Effects of *Schisandra sphenanthera* extract on the pharmacokinetics of midazolam in healthy volunteers

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- *Schisandra sphenanthera* extract (SchE) and tacrolimus are often co-administered in treating renal and liver transplant recipients in China.
- Our previous study has demonstrated that SchE can increase the oral bioavailability of tacrolimus.

WHAT THIS STUDY ADDS

- The results of this study show that SchE can markedly increase the blood concentration and oral bioavailability of midazolam in healthy volunteers.
- This finding suggests that SchE may be an inhibitor of CYP3A and is likely to alter the disposition of drugs that are metabolized by CYP3A.

AIMS

To assess the effect of *Schisandra sphenanthera* extract (SchE) on the pharmacokinetics of midazolam, a probe drug of CYP3A, and its metabolite 1'-hydroxy midazolam in healthy volunteers.

METHODS

Twelve healthy male volunteers were orally treated with SchE, three capsules twice daily for 7 days. Pharmacokinetic investigations of oral midazolam administration at 15 mg were performed both before and at the end of the SchE treatment period. The plasma midazolam and 1'-hydroxy midazolam concentrations were determined by high-performance liquid chromatography–tandem mass spectrometry. Estimated pharmacokinetic parameters before and with SchE were calculated with noncompartmental techniques.

RESULTS

Following administration of SchE, the average increases (%) of individual increases in AUC, AUMC and $C_{\max}$ of midazolam were 119.4% [95% confidence interval (CI) 83.9, 155.0], 183.4% (95% CI 120.5, 246.2) and 85.6% (95% CI 14.4, 156.9), respectively ($P < 0.01$ or 0.05). On average, there was a 133.3% (95% CI 8.9, 257.7) increase in midazolam $t_{\max}$ ($P < 0.01$). The average decrease (%) in CL/F was 52.1% (95% CI 44.9, 59.4) ($P < 0.01$). No significant changes were seen in midazolam half-life. After co-administration of SchE, the average increase (%) in $t_{\max}$ of 1'-hydroxy midazolam was 150.0% (95% CI 22.2, 277.8) ($P < 0.05$). No significant differences were observed in the other pharmacokinetic parameters of 1'-hydroxy midazolam.

CONCLUSIONS

SchE can markedly increase the oral bioavailability of midazolam in healthy volunteers. SchE is an inhibitor of CYP3A and has a high susceptibility to alter the disposition of drugs metabolized by CYP3A.

Introduction

Schisandra sphenanthera extract (SchE) is a prescribed drug rather than a herbal supplement that has been widely used to treat viral and drug-induced hepatitis in China. It has shown that several constituents isolated from *S. sphenanthera* can interact with CYP3A and/or P-glycoprotein (P-gp) [1–4]. Tacrolimus (FK506) is a widely used immunosuppressive drug in various solid-organ transplantations and is co-medicated with multiple drugs under clinical conditions [5, 6]. SchE and tacrolimus are often co-administered when drug-induced hepatitis occurs in transplant recipients. Tacrolimus is a substrate for both CYP3A and P-gp [7, 8]. Therefore interaction between SchE and tacrolimus may occur when the two drugs are co-administered. Our previous work has demonstrated that SchE treatment can result in a substantial increase in the relative bioavailability of tacrolimus [9]. However, the mechanism of this interaction is still unknown.

Since tacrolimus is primarily metabolized by CYP3A, we aimed to investigate whether SchE increases the bioavailability of tacrolimus by inhibiting CYP3A. The use of select drugs as 'probes' to assess *in vivo* CYP activity has been the subject of intense interest for over a decade [10]. Midazolam is a benzodiazepine adaptable for preoperative sedation, anxiolysis and anterograde amnesia. Midazolam is metabolized to 1'-hydroxy and 4-hydroxy midazolam by CYP3A4 and CYP3A5 and has been suggested to be an ideal probe for the activity of CYP3A [11–13].

The goal of the current study was to evaluate the effects of SchE on hepatic and intestinal CYP3A catalytic activity. Midazolam was employed in this study as an *in vivo* probe for CYP3A hepatic and intestinal activity. The effect of SchE on the AUC and other pharmacokinetic parameters of midazolam and the metabolite 1'-hydroxy midazolam was studied in healthy volunteers.

Materials and methods

Subjects

Twelve healthy male volunteers, 22.5 ± 0.9 years old, 172.6 ± 5.0 cm in height and weighing 63.1 ± 6.1 kg, participated in the trials. Before entering the study, each subject underwent routine physical examination and laboratory investigations. Laboratory indices included liver and renal function, blood routine and blood glucose. Volunteers were considered to be healthy if physical examination, medical history, and standard biochemical blood tests were normal. Smokers and those taking medication were excluded. Informed consent was obtained according to the Declaration of Helsinki. The study was approved by the Ethics Committee of Wuhan General Hospital, ethics approval no. 2008005.

Trial design and blood sampling

In the morning of day 1, each volunteer received 15 mg midazolam (Shanghai Roche Pharmaceuticals Ltd, China) orally along with 150 ml water. Venous blood samples (2 ml) were taken from an indwelling venous catheter into tubes containing heparin sodium at 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 9, 12 and 24 h postdose. After last sampling, subjects received three capsules of SchE (also called Wu-Zhi capsule) (Hezheng Pharmaceutical Company, Chengdu, China, each capsule containing 11.25 mg deoxyschizandrin) twice daily for 7 days. On day 9, subjects were given another single oral dose of 15 mg midazolam, followed by three SchE capsules. The sampling times were the same as on day 1. On day 16, venous blood samples were taken to determine liver and renal function, blood routine and blood glucose.

Diet

Volunteers fasted from midnight until receiving their morning dose of midazolam on day 1 and day 9. Alcohol, caffeine-containing foods and beverages were not allowed from 72 h before the study until the end of the trial. Subjects received standardized meals at specified times: lunch 4 h postdose (at about 12.00 h) and dinner 10 h postdose (at about 18.00 h) on both midazolam administration days.

Drug and metabolite analysis

Plasma concentrations of midazolam and 1'-hydroxy midazolam were determined by a liquid chromatography–tandem mass spectrometry (LC/MS/MS) method simultaneously. The analysis was carried out on a TSQ Quantum Ultra mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with a Agilent 1200 high-performance liquid chromatography (HPLC) system (Agilent, Munich, Germany).

Plasma samples were assayed for parent midazolam and for 1'-hydroxy midazolam. To 0.5 ml plasma, 50 μ l internal standard (800 ng ml⁻¹ tamsulosin), 100 μ l methanol:water (80:20, v/v) and 100 μ l NaOH (1 mol l⁻¹) were added and then mixed. After the addition of 4 ml hexane:trichloromethane (1:1, v/v), the samples were shaken for 4 min and centrifuged. The organic phase was extracted and evaporated to dryness at 40°C. The residue was dissolved in 100 μ l of mobile phase, and 10 μ l was used for analysis with HPLC-MS/MS. Analytes were separated using an Inertsil ODS-3, 100 $\times$ 2.1 mm column with methanol:1% formic acid (70:30) as the mobile phase. Midazolam was monitored in positive ion mode with the transition of m/z 326.0 to m/z 291.0, 1'-hydroxy midazolam and tamsulosin with the transition of m/z 342.0 to m/z 324.0 and m/z 409.2 to m/z 228.0, respectively. The lower limit of quantification of both midazolam and 1'-hydroxy midazolam was 0.25 ng ml⁻¹. For midazolam a total of six batches, each containing three different concentrations (0.5, 50 and 350 ng ml⁻¹) were analysed, yielding an accu-

racy ranging from 95.6 to 105.0% with a coefficient of variation from 5.32 to 8.81%. For 1'-hydroxy midazolam a total of six batches, each containing three different concentrations (0.5, 50 and 350 ng ml⁻¹) were analysed, yielding an accuracy ranging from 95.9 to 103.0% with a coefficient of variation from 6.72 to 9.32%.

Pharmacokinetic calculations

The pharmacokinetic parameters included: area under the blood concentration–time curve (AUC), area under the moment curve (AUMC), elimination half life ($t_{1/2}$), maximum blood drug concentration (C_{max}), time to C_{max} (t_{max}), and apparent oral clearance (CL/F). C_{max} and t_{max} were determined by visual inspection of the blood concentration–time curves. The other parameters such as $t_{1/2}$ were calculated using the noncompartmental model with Pharmacokinetic Program DAS 2.0 (Chinese Pharmacologic Society, Beijing, China). For the above parameters, a 95% confidence interval (CI) for the increase (%) or decrease (%) of treatment before SchE compared with that after SchE was estimated. The 90% CI for the ratio of midazolam AUC after SchE to that before SchE was constructed. Drug–drug interaction was concluded if the 90% CI did not fall within the prespecified interval of 80–125%.

Statistical analysis

Statistical calculations were carried out using SPSS software 10.0 (SPSS Inc., Chicago, IL, USA). AUC and C_{max} were log transformed prior to analysis with paired Student's *t*-test. All other parameters were assessed using paired Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

Results

The plasma concentration–time curves of midazolam and its 1'-hydroxy metabolite prior to and following co-administration of SchE in 12 healthy volunteers are shown in Figures 1 and 2. Pharmacokinetic parameters of midazolam and 1'-hydroxy midazolam in 12 healthy volunteers before and after co-administration with SchE are given in Tables 1 and 2. Individual data for the effect of SchE on midazolam AUC are shown in Figure 3.

After co-administration of SchE, the average increases (%) of individual increases in AUC, AUMC and C_{max} of midazolam were 119.4% (95% CI 83.9, 155.0), 183.4% (95% CI 120.5, 246.2) and 85.6% (95% CI 14.4, 156.9), respectively ($P < 0.01$ or 0.05). The 90% CI for the ratio of midazolam AUC after SchE to that before SchE was 191.7, 250.1. On average, there was a 133.3% (95% CI 8.9, 257.7) increase in midazolam t_{max} ($P < 0.01$). The average decrease (%) in CL/F was 52.1% (95% CI 44.9, 59.4) ($P < 0.01$). No significant changes were seen in midazolam half-life.

After co-administration of SchE, the average increases (%) in t_{max} of 1'-hydroxy midazolam were 150.0% (95% CI

Figure 1

Mean blood concentration–time curves of midazolam in 12 healthy volunteers before and after co-administration with *Schisandra spheanthera* extract (SchE). Before SchE (—◆—); After SchE (—◇—)

Figure 2

Mean blood concentration–time curves of 1'-hydroxy midazolam in 12 healthy volunteers before and after co-administration with *Schisandra spheanthera* extract (SchE). Before SchE (—◆—); After SchE (—◇—)

22.2, 277.8) ($P < 0.05$). No significant differences were observed in the other pharmacokinetic parameters of 1'-hydroxy midazolam.

The plasma 1'-hydroxy midazolam/midazolam concentration ratios of 1 h after midazolam dosing in 12 healthy volunteers before and after co-administration with SchE were 0.28 ± 0.09 and 0.12 ± 0.04 , respectively ($P < 0.05$).

Measurements for hepatic and renal functions and other indices are shown in Table 3. These laboratory indices include serum total bilirubin (TB), direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase, urea nitrogen, creatinine (Cr), blood glucose (GLU), white blood cell, red blood cell, haemoglobin (Hb) and platelet. Compared with before co-administration of SchE, the mean TB and Cr levels with SchE were higher and the mean ALT, GLU

Table 1

The average midazolam pharmacokinetic parameters of 12 healthy volunteers before and after co-administration with SchE

	AUC ₀₋₂₄ (μg l ⁻¹ h ⁻¹)	AUMC ₀₋₂₄ (μg l ⁻¹ h ⁻¹)	CL(s) (l h ⁻¹)	T _{1/2} (h)	T _{max} (h)	C _{max} (μg l ⁻¹)
Before SchE						
Mean	577.72	2387.52	30.87	3.39	0.81	171.35
SD	226.83	1288.89	16.71	1.04	0.47	69.84
After SchE						
Mean	1185.61**	6163.42**	13.77**	3.55	1.63**	282.25*
SD	319.82	2713.08	5.75	1.13	1.17	152.18

Compared with before co-administration with SchE, *P < 0.05; **P < 0.01.

Table 2

The average 1'-hydroxy midazolam pharmacokinetic parameters of 12 healthy volunteers before and after co-administration with SchE

	AUC ₀₋₂₄ (μg l ⁻¹ h ⁻¹)	AUMC ₀₋₂₄ (μg l ⁻¹ h ⁻¹)	CL(s) (l h ⁻¹)	T _{1/2} (h)	T _{max} (h)	C _{max} (μg l ⁻¹)
Before SchE						
Mean	140.86	561.37	129.74	5.59	0.75	47.85
SD	69.29	290.83	67.57	3.25	0.26	23.17
After SchE						
Mean	129.15	677.82	134.41	4.99	1.63*	36.33
SD	48.74	245.96	71.57	2.14	1.17	28.05

Compared with before co-administration with SchE, *P < 0.05.

Figure 3

Individual data for the effect of *Schisandra sphenanthera* extract (SchE) on midazolam AUC

and Hb values were lower in the presence of SchE ($P < 0.05$ or 0.01), but the values were still within the normal range. No significant changes were observed in the other indices.

Table 3

Liver function and renal function as well as other indices in 12 healthy volunteers before and after co-administration with SchE (mean $\pm$ SD)

Index	Before co-administration with SchE	After co-administration with SchE
TB (μmol l ⁻¹)	11.97 $\pm$ 3.44	15.20 $\pm$ 6.05*
DB (μmol l ⁻¹)	3.30 $\pm$ 0.95	2.53 $\pm$ 1.35
ALT (U l ⁻¹)	18.58 $\pm$ 12.31	13.83 $\pm$ 7.55*
AST (U l ⁻¹)	18.42 $\pm$ 4.56	17.75 $\pm$ 5.55
BUN (mmol l ⁻¹)	3.85 $\pm$ 0.93	3.97 $\pm$ 0.75
Cr (μmol l ⁻¹)	72.83 $\pm$ 9.54	77.53 $\pm$ 11.87*
GLU (mmol l ⁻¹)	5.00 $\pm$ 0.33	4.41 $\pm$ 0.33**
WBC ($\times 10^9$ l ⁻¹)	5.68 $\pm$ 0.67	5.96 $\pm$ 0.70
RBC ($\times 10^{12}$ l ⁻¹)	4.83 $\pm$ 0.26	4.74 $\pm$ 0.36
Hb (g l ⁻¹)	148.50 $\pm$ 10.36	144.67 $\pm$ 9.28*
Plt ($\times 10^9$ l ⁻¹)	230.33 $\pm$ 35.14	224.42 $\pm$ 32.94

Compared with before co-administration with SchE, *P < 0.05; **P < 0.01. TB, total bilirubin; DB, direct bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, urea nitrogen; Cr, creatinine; GLU, blood glucose; Hb, haemoglobin; Plt, platelets; RBC, red blood cells; WBC, white blood cells.

During administration of midazolam alone, all subjects felt drowsy about 30 min after administration of midazolam and then went to sleep. They woke up 2–4 h later. Besides drowsiness, they reported a total of 16 adverse events, which were either mild or moderate in intensity. These symptoms were headache ($n = 2$), dizziness ($n = 4$) and fatigue ($n = 10$). During administration of SchE alone for 7 days, no adverse events were reported. During concomitant administration of SchE and midazolam, all volunteers also went to sleep about 30 min after orally taking midazolam. Their sleep time was about 3–5 h longer than in the period of administration of midazolam alone. Moreover, a total of 22 adverse events were reported. These adverse events included headache ($n = 3$), dizziness ($n = 7$), fatigue ($n = 11$) and postural hypotension ($n = 1$). All adverse events resolved completely within 24 h and none resulted in withdrawal from the study.

Discussion

The combined use of several drugs may produce interactions based on the pharmacological effects of individual drugs. In addition to interactions between synthetic drugs, drug–herbal medicine or supplement interactions have also been observed [14]. Some reported studies on herbal–drug interactions have revealed that the extract of herbal supplements including ginkgo leaf, echinacea, and milkthistle inhibited CYP3A4 activity in human liver microsomes [15–17]. Interaction between SchE and tacrolimus has been reported in our previous study [9]. The present study was conducted to clarify whether SchE may interact with tacrolimus via CYP3A. Our results have shown that 1 week’s ingestion of SchE caused significant

increases in AUC, $C_{\max}$ and $t_{\max}$ and declines in CL/F of midazolam, whereas there was no change in the elimination half-life. Since the 90% CI for the AUC was much higher than the prespecified interval of 0.8, 1.25, the interaction between SchE and midazolam must be considered to be clinically significant.

One study has shown that grapefruit juice could increase the bioavailability of midazolam and led to a 79% increase in $t_{\max}$ of midazolam and a 105% increase in $t_{\max}$ of 1'-hydroxy midazolam [18]. In this study, $t_{\max}$ of midazolam and 1'-hydroxy midazolam was also significantly increased by pretreatment of SchE. The increase in $t_{\max}$ of 1'-hydroxy midazolam should be caused by the increase in $t_{\max}$ of midazolam. However, the exact mechanism for the increase in $t_{\max}$ of midazolam is unclear and should be studied further.

Several studies have demonstrated that there was no change in the elimination half-life of midazolam, whereas AUC and $C_{\max}$ were significantly changed [17, 19]. It has also been reported that even though there was no change in $t_{1/2}$ of midazolam, inhibition of hepatic and/or intestinal CYP3A was considered [17]. Our present study had similar results. Therefore, although no change in $t_{1/2}$ of midazolam was found in our study, the inhibition of hepatic and/or intestinal CYP3A by SchE was still considered. According to the classification system of CYP3A inhibitors as in the September 2006 Food and Drug Administration guidance on drug interaction studies, a moderate inhibitor is one that causes a two or more but less than fivefold increase in the AUC values in clinical evaluations. Based on the results of this study, we can conclude that SchE is a moderate CYP3A inhibitor.

It has been reported that the plasma 1'-hydroxy midazolam/midazolam concentration ratio of 0.5 or 1 h after midazolam administration can be used to predict CYP3A activity [20, 21]. The use of a single blood sample to predict changes in CYP3A activity is particularly useful and important in a drug interaction study. In the present study, the ratio mentioned above after co-administration with SchE was decreased significantly. This result further demonstrates that SchE can inhibit the CYP3A activity and also indicates that a single blood sample at 1 h after midazolam dosing can be used to confirm CYP3A activity.

After co-administration with SchE, the sleep time in 12 volunteers became longer and the adverse effects increased, although these symptoms were part of the expected adverse event profile of midazolam. The enhanced sedative effect should be due to a considerable increase in the blood concentration of midazolam.

The effects of SchE on the function of P-gp may also be important when considering the mechanism of the changes in the pharmacokinetics of orally administered drugs during the absorption process, but it can be excluded in this case, since midazolam is not a substrate for P-gp [22]. Thus, the enhanced oral bioavailability of midazolam caused by SchE could result from the decreased

activity of CYP3A. It has been reported that the relationship between the disposition of midazolam administered orally and hepatic CYP3A content is weaker than that after intravenous administration, indicating the importance of the contribution of intestinal CYP3A to the *in vivo* disposition of midazolam administered orally [23]. Therefore, the increased bioavailability of midazolam by SchE may mainly come from the decreased activity of intestinal CYP3A.

In fact, one study has indicated that Schisandra fruit components could inhibit the activity of CYP3A4 *in vitro* [4]. That study was conducted to identify Schisandra fruit components having inhibitory effects on CYP3A4 by surveying the effect on human liver microsomal erythromycin N-demethylation activity. Known components of Schisandra fruit, gomisins B, C, G and N and gamma-shizandrin, showed inhibitory effects on N-demethylation activity. Among these components, gomisin C displayed the most potent and competitive inhibitory effect. Furthermore, the inhibitory effect of gomisin C was stronger than that of ketoconazole, a known potent CYP3A4 inhibitor. The human liver microsomal erythromycin N-demethylation activity inactivated by gomisin C did not recover on dialysis of the microsomes. These results indicate that gomisin C is a mechanism-based inhibitor that not only competitively inhibits but irreversibly inactivates CYP3A4. Our results *in vivo* are consistent with the above study *in vitro* [4]. However, which components of SchE play important roles in reduced CYP3A activity is still unclear.

The inhibition of CYP3A may result in enhanced blood and tissue concentrations of CYP3A substrates, and caused toxicity. Our results have shown the possible interaction between SchE and medicines metabolized by CYP3A. Thus, frequent monitoring of blood concentration and dose adjustments of CYP3A substrates are highly recommended when co-administration with SchE is unavoidable.

In conclusion, the results of this study have shown that SchE can markedly increase the blood concentration and oral bioavailability of midazolam in healthy men. This finding suggests that SchE is an inhibitor of CYP3A and has a high susceptibility to alter the disposition of drugs metabolized by CYP3A (e.g. tacrolimus).

Competing interests

None declared.

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