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Original Article

The effects of *Andrographis paniculata* (Burm.f.) Nees on the pharmacokinetics and pharmacodynamics of midazolam in healthy volunteers

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Abstract

Andrographis paniculata (Burm.f.) Nees has been widely used for centuries in Asia for the treatment of common cold and diarrhea. Although it was previously reported to inhibit cytochrome P450 *in vitro*, the potential to cause herb-drug interaction has been questioned. The purpose of this study was to evaluate the effects of *A. paniculata* on the pharmacokinetics and pharmacodynamics of midazolam, a CYP3A4 probe drug, in normal healthy volunteers. The study was an open-label, randomized, 2-phase crossover design with a 2-weeks washout period. Twelve healthy male volunteers received 4 capsules of 250 mg *A. paniculata* 3 times a day orally for 7 days. Midazolam plasma concentration time profiles were characterized after a single oral dose of 7.5 mg midazolam on the day before and after *A. paniculata* medication. Pharmacodynamics of midazolam were also evaluated. The results demonstrated that pretreatment with *A. paniculata* did not change mean pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-12}, $AUC_{0-\infty}$, $T_{1/2}$, Cl/F) of oral midazolam. Since midazolam is the most sensitive substrate for CYP3A4, thus, herb-drug interaction caused by CYP3A4 inhibition after *A. paniculata* in healthy volunteers was considered not clinically relevant. However, *A. paniculata* potentiated the effect of midazolam in lowering blood pressure and pulse rate. Therefore, co-administration of *A. paniculata* with midazolam should be warranted.

Keywords: *Andrographis paniculata*, andrographolide, midazolam, herb-drug interaction, CYP3A4

1. Introduction

During the past decade, the use of herbal medicines among the general public has been increased dramatically. Many reports indicated that herbal medicines were often taken concurrently with conventional therapy (Lin *et al.*, 2004). Although herbal medicines are generally perceived as safe when used alone at the recommended dose and duration, there is increasing evidence of herb-drug interactions which may lead to serious adverse reactions or failure of therapy with conventional medicines, such as bleeding tendency caused by ginkgo and aspirin interaction or graft rejection in patient taking immunosuppressant, cyclosporine, with St John's wort (Izzo, 2004).

Andrographis paniculata (Burm.f.) Nees (Fa-ta-lai-jone) belongs to Family Acanthaceae. It has been widely used for centuries in Asia to treat common cold, diarrhea, and fever (Farnsworth and Bunyapraphatsara, 1992). The three major active components in *A. paniculata* are andrographolide, neoandrographolide and 14-deoxy-11,12-didehydro-andro-

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grapholide. Many studies have shown that *A. paniculata* and its major components, andrographolide, have various pharmacological activities, such as anti-inflammatory, anti-cancer, anti-platelet aggregation, anti-viral and hepatoprotective effects (Mishra *et al.*, 2007). Previous *in vitro* studies demonstrated that *A. paniculata* extracts and andrographolide inhibited activity and mRNA expression of various cytochrome P450 (CYP) isozymes including CYP3A4, CYP2D6 and CYP2C in human and rat liver microsomes (Usia *et al.*, 2006; Pekthong *et al.*, 2009), while CYP1A inducing activity was also reported (Jaruchotikamol *et al.*, 2007). These data suggested that *A. paniculata* might cause herb-drug interaction when used in patients. However, an *in vitro* or even *in vivo* experiment in animals cannot be precisely extrapolated to the clinical situation. To our knowledge, there have been no clinical studies relating to the interaction of *A. paniculata* on drugs metabolized by CYP.

Midazolam is a short-acting benzodiazepine. It has anxiolytic, sedative, hypnotic, anticonvulsant, muscle relaxant and amnestic effects. After oral administration of 15 mg midazolam tablet in human, plasma concentration peaked between 0.5-1.5 h at an average concentration of 0.05 mg/l. The elimination half-life was 1.9 h, the mean clearance was 0.52 l/h/kg and the oral bioavailability was 49% (Langlois *et al.*, 1987). Midazolam is extensively metabolized in both the liver and the intestine via CYP3A4 and CYP3A5 to 1'-hydroxymidazolam and 4-hydroxymidazolam, which undergo rapid conjugation with glucuronic acid and are then excreted into the urine within 24 h. Many drugs and herbs have been reported to cause pharmacokinetic drug interaction with midazolam by the inhibition of CYP3A4, such as itraconazole, cimetidine and grapefruit juice, which can increase the risk of side effects such as drowsiness, confusion, memory loss, hypotension or respiratory depression, or the induction of CYP3A4 leading to the loss of clinical effects, such as rifampin, nevirapine and St John's wort (Yuan *et al.*, 1999, Goho, 2001, Wang *et al.*, 2001). In the present study, we used midazolam as a probe drug because it is the most sensitive substrate for CYP3A and it is suggested for the study of the relevance of inhibition or induction by an investigational drug (Huang *et al.,* 2007).

2. Objectives

The purpose of this study was to evaluate the effects of *A. paniculata* on pharmacokinetics and pharmacodynamics of midazolam, which is the substrate of CYP3A4, in healthy volunteers.

3. Materials and Methods

3.1 Subjects

Twelve healthy male, non-smoking and non-alcoholic, volunteers with a mean age of 27.5±6.1 years, height of 164.6±5.0 cm, weight of 56.8±7.0 kg, and body mass index of 20.9±1.9 kg/m^2 participated in the study. Participants were determined to be healthy on the basis of medical history, physical examination, clinical chemistry, and hematologic screening. One month before the start of the study and during the study, all subjects were not allowed to drink alcohol or take any medicines or herbs. Participants provided their written informed consent before participation in the study. The study was approved by the ethics committee of the Faculty of Science, Prince of Songkla University (0521.1.09/1031).

3.2 Study design

The study was an open-label, randomized, two-phase crossover design with a 2-weeks washout period, of single-dose (midazolam) and multiple-dose (*A. paniculata*) drug-drug interaction study. The subjects received both treatments in random sequence. Treatment A: after an overnight fast, volunteers received a single oral dose of 7.5 mg midazolam (Dormicum®, Hoffmann-La Roche Ltd, Basel, Switzerland) with 200 ml of water. Baseline pharmacokinetics of midazolam were evaluated. Treatment B: a midazolam pharmacokinetic characterization was performed on day 8 of the *A. paniculata* medication period with the regimen presented as below. Both phases of midazolam pharmacokinetic study were conducted identically. Blood samples were taken through a venous catheter before administration of midazolam (T0) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, and 12 h post dose. The heparinized plasma was collected and stored at -20°C until analysis.

3.3 *A. paniculata* medication

Each volunteer received 4 capsules of 250 mg *A. paniculata* t.i.d. orally for 7 days and 4 capsules once in the morning on day 8, 1 hour before midazolam administration. *A. paniculata* capsules (Lot no. CAP030) were manufactured by the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. Analysis of the *A. paniculata* main constituent (andrographolide) in the capsules was performed as described previously (Jain *et al.*, 2000). The content of andrographolide per capsule was 8.36±0.06 mg.

3.4 Midazolam analysis

Midazolam concentration in plasma was determined by HPLC method (Lehmann and Boulieu, 1995). The 500 µl of sample was added with 1 ml of diethyl ether. The mixture was vortexed for 5 minutes and centrifuged at 1,000×g for 10 minutes. The 800 µl of upper organic phase was separated and evaporated at 35°C under air flow. The residue was reconstituted in 100 µl of the mobile phase and 50 µl was injected into the HPLC system (Agilent Technologies) using C8

reversed-phase column (Phenomenex Inc, USA). The mobile phase consisted of 0.05M KH_2PO_4 (pH 4.0): acetonitrile: methanol (55:30:15 v/v/v), at a flow rate of 1.2 ml.min^{-1}. The peak was detected using a UV detector set at 210 nm. The standard midazolam was eluted at around 11 min (Figure 1, 2). The assay was validated according to the guideline (US. FDA. 2001) with the LLOQ of 4.64 ng/ml, recoveries of 88-98%, inter-/intraday CV and accuracy of 1.66/2.54% and 100.27-99.46 %, respectively.

3.5 Andrographolide analysis

Andrographolide concentration in plasma samples from all subjects at 1 h after oral administration of the last dose of *A. paniculata* was determined as described previously (Suo *et al*., 2007) with some modifications. The 500 µl of sample and internal standard 50 µl (carbamazepine in methanol, 5 µg/ml), were added with 1.2 ml of chloroform. The mixture was vortexed for 15 minutes and centrifuged at 1,000×g for 10 minutes. The 1000 µl of organic phase was separated and evaporated at 40°C under air flow. The residue was reconstituted in 100 µl of the mobile phase and 30 µl was injected into the HPLC system (Agilent Technologies) using C18 reversed-phase column (Eclipse XDB). The mobile phase consisted of methanol: water (52:48 v/v), at a flow rate of 0.8 ml/min. The peak was detected using a UV detector set at 225 nm. The standard andrographolide and internal standard were eluted at around 6 and 8 min, respectively (Figure 3). The assay was validated according to the guideline (US. FDA. 2001) with recoveries of 76-111%, LLOQ of 24.29 ng/ml, inter-/intraday CV and accuracy of 2.31/3.76 % and 105.91-107.7 %, respectively.

3.6 Subject monitoring

After drug administration, vital signs (blood pressure, pulse rate, respiration rate and body temperature) were monitored at 1, 1.5, 2, 4, 8 and 12 h. Subjects were asked for unusual symptoms periodically.

3.7 Meausurement of pharmacodynamics of midazolam

The pharmacodynamics of midazolam were estimated predosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10 and 12 h post dose. The alertness score (Langlois *et al.*, 1987) was chosen as the pharmacodynamic index, using the score: 4 = alert, 3 = drowsy, 2 = ataxia+dysarthria, 1 = asleep (rousable by voice), and 0 = asleep (not rousable by voice).

3.8 Pharmacokinetics analysis

Pharmacokinetic parameters (C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, $T_{1/2}$, and Cl/F) of midazolam were analyzed by non-compartment model with the use of WinNonlin professional Software Version 1.1 (Pharsight, Mountain View, CA).

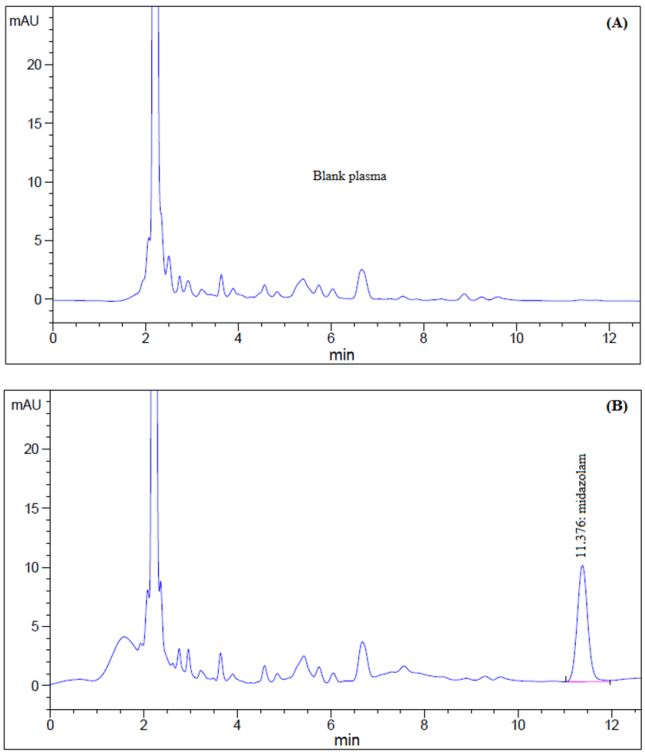

Figure 1. Chromatogram of (A) blank plasma, (B) blank plasma spiked with standard midazolam (150 ng/mL).

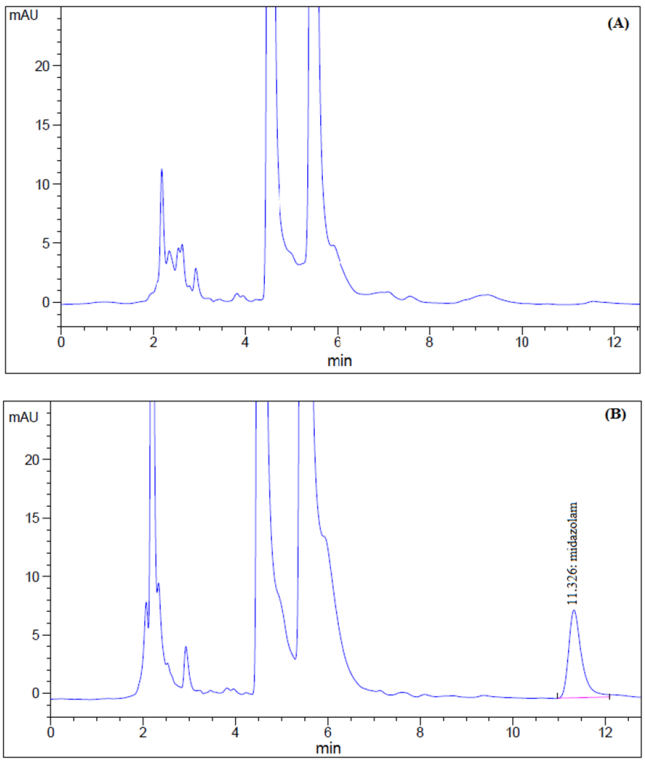

Figure 2. Chromatogram of (A) plasma obtained from a subject before treatment with midazolam, (B) plasma obtained from a subject receiving a single oral dose of 7.5 mg midazolam after oral administration at 0.25 h

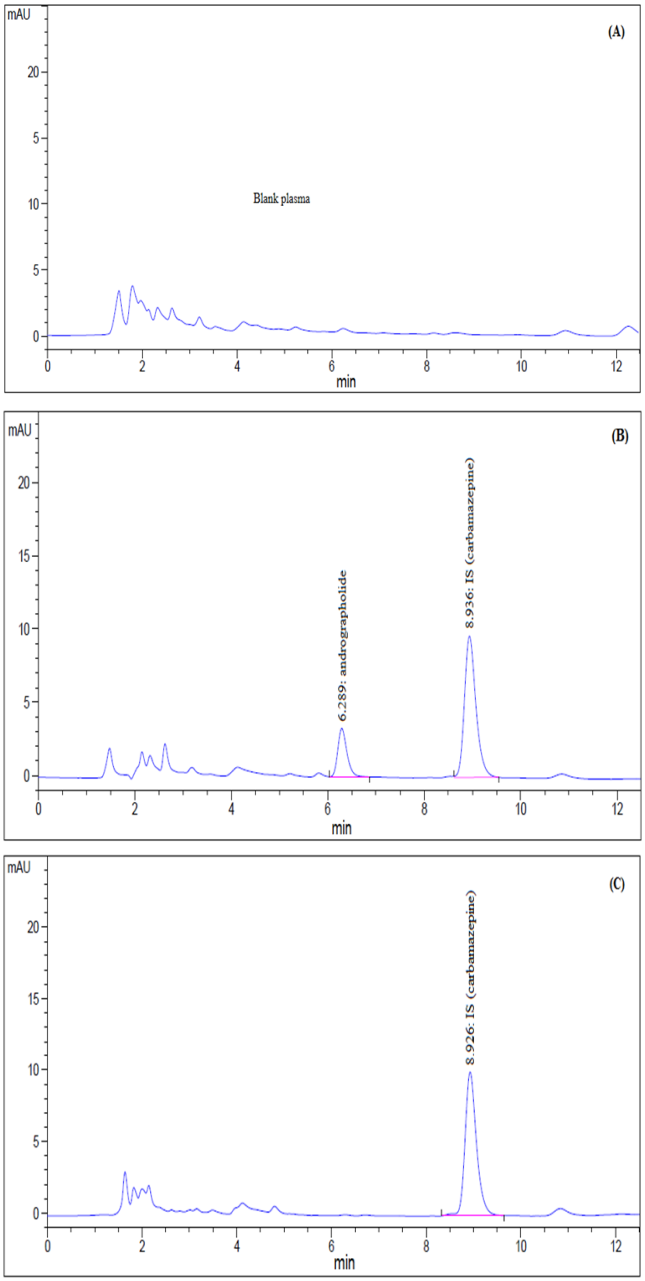

Figure 3. Chromatogram of (A) blank plasma, (B) blank plasma spiked with standard andrographolide (300 ng/mL) and carbamazepine (internal standard, 5 µg/mL), (C) plasma obtained from a subject at 1 h. after oral administration of 4 capsules of 250 mg *A. paniculata*.

3.9 Statistical analysis

The results were presented as the mean±standard deviation ($\overline{X}$ ±SD). All data were analyzed by one-way ANOVA followed by LSD or paired *t-test*, except for the alertness scores, which were analyzed by Wilcoxon signed-rank test. A difference was considered significant at $p<0.05$. The software used was the SPSS (Version 11.5, SPSS Inc, Chicago, IL, USA.).

4. Results

4.1 Midazolam pharmacokinetic study

Mean plasma concentration-time profiles of midazolam before and after treatment with *A. paniculata* are shown in Figure 4. The mean C_{max}, T_{max}, $AUC_{0\text{-}12h}$, $AUC_{0\text{-}\infty}$, $T_{1/2}$ and Cl/F of midazolam were not changed significantly ($p>0.05$), as shown in Table 1.

4.2 Midazolam pharmacodynamic study

The mean arterial pressure, pulse rate, respiration rate and body temperature are shown in Table 2. In subjects receiving oral 7.5 mg midazolam after *A. paniculata* (Treatment B), the mean arterial pressure and pulse rate were significantly decreased when compared with midazolam alone (Treatment A) at 2 h and 1 h post-dose, respectively ($p<0.05$), while the mean respiration rate and body temperature were not changed. The mean alertness score was slightly decreased

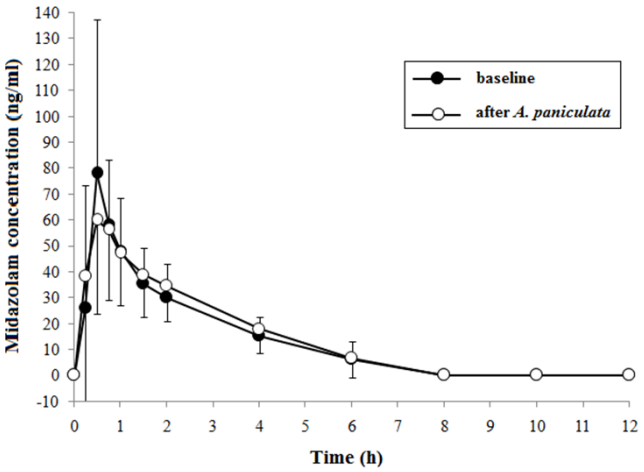

Figure 4. Mean plasma concentration time-profiles of midazolam after a single oral dose of 7.5 mg midazolam before (baseline, —●—) and after pretreatment with 4 capsules of 250 mg *A. paniculata* t.i.d. orally for 7 days (—○—).

Table 1. Pharmacokinetic parameters ($\overline{X}$ ± SD) of midazolam in 12 subjects receiving a single oral dose of 7.5 mg midazolam (phase 1) compared with after pretreatment with 4 capsules of 250 mg *A. paniculata* t.i.d. orally for 7 days (phase 2)

Parameters	Phase 1	Phase 2	*p*-value
C_{max} (ng/ml)	94.76±55.28	74.78±52.02	0.132
$AUC_{0\text{-}12}$ (ng.h/ml)	151.58±65.66	163.14±92.68	0.445
$AUC_{0\text{-}\infty}$ (ng.h/ml)	181.24±68.51	194.47±101.07	0.476
T_{max} (h)	0.583±0.19	0.583±0.22	1.000
$T_{1/2}$ (h)	2.05±0.63	2.09±0.60	0.808
Cl/F (l/h/kg)	0.047±0.02	0.046±0.02	0.638

Table 2. The pharmacodynamic parameters of 12 subjects receiving a single oral dose of 7.5 mg midazolam (phase 1) compared with after pretreatment with 4 capsules of 250 mg *A. paniculata* t.i.d. orally for 7 days (phase 2)

Time (h)	Mean arterial pressure (mmHg) ($\overline{X} \pm SD$)		
	Phase 1	Phase 2	*p*-value
0	89.78±10.84	89.56±7.42	0.904
1	85.37±9.43	76.13±8.55**	0.058
1.5	84.78±6.57	77.92±10.19**	0.051
2	85.36±7.80	76.69±7.04**	0.014[a]
4	82.67±5.03*	80.11±6.91**	0.383
8	83.06±5.88	78.97±3.99	0.098
12	87.14±7.66	85.75±7.70	0.645
Time (h)	Pulse rate (beats/min) ($\overline{X} \pm SD$)		
	Phase 1	Phase 2	*p*-value
0	65.33±10.46	66.92±6.01	0.566
1	61.70±5.48	59.00±5.16**	0.012[a]
1.5	59.00±10.39	58.92±7.55**	0.972
2	56.83±5.70*	59.08±7.74**	0.409
4	66.17±8.82	64.17±6.73	0.425
8	62.75±7.77	62.92±8.87	0.956
12	65.67±6.71	68.25±7.48	0.251
Time (h)	Respiration rate (breaths/min) ($\overline{X} \pm SD$)		
	Phase 1	Phase 2	*p*-value
0	18.33±2.39	17.67±2.06	0.560
1	17.60±1.58	18.20±0.63	0.273
1.5	17.40±1.35	18.00±0.94	0.343
2	17.67±1.44	18.00±0.85	0.551
4	19.17±1.03	18.50±1.24	0.266
8	18.50±0.90	18.00±0.85	0.191
12	18.83±1.03	18.50±1.24	0.586
Time (h)	Body temperature (°C) ($\overline{X} \pm SD$)		
	Phase 1	Phase 2	*p*-value
0	36.24±0.45	36.38±0.35	0.421
1	36.42±0.34	36.65±0.11	0.065
1.5	36.45±0.35	36.67±0.13	0.118
2	36.54±0.31	36.68±0.08	0.160
4	36.65±0.25	36.70±0.07	0.541
8	36.64±0.21	36.68±0.11	0.586
12	36.67±0.16	36.68±0.09	0.874

* Significantly different from T= 0 h of phase 1 at $p<0.05$
** Significantly different from T= 0 h of phase 2 at $p<0.05$
[a] Significantly different when compared between phase 1 and phase 2 at $p<0.05$

(1.75 VS 1.00, at 0.75-1 h post dose) but was not significantly different ($p>0.05$) (Table 3). No other adverse effects were found after drug administration.

4.3 The concentration of andrographolide in plasma

Andrographolide could not be detected in plasma samples from any subject at 1 h after oral administration of the last dose of *A. paniculata* with the LLOQ of 24.29 ng/ml.

5. Discussion

A. paniculata is one of the most well known herbal medicines in Thailand for relief of sore throat (Satyapan *et al.,* 2010). It has been placed in the National List of Essential Medicines for treatment of non-infectious diarrhea and common cold since 1999 (National Drug Committee, 2011). Although it is a herbal medicine developed from a single plant with the evidence indicating its safety for use in humans, according to the criteria of The National Drug Committee, data concerning interaction of *A. paniculata* with other medicines used in conventional therapy has never been reported. In the present study, we demonstrated that pretreatment with 4 capsules of 250 mg *A. paniculata* t.i.d. for 7 days in 12 normal volunteers did not significantly change the mean pharmacokinetic parameters (C_{max}, T_{max}, $AUC_{0\text{-}12h}$, $AUC_{0\text{-}\infty}$, $T_{1/2}$, Cl/F) of oral midazolam. Each capsule of *A. paniculata* used in the study contained about 8.36±0.06 mg of andrographolide. The dosage regimen of *A. paniculata* used in this study corresponds to about 1.8 mg/kg/day of the active principle andrographolide (12 capsules of 8.36 mg of andrographolide per day and average weight of the subjects was 56.8 kg) and is close to that recommended for treatment of respiratory diseases (4 capsules of 250 mg q.i.d. orally for 7 days) (National Drug Committee, 2011). Since we realized that andrographolide has a short half-life (3.9 h.) (Panossian *et al.*, 2000), another 4 capsules of *A. paniculata* were given to the subjects 1 h before midazolam administration after 7 days of

Table 3. The mean alertness scores of 12 subjects receiving a single oral dose of 7.5 mg midazolam (phase 1) compared with after pretreatment with 4 capsules of 250 mg *A. paniculata* t.i.d. orally for 7 days (phase 2)

Time (h)	Alertness score ($\overline{X} \pm SD$)		
	Phase 1	Phase 2	*p*-value
0.25	3.67±0.49	3.42±1.00	0.317
0.5	2.58±1.31	2.08±1.16	0.319
0.75	1.75±1.29	1.00±1.04	0.107
1	1.75±1.29	1.00±1.04	0.142
1.5	1.67±1.44	1.25±0.87	0.339
2	1.92±1.56	1.58±1.31	0.490
4	2.92±1.44	3.58±0.90	0.131
6	3.50±0.90	3.83±0.39	0.102

Alertness scores: 0 = unrousable to voice, 1 = rousable to voice, 2 = ataxia+dysarthria, 3 = drowsy, 4 = alert

A. paniculata medication, in order to assure the inhibitory effect of *A. paniculata* and to determine the plasma concentration of andrographolide. However, it is interesting to note that plasma level of andrographolide was negligible (less than the LLOQ of 24.29 ng/ml). This might be because of the poor oral bioavailability (2.67%) of andrographolide as reported by Xu *et al.* (2009) and Wangboonskul *et al.* (2006). On the contrary, according to Panossian *et al.* (2000), using Kan-Jang tablets which contained the extracts of *A. paniculata* and *A. senticosus*, the calculated steady state plasma concentration of andrographolide for multiple doses of *A. paniculata* after the normal therapeutic dose regimen (3x4 tablets of Kan Jang/day, about 1 mg andrographolide/kg/day) was approximately 1.9 μM (660 ng/ml), which is, however, less than the concentration affecting CYP activities *in vitro* (12.5-50 μM), as reported previously (Usia *et al.*, 2006; Pekthong *et al.*, 2009; Jaruchotikamol *et al.*, 2007). This could be the explanation of no clinical effect of *A. paniculata* on pharmacokinetics of midazolam in our study. Oral midazolam was used as a CYP3A probe drug since it is the most sensitive substrate for both intestinal and hepatic CYP3A (Chung *et al.*, 2006). Regulatory authorities suggest that in case of a negative drug interaction with the most sensitive substrate, it can be presumed that less sensitive substrates would also be unaffected (Huang *et al.*, 2007). Thus, it is indicated that pharmacokinetic drug interaction between *A. paniculata* and CYP3A4 substrate is clinically insignificant.

We also monitored the effect of *A. paniculata* on the pharmacodynamics of midazolam. It was demonstrated that *A. paniculata* when coadministered with midazolam decreased mean arterial pressure and pulse rate, whereas the respiratory rate and body temperature were not changed when compared with a single oral 7.5 mg midazolam alone. Although the side effect of *A. paniculata* on cardiovascular system or central nervous system in human is rarely reported. *A. paniculata* has been reported to decrease blood pressure and pulse rate in rat (Huang, 1987; Zhang and Tan, 1997; Yoopan *et al.*, 2007). Thus, it is possible that *A. paniculata* has an additive/synergistic effect on blood pressure and pulse rate with midazolam. Moreover, *A. paniculata* has been reported to potentiate the hypnotic effect of pentobarbitone in rat, which indicates that it may act at the barbiturate receptors in the brain (Mandal *et al.*, 2001). *A. paniculata* also produced hypothermia, and exhibited motor incoordination and muscle relaxant activity (Mandal *et al.*, 2001). According to the above data, it is suggested that *A. paniculata* extract has CNS-depressant action. In our study, the mean alertness score in the subjects who received midazolam after *A. paniculata* was slightly lower than after midazolam alone but the difference was not statistically significant. It is noted that the dose of midazolam used in this study was 7.5 mg which is the minimum therapeutic dose. It is possible that at the maximum therapeutic dose of 15 mg midazolam, the additive effect of *A. paniculata* on CNS depression might be more obvious which may lead to serious side effects.

6. Study limitation

In the present study, the method of midazolam analysis had no internal standard since we could not find a suitable one. However, we could get good results in the method validation which are in the acceptable range according to the US FDA guideline.

7. Further study

An ongoing project is studying the interaction between *A. paniculata* and other herbs which increase the absorption of andrographolide and the correlation with CYP inhibition *in vivo*.

8. Conclusion

No significant changes in pharmacokinetics of oral midazolam were demonstrated after medication with *A. paniculata* at the recommended dosage regimen for the treatment of respiratory tract infection. It is indicated that there is no clinically relevant CYP3A4 inhibition after *A. paniculata* treatment in healthy volunteers. Thus, pharmacokinetic interaction between *A. paniculata* and CYP3A4 substrates is considered clinically insignificant. However, *A. paniculata* affected the pharmacodynamics of oral midazolam. Therefore, the risk associated with the additive or potentiative effects of *A. paniculata* and CNS depressants (e.g., alcohol, barbiturates, benzodiazepines) should be warranted.

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