92)*	0.77 (0.70, 0.85)*	0.76 (0.64, 0.88)*	0.88 (0.738, 0.98)*	0.77 (0.67, 0.88)*	0.85 (0.78, 0.91)*	0.81 (0.78, 0.84)*
F _{renal}	D1	0.25 \pm 0.07	0.30 \pm 0.10	0.26 \pm 0.02	0.28 \pm 0.06	0.27 \pm 0.06	0.30 \pm 0.07	0.28 \pm 0.06
	D11	0.15 \pm 0.03	0.18 \pm 0.07	0.19 \pm 0.03	0.18 \pm 0.05	0.23 \pm 0.07	0.19 \pm 0.09	0.19 \pm 0.06
	D11/D1	0.62 (0.38, 0.85)*	0.59 (0.42, 0.76)*	0.72 (0.58, 0.86)*	0.64 (0.46, 0.82)*	0.86 (0.52, 1.20)	0.66 (0.24, 1.08)	0.68 (0.60, 0.76)*

D1, day 1; D11, day 11.

*Significantly different from hypothesized mean of 1 ($P < .05$).

11 to day 1 were similar among the 6 ethnic groups. Midazolam CL_{oral} with SJW was about 3 times higher than that of midazolam alone based on all subjects. Caucasians' CL_{oral} was approximately 2 times higher than that of Hispanics, Chinese, and Indians before SJW administration, whereas the only significant difference was observed between Caucasians and Chinese on day 11. The CL_{oral} ratios of day 11 to day 1 were not different among the ethnic groups. Midazolam F on day 11 was only 55% of that on day 1 (Table I), in which F_H decreased by 19% and F_C decreased by 32% with SJW based on all subjects. Mean F_H was about 2- to 4-fold higher than F_C before and after SJW for all subjects. Six ethnic populations showed similarity of midazolam F and F_C for both day 1 and day 11. Caucasians' F_H was significantly lower than that of Chinese, Hispanics, and Indians on day 1 and also significantly lower than that of Chinese on day 11. Nevertheless, there were no significant differences among the ethnic groups for the ratios of day 11 to day 1 for F , F_H , and F_C .

Genotype-Phenotype Association

The genes and alleles tested for all subjects were CYP3A4 (*2 to *19), CYP3A5 (1, *3, *4, *6, and *7), Pgp (G3435T-exon26, G2677T, and G2677A-exon21), and PXR (G1108A, G418A, A488G, G52A, C79T (*2), G106A (*3), and G365A (*4)). All Asian subjects had 1 or 2 variant alleles in G2677T; only 20% non-Asians were wild-type G2677T, whereas 13% non-Asians and 46% Asians were wild-type G3435T. All subjects were wild-type CYP3A4, except 1 within CYP3A4*10. Three Asian subjects had heterozygous PXR variants. None of the individual genotypes was significantly correlated with fexofenadine or midazolam PK parameters.

DISCUSSION

In the current study, fexofenadine CL_{oral} was approximately doubled based on all subjects after a 10-day treatment with SJW. Although the CL_{oral} substantially increased in all ethnic groups, a statistical significance was not always observed mainly due to considerable interindividual variability and small sample size. Fexofenadine $t_{1/2}$ and CL_R were not influenced by SJW. This may be due to the fact that SJW can induce intestinal Pgp,¹ which in turn can decrease the bioavailability of fexofenadine but not its systemic clearance. Our observations are similar to recent reports of fexofenadine and SJW interaction studies.^{10,11}

Caucasians showed higher mean CL_{oral} of fexofenadine than in Asian populations both before and after SJW treatment. However, there were large overlaps

for individual values across the 6 ethnic groups, resulting in no significant differences in fexofenadine CL_{oral} among studied groups. These results indicate that interindividual variability within the group is comparable to interethnic variability and imply that Pgp activity is not likely to be significantly different among these ethnic groups. Moreover, because the ratios (D11/D1) of PK parameters of fexofenadine were similar, the degree of induction of Pgp by SJW appears to be comparable among the ethnic groups evaluated.

Midazolam CL_{IV} and CL_{oral} significantly increased by SJW in the 6 ethnic groups, without change in V_{ss} . Both F_H and F_C significantly decreased after SJW treatment. These results support the premise that SJW increases first-pass elimination of midazolam in both the intestine and liver due to the induction of intestinal and hepatic CYP3A4 enzyme activities.¹¹ Our study results demonstrate a larger decrease in F_C compared to the decrease in F_H , providing evidence that CYP3A4 enzyme activity was induced to a greater extent in the intestinal tract. Higher hepatic availability in comparison with intestinal availability was found, indicating that oral first-pass elimination played an important and determining role in midazolam disposition.¹³⁻¹⁵ It has been found that the intestine is a major site of interaction between midazolam and clarithromycin.¹⁶

Our results suggest that Caucasians have significantly higher clearances (CL_{IV} and CL_{oral}) and lower midazolam F_H than those of Chinese, Hispanics, and Indians prior to enzyme induction. The reason might be that Caucasians have higher intrinsically hepatic CYP3A4 enzyme activity. Ethnic differences of CYP3A4 activity have been studied in different populations for different CYP3A4 substrates,^{6,17} and different results were reported.^{8,18,19} Therefore, ethnic differences in hepatic metabolism seem to be unpredictable by race and specific enzyme, and ethnic variation caused by CYP3A4 activity may be dependent on the substrates evaluated. Midazolam CL and F_H ratios were similar among the 6 ethnic groups, indicating that hepatic CYP3A4 enzyme inducibility was comparable. The results also demonstrated that F and F_C of midazolam with and without SJW were comparable among the evaluated 6 ethnic groups, further supporting that inducibility of CYP3A4 enzyme activity was similar.

In conclusion, this study showed that the co-administration of SJW resulted in a significant increase in the clearances of a Pgp substrate (fexofenadine) and a CYP3A substrate (midazolam) in 6 ethnic groups. Both intestinal and hepatic CYP3A4 activities are induced. However, the induction of intestinal CYP3A4 activity was significantly higher than that of hepatic CYP3A4 activity. There is an indication that the extent

of Pgp and CYP3A4 induction was comparable among the 6 evaluated ethnic groups.

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