

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Membrane Transport of Andrographolide in Artificial Membrane and Rat Small Intestine

¹Supawadee Daodee, ¹Jinda Wangboonskul, ¹Kanokwan Jarukamjorn,
¹Bung-orn Sripanidkulchai and ²Teruo Murakami

¹Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand

²Faculty of Pharmaceutical Sciences, Hiroshima International University, Kure, Japan

Abstract: In the present study, the possible drug interactions of andrographolide with co-administering drugs such as acetaminophen, amoxycillin, aspirin, chlorpheniramine and norfloxacin to treat various infectious and inflammatory diseases that may be induced during absorption process were examined using artificial lipophilic membrane and everted rat intestine. The membrane transport of andrographolide across the artificial membrane was not affected by different pH of the medium (simulated gastric and intestinal fluids), different concentrations of andrographolide and co-administered drugs examined. In everted rat intestine, above co-administered drugs examined showed no significant effect on andrographolide membrane transport. The participation of efflux transporters such as P-glycoprotein and MRP2 in andrographolide transport was then examined, since andrographolide is a diterpene compound and some diterpene compounds are known as P-glycoprotein substrates. Cyclosporine, a P-glycoprotein/MRP2 inhibitor, significantly suppressed the efflux transport of andrographolide in distal region of intestine, whereas probenecid, an MRP inhibitor, showed no significant effect in both proximal and distal regions of intestine. These results suggest that P-glycoprotein, but not MRP, is participated in the intestinal absorption of andrographolide and P-glycoprotein-mediated drug interactions occur depending on the co-administered drugs and its concentrations.

Key words: Andrographolide, membrane transport, drug interaction, p-glycoprotein, MRP2, rat small intestine

INTRODUCTION

Andrographis paniculata (Burm.f.) Nees, also known as King of bitters, is a member of the plant of Acanthaceae family and has been used for centuries to treat gastrointestinal tract and upper respiratory infections, fever, herpes, sore throat and a variety of other chronic infectious diseases (Anonymous, 1999). Moreover, Kan Jang, which is the extracts from *A. paniculata* and *Eleutherococcus senticosus*, has been used as a non-herbal medicinal product for treatment of non-complicated upper respiratory tract infection (Caceres *et al.*, 1995, 1997; Melchior *et al.*, 1996, 2000; Kulichenko *et al.*, 2003; Spasov *et al.*, 2004). Andrographolide, the main active component in *A. paniculata* (Sharma *et al.*, 1992), enhances functionalities of the immune system and inhibits the proliferation of tumor cell lines including various human cancers (Sriram *et al.*, 2003; Kumar *et al.*, 2004). Significant anti-inflammatory, anti-allergic and antipyretic effects have also been demonstrated by using the pure andrographolide, as well as the extracts of *A. paniculata* (Madav *et al.*, 1996; Gupta *et al.*, 1998).

In clinical pharmacotherapy andrographolide, or extracts of *A. paniculata*, is commonly used in combination with other drugs such as acetaminophen (analgesic and antipyretic), amoxycillin (antibiotic), aspirin (analgesic and antipyretic), chlorpheniramine maleate (antihistamine) and norfloxacin (antibiotic) (Budavari, 1996; Shannon *et al.*, 2001). In the present study, the possibility of drug interactions of andrographolide with above co-administering drugs was examined in artificial lipophilic membrane and everted rat intestine, since such information is lacking. Also, in rat small intestine, the participation of ATP-dependent efflux transporters such as P-glycoprotein and multidrug resistance-associated protein 2 (MRP2) in andrographolide transport was also examined, since andrographolide is a diterpene lactone compound and some diterpene compounds such as taxol (or paclitaxel) and taxotere (docetaxel) are known to be substrates of P-glycoprotein and/or MRP2 (Dantzig *et al.*, 2001; Huisman *et al.*, 2005; Vaclavikova *et al.*, 2006). These efflux transporters are expressed on the apical (brush-border) membrane of enterocytes and limit the intestinal absorption by facilitating the efflux to prevent the intracellular accumulation of various

substrate drugs. A variety of structurally and pharmacologically unrelated hydrophobic compounds are transported by P-glycoprotein and relatively hydrophilic acidic compounds including glucuronide-, glutathion- and sulfate-conjugated compounds are transported by MRP2 (Hunter *et al.*, 1993; Terao *et al.*, 1996; Suzuki and Sugiyama, 2002; Chan *et al.*, 2004; Takano *et al.*, 2006). In the present study, the participation of such ATP-dependent efflux transporters in membrane transport of andrographolide was examined in proximal and distal regions of rat small intestine, since P-glycoprotein is expressed mostly in the distal intestine (Terao *et al.*, 1996; Takano *et al.*, 2006) and MRP2 is in the proximal intestine (Chan *et al.*, 2004). To detect P-glycoprotein- and/or MRP-mediated transport of andrographolide, cyclosporine and probenecid were employed as inhibitors of P-glycoprotein and MRP2, respectively (Yumoto *et al.*, 1999; Horikawa *et al.*, 2002; Naruhashi *et al.*, 2002).

MATERIALS AND METHODS

Materials: Andrographolide was extracted from *A. paniculata* dried powder and identified by TLC, HPLC, proton NMR and mass spectrometer. All solvents were purchased from Merck (Darmstadt, Germany). Acetaminophen, chlorpheniramine maleate, norfloxacin, amoxycillin and probenecid were purchased from Sigma-Aldrich (Germany). Aspirin and cyclosporine were from Fluka (Switzerland) and Novartis (Thailand), respectively.

Transport study of andrographolide using the artificial membrane: A side-by-side diffusion cell (Crown Glass Company, USA) with a diffusion area of 0.936 cm² and each diffusion cell volume of 3 mL was used for the transport study of andrographolide across the artificial membrane. A cellulose nitrate membrane filter with 0.2 µm pore size (Sartorius, Germany) was immersed into octanol for one day prior to the transport study and used as the artificial lipid membrane. The filter-disc membrane was then mounted in the diffusion cell and equilibrated with either the simulated intestinal fluid (pH 7, 0.05 mole L⁻¹ phosphate buffer solution) or the simulated gastric fluid (pH 2, 0.01 mol L⁻¹ HCl, 2.5 g sodium lauryl sulphate, 2 g NaCl) for 30 min. In the experiments, the effects of different medium pHs (simulated pH 2 gastric and pH 7 intestinal fluids), different concentrations of andrographolide and co-administering drugs on membrane transport of andrographolide were examined. Andrographolide was dissolved at a concentration of 16.0, 68.0, 154.7 or 253.0 µg mL⁻¹ in simulated intestinal fluid or at a concentration of 13.3, 33.3, 66.7 or 128.0 µg mL⁻¹ in simulated gastric fluid. As commonly administered drugs together with andrographolide in

usual pharmacotherapy, acetaminophen (12 mg mL⁻¹), chlorpheniramine maleate (0.016 mg mL⁻¹), amoxycillin (2 mg mL⁻¹), aspirin (1.2 mg mL⁻¹) and norfloxacin (1.6 mg mL⁻¹) were employed, where the concentration of co-administered drug was determined according to their oral dosages. Andrographolide solution (0.2 mg mL⁻¹, 1 mL), above coadministering drug solutions (1 mL) and simulated intestinal or gastric fluid (1 mL) were mixed in the donor cell. To receiver cell, 3 mL of simulated intestinal or gastric fluid was placed. Sampling (2.5 mL) was made from receiver cell periodically for 120 min and an equal volume of fresh simulated fluid was re-supplied each time.

Transport study of andrographolide using rat everted intestine:

Male Sprague-Dawley rats (250-350 g) were fasted one night prior to the experiment. The protocol of animal study was reviewed and approved by the Animal Ethics Committee of Khon Kaen University based on the Ethic of Animal Experimentation of National Research Council of Thailand. Rats were anaesthetized with ether and killed by decapitation. Proximal and distal parts of small intestine were isolated and the luminal contents were flushed with ice-cold normal saline. The intestine was everted and two everted sacs of approximately 10 cm long were made from each proximal and distal rejoin of the intestine. One everted sac was used as a control and the other one was used for test compounds to avoid inter-individual differences in P-glycoprotein or MRP2 function. The everted sac was incubated in 8 mL of pH 7.4, Dulbecco's phosphate buffer solution (D-PBS), which was saturated with a 95/5% O₂/CO₂ gas in advance at 37°C for 20 min. Andrographolide was dissolved in D-PBS at a concentration of 0.1 mmol L⁻¹ and the solution (0.5 mL) was introduced into the inside (serosal side) of the everted sac. Sampling (0.5 mL) of mucosal side medium was made periodically for 120 min and equal volume (0.5 mL) of fresh D-PBS was re-supplied each time.

The effect of commonly administered drugs on andrographolide efflux transport was also examined in everted rat intestine using acetaminophen (1 mM), chlorpheniramine maleate (1 mM), amoxycillin (1 mM), aspirin (1 mM) and norfloxacin (1 mM). In addition, the participation of ATP-dependent efflux transporters in membrane transport of andrographolide was examined by using cyclosporine as a P-glycoprotein and MRP2 inhibitor and probenecid as an MRP2 inhibitor in proximal and distal everted intestine. These compounds were added into the mucosal side of everted sac at a concentration of 1 mM and the efflux transport from serosal to mucosal surfaces of andrographolide was determined periodically.

Analytical method of andrographolide: To each aqueous sample (500 μL) containing andrographolide, 10 μL of naproxen sodium solution (100 $\mu\text{g mL}^{-1}$) as an internal standard and 490 μL of acetonitrile were added. The mixture solution was analysed by HPLC (A Hewlett Packard LC-1100 liquid chromatography instrument, equipped with an autosampler system) on a C18 reversed phase column (a Hypersil[®] ODS column, 250 \times 4.0 mm, 5 μm particle). Mobile phase used was a mixture of acetonitrile and 10 mM phosphate buffer solution (50:50 %v/v) adjusted to pH 2.0 with phosphoric acid. Andrographolide was monitored at a wavelength of 230 nm.

Data analysis: The transport rate of andrographolide across artificial membrane and rat everted intestine was calculated from the slope of the transported amount plotted against the time. The transport rate constant of andrographolide was estimated by dividing the transport rate by a product of the exposed area of the membrane (0.936 cm^2) and the initial concentration of andrographolide in donor cell.

Results were expressed as the mean \pm the standard deviation. The analysis of variance (ANOVA) was applied to evaluate the significance of difference observed. p-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Transport of andrographolide across the artificial membrane: The time course of andrographolide transport across the artificial membrane from pH 7 simulated intestinal fluids and pH 2 simulated gastric fluids are shown in Fig. 1 and 2, respectively. The transported amount of andrographolide increased with time in a zero-order fashion and the transport rate of andrographolide increased almost in parallel with increase in the initial concentrations in the simulated fluids as follows: $0.0940\pm0.0017 \mu\text{g min}^{-1}$ at $16.0 \mu\text{g mL}^{-1}$ to $1.7570\pm0.0058 \mu\text{g min}^{-1}$ at $253.0 \mu\text{g mL}^{-1}$ in simulated intestinal fluid and $0.0774\pm0.0018 \mu\text{g min}^{-1}$ at $13.3 \mu\text{g mL}^{-1}$ to $1.0525\pm0.1134 \mu\text{g min}^{-1}$ at $128.0 \mu\text{g mL}^{-1}$ in simulated gastric fluid. That is, the transport rate constants of andrographolide, estimated by dividing the transport rate by a product of initial concentration of andrographolide and exposure area of artificial membrane, were comparable between different pHs of medium (simulated gastric and intestinal fluids) and among different concentrations of andrographolide. Collectively, it was confirmed that the transport of andrographolide across artificial lipid membrane follows passive diffusion and the transport rate constant of andrographolide is constant among different pHs of medium, because andrographolide is an uncharged neutral compound.

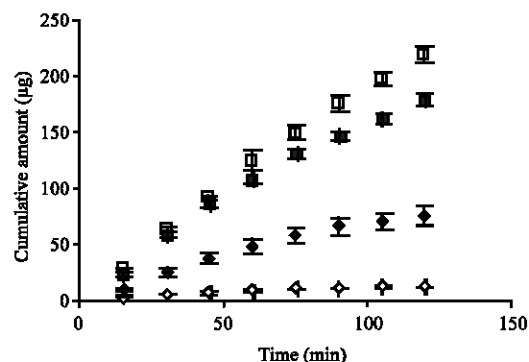


Fig. 1: Transport profiles of andrographolide from the simulated intestinal fluid across the artificial membrane. The concentration of andrographolide in donor cell: (\diamond) $16.00 \mu\text{g mL}^{-1}$; (\blacklozenge) $68.00 \mu\text{g mL}^{-1}$; (\blacksquare) $154.7 \mu\text{g mL}^{-1}$ and (\square) $253.0 \mu\text{g mL}^{-1}$. Each value represents the mean \pm SD (n = 3)

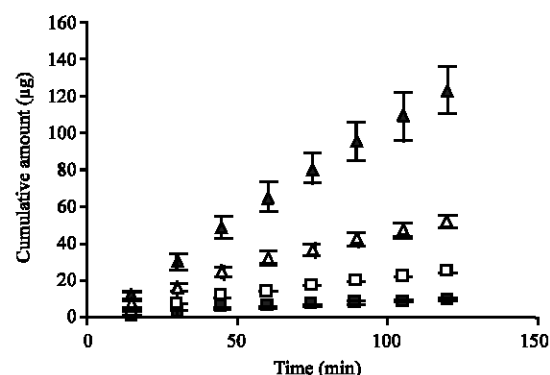


Fig. 2: Transport profiles of andrographolide from simulated gastric fluid across artificial membrane. The concentration of andrographolide in donor cell: (\blacksquare) $13.33 \mu\text{g mL}^{-1}$; (\square) $33.33 \mu\text{g mL}^{-1}$; (\blacktriangle) $66.67 \mu\text{g mL}^{-1}$ and (\triangle) $128.0 \mu\text{g mL}^{-1}$. Each value represents the mean \pm SD (n = 3)

In clinical pharmacotherapy andrographolide, or extracts of *A. paniculata*, is commonly used in combination with other drugs such as acetaminophen, amoxycillin, aspirin, chlorpheniramine maleate and norfloxacin, as already described in the introduction. The possibility of drug interactions of andrographolide with above co-administering drugs was examined. In artificial lipophilic membrane, physicochemical drug interactions such as chelation, ion pair formation, micelle formation, hydrogen bonding and stacking in the aqueous medium or lipid layer could be detected, since the transport rate of andrographolide, or apparent lipophilicity, would change if above-mentioned drug interactions occurred. Results are summarized in Table 1. The transport rate and the

Table 1: The transport rate constants of andrographolide across artificial lipid membrane in the absence (control) and presence of commonly co-administered drugs

Co-administered drug (concentration)	Transport rate constant of andrographolide (cm/sec)
Simulated intestinal fluid (pH 7)	
Control	2.0219E-04±3.99E-06
+acetaminophen (12 mg mL ⁻¹)	2.0891E-04±4.99E-06
+chlorpheniramine maleate (0.016 mg mL ⁻¹)	2.0502E-04±1.00E-05
+amoxicillin trihydrate (2 mg mL ⁻¹)	2.0673E-04±3.68E-06
+aspirin (1.2 mg mL ⁻¹)	2.0961E-04±3.23E-06
Simulated gastric fluid (pH 2)	
Control	2.0943E-04±4.74E-06
+acetaminophen (12 mg mL ⁻¹)	2.1370E-04±6.29E-06
+chlorpheniramine maleate (0.016 mg mL ⁻¹)	2.1164E-04±4.34E-06
+amoxicillin trihydrate (2 mg mL ⁻¹)	2.1180E-04±3.95E-06
+aspirin (1.2 mg mL ⁻¹)	2.1405E-04±1.01E-05
+norfloxacin (1.6 mg mL ⁻¹)	2.1813E-04±9.69E-06

Each value represents the mean±SD (n = 5)

transport rate constant of andrographolide were not affected by above co-administered drugs, suggesting that direct physicochemical interaction will not occur between andrographolide and commonly co-administered drugs such as acetaminophen, amoxycillin, aspirin, chlorpheniramine maleate and norfloxacin.

Transport of andrographolide across rat everted intestine: The possibility of drug interactions of andrographolide with above co-administering drugs was then examined in rat intestine. In biomembranes, it is expected that pharmacokinetic drug interactions such as metabolism-mediated and transporter-mediated drug interactions could be detected in addition to physicochemical drug interactions. In the present study, the proximal and distal regions of small intestine were employed, since the expression and function of P-glycoprotein and MRP2 are known to show regional differences (Terao *et al.*, 1996; Yumoto *et al.*, 1999; Chan *et al.*, 2004). In Fig. 3, the efflux transported amounts of andrographolide across everted intestine in the absence and presence of co-administered drugs are summarized. As shown in the Fig. 3, the values in the absence of co-administered drugs were scattered among different intestines (experiments), possibly due to the inter-individual variation of membrane permeability. However, examined co-administered drugs such as acetaminophen, amoxycillin, aspirin, chlorpheniramine maleate and norfloxacin showed no significant effect on andrographolide transport in each intestine. These results suggested that pharmacokinetic drug interaction, as well as physicochemical drug interaction, between andrographolide and above commonly co-administered drugs will not occur in the intestine *in vivo*. Acetaminophene and norfloxacin are reportedly absorbed mostly by passive diffusion (Goon and Klassen, 1990; Bermejo *et al.*, 2004; Schaiquevich *et al.*, 2004). Amoxycillin and aspirin are absorbed by both passive

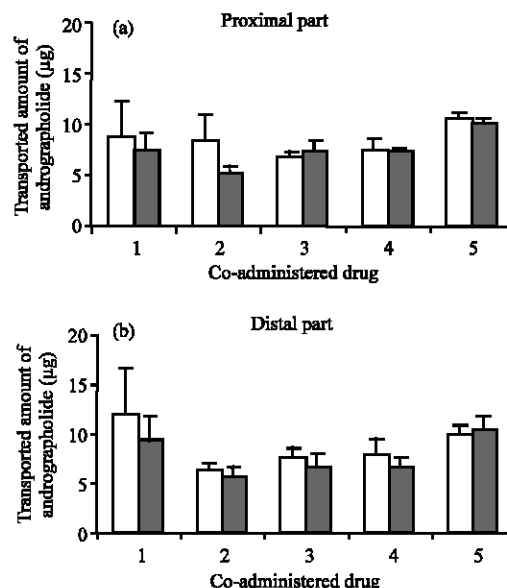


Fig. 3: Transported amounts of andrographolide across everted proximal (a) and distal (b) intestine in the absence and presence of commonly co-administered drugs during a 120 min incubation. The concentration of andrographolide was 0.1 mM. Open column: absence; Closed column, presence of co-administered drugs: 1, acetaminophen (1 mM); 2, chlorpheniramine (1 mM); 3, amoxicillin (1 mM); 4, aspirin (1 mM); 5, norfloxacin (1 mM). Each value represents the mean±SD (n = 3)

diffusion and active transport mediated by oligopeptide carrier 1 (Lennernas *et al.*, 2002) and monocarboxylic transporter (Katherine, 2002), respectively. In contrast, the intestinal transport mechanism of chlorpheniramine is obscure. For example, Kandimalla and Donovan (2005) recently reported that chlorpheniramine is a P-gp substrate, however, in other papers, modulators of P-glycoprotein and cytochrome P450 such as cyclosporin A and ketoconazole did not modify the intestinal absorption of this compound *in vitro* and *in situ* (Hiep *et al.*, 2001).

Though chlorpheniramine showed no significant effect on andrographolide efflux transport in the present study, the participation of efflux transporters such as P-glycoprotein and MRP2 in andrographolide transport was examined using cyclosporine and probenecid as inhibitors. Andrographolide is a diterpene lactone compound and some diterpene compounds such as taxol (or paclitaxel) and taxotere (docetaxel) are known to be substrates of P-glycoprotein and/or MRP2 (Dantzig *et al.*, 2001; Huisman *et al.*, 2005; Vaclavikova *et al.*, 2006).

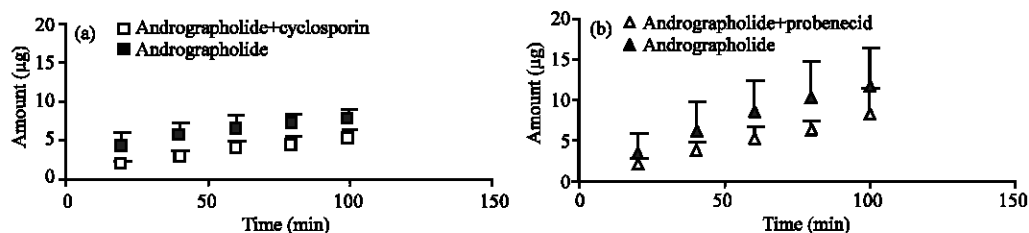


Fig. 4: The transport profiles of andrographolide across everted proximal intestine in the absence and presence of cyclosporine A (a) or probenecid (b). Andrographolide was added into the serosal-side medium of a 10 cm long everted intestine at a concentration of 0.1 mM (0.5 mL). Cyclosporin A or probenecid was added to mucosal-side medium at a concentration of 1 mM (8 mL). Each value represents the mean \pm SD (n = 3)

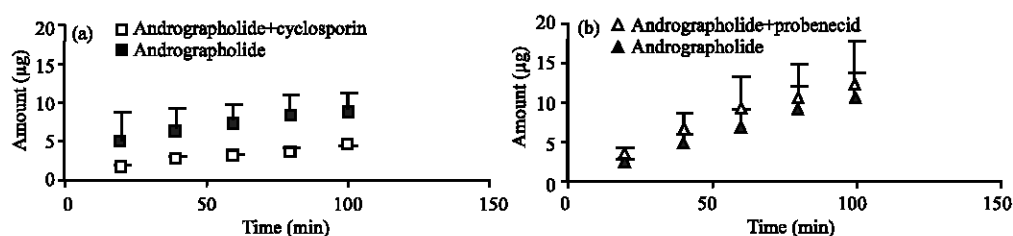


Fig. 5: The transport profiles of andrographolide across everted distal intestine in the absence and presence of cyclosporine A (a) or probenecid (b). Andrographolide was added into the serosal-side medium of a 10 cm long everted intestine at a concentration of 0.1 mM (0.5 mL). Cyclosporin A or probenecid was added to mucosal-side medium at a concentration of 1 mM (8 mL). Each value represents the mean \pm SD (n = 3)

Also, some diterpene compounds are known to inhibit P-glycoprotein (Ferreira *et al.*, 2005a, b). P-glycoprotein is highly expressed in the distal intestine, but not in the proximal region (Terao *et al.*, 1996; Yumoto *et al.*, 1999; Takano *et al.*, 2006) and MRP2 is highly expressed in the proximal intestine, but not in distal region (Chan *et al.*, 2004). In the proximal intestine, cyclosporine and probenecid suppressed the efflux transport of andrographolide a little, but not significantly (Fig. 4). In contrast, in the distal intestine, the efflux transport of andrographolide was significantly suppressed by cyclosporine A, though probenecid showed no significant effect (Fig. 5). Thus, it was suggested that P-glycoprotein, but not MRP, is participated in the intestinal absorption of andrographolide and P-glycoprotein-mediated drug interactions may occur depending on the co-administered drugs and its concentrations. Further *in vivo* studies including multiple treatments with commonly co-administered drugs would be necessary to reveal, predict and prevent the P-glycoprotein or MRP2-mediated drug interaction of andrographolide, since, for example, it is reported that acetaminophen significantly increased the expression of both MRP-2 and P-glycoprotein (Ghanam *et al.*, 2004).

At present, it is well recognized that natural products such as grapefruit juice, several components of grapefruit juice, St. John's wort, curcuminoids, green tea

extract, salvinal and flavonoid constituents also modify efflux transporters functions (Chearwae *et al.*, 2006; Ferreira *et al.*, 2005a and b; Netsch *et al.*, 2005; Panchagnula *et al.*, 2005; Singh 2005; Wang *et al.*, 2005; Chang *et al.*, 2004; Honda *et al.*, 2004). As well, the intake of *Andrographis paniculate* extracts, or andrographolide, may cause efflux transporter-mediated drug interaction by modulating their functions, as demonstrated in the present study. The analysis of transport property, or drug interaction, of andrographolide would be important information for pharmacotherapy with andrographolide or *A. paniculata* (He *et al.*, 2003; Imanidis *et al.*, 1995; Jonker, 2002; Legen *et al.*, 2003).

CONCLUSIONS

In the present study, the possible drug interaction of andrographolide with commonly used drugs such as acetaminophen, amoxycillin, aspirin, chlorpheniramine and norfloxacin were examined using artificial lipophilic membrane and everted rat intestine. In former experimental system, direct physicochemical drug interactions and in latter experimental system, pharmacokinetic drug interactions would be detected. Above co-administered drugs examined showed no significant effect on andrographolide transport in both artificial lipophilic membrane and everted rat intestine. Then, the

participation of ATP-dependent efflux transporters such as P-glycoprotein and MRP2 in andrographolide transport was examined in rat intestine *in vivo*. As a result andrographolide, a diterpene compound, was found to be related to P-glycoprotein and caused P-glycoprotein-mediated drug interaction. It will be necessary to pay attention in clinical pharmacotherapy with andrographolide from the viewpoint of P-glycoprotein-mediated drug interaction. In addition, it was suggested that the combined experimental system of absorption simulation using artificial lipophilic membrane and the transport study in everted intestine are useful in predicting some types of drug interaction that may occur in the intestine *in vivo*, though there was no significant drug interaction with commonly used drugs such as acetaminophen, amoxycillin, aspirin, chlorpheniramine and norfloxacin in the present study.

ACKNOWLEDGMENTS

The authors would like to thank the Graduate School of Khon Kaen University and the Central for Research and Development of Herbal Health Product, Khon Kaen University for their financial support.

REFERENCES

- Anonymous, 1999. Standard of Thai herbal medicine: *Andrographis paniculata* (Burm.f.) Nees. Thailand: The War Veterans Organization Press.
- Bermejo, M., A. Avdeef, A. Ruiz, R. Nalda, J.A. Ruell, O. Tsinman *et al.*, 2004. AMPA-a drug absorption *in vitro* model. Eur. J. Pharm. Sci., 21: 429-441.
- Budavari, S., 1996. The Merck Index. 12th Edn. United State of America: Merck and Co Inc.
- Caceres, D.D., J.L. Hancke and R.A. Burgos, 1995. Double blind study with a new monodrug Kan Jang: Decrease of symptoms and improvement in the recovery from common colds. Phytother. Res., 9: 559-562.
- Caceres, D.D., J.L. Hancke, R.A. Burgos and G.K. Wikman, 1997. Prevention of common colds with *Andrographis paniculata* dried extract: A pilot double blind study. Phytomedicine, 4: 101-104.
- Chan, L.M.S., S. Lowes and B.H. Hirst, 2004. The ABCs of drug transport in intestine and liver: Efflux proteins limiting drug absorption and bioavailability. Eur. J. Pharm. Sci., 21: 25-51.
- Chang, J.Y., C.Y. Chang, C.C. Kuo, L.T. Chen, Y.S. Wein and Y.H. Kuo, 2004. Salvinal, a novel microtubule inhibitor isolated from *Salvia miltiorrhizae* Bunge (Danshen), with antimitotic activity in multidrug-sensitive and -resistant human tumor cells. Mol. Pharmacol., 65: 77-84.
- Chearwae, W., S. Anuchapreeda, K. Nandigama, S.V. Ambudkar and P. Limtrakul, 2004. Biochemical mechanism of modulation of human P-glycoprotein (ABCB1) by curcumin I, II and III purified from Turmeric powder. Biochem. Pharmacol., 68: 2043-2052.
- Dantzig, A.H., K.L. Law, J. Cao and J.J. Starling, 2001. Reversal of multidrug resistance by the P-glycoprotein modulator, LY335979, from the bench to the clinic. Curr. Med. Chem., 8: 39-50.
- Ferreira, M.J., N. Gyemant, A.M. Madureira and J. Molnar, 2005a. Inhibition of P-glycoprotein transport activity in a resistant mouse lymphoma cell line by diterpenic lactones. Anticancer Res., 25: 3259-3262.
- Ferreira, M.J., N. Gyemant, A.M. Madureira, M. Tanaka, K. Koos, R. Didziapetris and J. Molnar, 2005b. The effects of jatropha derivatives on the reversion of MDR1- and MRP-mediated multidrug resistance in the MDA-MB-231 (HTB-26) cell line. Anticancer Res., 25: 4173-4178.
- Ghanam, C.I., P.C. Gomez, M.C. Arana, M. Perassolo, M.L. Ruiz, S.S.M. Villanueva *et al.*, 2004. Effect of acetaminophen on expression and activity of rat liver multidrug resistance-associated protein 2 and P-glycoprotein. Biochem. Pharmacol., 68: 791-798.
- Goon, D. and C.D. Klaassen, 1990. Dose-dependent intestinal glucuronidation and sulfation of acetaminophene in the rat *in situ*. J. Pharmacol. Exp. Ther., 252: 201-207.
- Gupta, P.P., J.S. Tandon and G.K. Patnaik, 1998. Antiallergic activity of andrographolides isolated from *Andrographis paniculata* (Burm. F) Wall. Pharm. Biol., 36: 72-74.
- He, X., M. Sugawara, M. Kobayashi, Y. Takekuma and K. Miyazaki, 2003. An *in vitro* system for prediction of oral absorption of relatively water-soluble drugs and ester prodrugs. Int. J. Pharm., 263: 35-44.
- Hiep, B.T., C. Fernandez, M. Tod, H. Banide, A. Thuillier, B. Lacour, R. Farinotti and F. Gimenez, 2001. Intestinal absorption and metabolism of chlorpheniramine enantiomers in rats. Chirality, 13: 207-213.
- Honda, Y., F. Ushigome, N. Koyabu, S. Morimoto, Y. Shoyama, T. Uchiumi, M. Kuwano, H. Ohtani and Y. Sawada, 2004. Effects of grapefruit juice and orange juice components on P-glycoprotein- and MRP2-mediated drug efflux. Br. J. Pharmacol., 143: 856-864.
- Horikawa, M., Y. Kato and Y. Sugiyama, 2002. Reduced gastrointestinal toxicity following inhibition of the biliary excretion of irinotecan and its metabolites by probenecid in rats. Pharm. Res., 19: 1345-1353.
- Hunter, J., B.H. Hirst and N.L. Simmons, 1993. Drug absorption limited by P-glycoprotein-mediated secretory drug transport in human intestinal epithelial Caco-2 cell layers. Pharm. Res., 10: 743-749.

- Huisman, M.T., A.A. Chhatta, O.V. Telling, J.H. Beijnen and A.H. Schinkel, 2005. MRP2 (ABCC2) transports taxanes and confers paclitaxel resistance and both processes are stimulated by probenecid. *Int. J. Cancer.*, 116: 824-829.
- Imanidis, G., K.C. Hartner and N.A. Mazer, 1995. Intestinal permeation and metabolism of a model peptide (leuprolide) and mechanisms of permeation enhancement by non-ionic surfactants. *Int. J. Pharm.*, 120: 41-50.
- Jonker, C., J.H. Hamman and A.F. Kotze, 2002. Intestinal paracellular permeation enhancement with quaternised chitosan: *In situ* and *in vitro* evaluation. *Int. J. Pharm.*, 238: 205-213.
- Katherine, M.D., 2002. The mechanism of aspirin absorption in the Caco-2 model of intestinal mucosa Ph.D. Thesis in Pharmaceutical Science and Cell Biology/Biophysics. USA: University of Missouri-Kansas City.
- Kandimalla, K.K. and M.D. Donovan, 2005. Transport of hydroxyzine and triprolidine across bovine olfactory mucosa: Role of passive diffusion in the direct nose-to-brain uptake of small molecules. *Int. J. Pharm.*, 302: 133-144.
- Kulichenko, L.L., L.V. Kireyeva, E.N. Malysheva and G. Wikman, 2003. A randomized, controlled study of Kan Jang versus amantadine in the treatment of influenza in Volgograd. *J. Herb. Pharm. Ther.*, 3: 77-93.
- Kumar, R.A., K. Sridevi, N.V. Kumar, S. Nanduri and S. Rajagopal, 2004. Anticancer and immunostimulatory compounds from *Andrographis paniculata*. *J. Ethnopharm.*, 92: 291-295.
- Legen, I., S. Zakelj and A. Kristl, 2003. Polarised transport of monocarboxylic acid type drugs across rat jejunum *in vitro*: The effect of mucolysis and ATP-depletion. *Int. J. Pharm.*, 256: 161-166.
- Lennernas, H., L. Knutson, T. Knutson, A. Hussain, L. Lesko and T. Salomonson *et al.*, 2002. The effect of amiloride on the *in vivo* effective transport of amoxicillin in human jejunum: Experience from a regional perfusion technique. *Eur. J. Pharm. Sci.*, 15: 271-277.
- Madav, S., S.K. Tandan, J. Lal and H.C. Tripathi, 1996. Anti-inflammatory activity of andrographolide. *Fitoterapia*, 67: 452-458.
- Melchior, J., S. Palm and G. Wikman, 1996. Controlled clinical study of standardized Andrographis paniculata extract in common cold a pilot trial. *Phytomedicine*, 3: 315-318.
- Melchior, J., A.A. Spasov, O.V. Ostrovskij, A.E. Bulanov and G. Wikman, 2000. Double-blind, placebo-controlled pilot and phase III study of activity of standardized *Andrographis paniculata* fixed combination Kan Jang in rats and human. *Phytomedicine*, 7: 341-364.
- Narumashi, K., I. Tamai, N. Inoue, H. Muraoka, Y. Sai, N. Suzuki and A. Tsuji, 2002. Involvement of multidrug resistance-associated protein 2 in intestinal secretion of grepafloxacin in rats. *Antimicrob. Agents Chemother.*, 46: 344-349.
- Netsch, M.I., H. Gutmann, S. Luescher, S. Brill, C.B. Schmidlin, M.H. Kreuter and J. Drewe, 2005. Inhibitory activity of a green tea extract and some of its constituents on multidrug resistance-associated protein 2 functionality. *Planta Med.*, 71: 135-141.
- Panchagnula, R., T. Bansal, M.V. Varma and C.L. Kaul, 2005. Co-treatment with grapefruit juice inhibits while chronic administration activates intestinal P-glycoprotein-mediated drug efflux. *Pharmazie*, 60: 922-927.
- Schachuevich, P., N. Viviana, T. Omar and R. Modesto, 2004. Evaluation of acetaminophen P-glycoprotein-mediated salivary secretion by rat submandibular glands. *Arch. Oral. Biol.*, 49: 895-901.
- Shannon, M.T., B.A. Wilson and C.L. Stang, 2001. Health Professional's Drug Guide. USA: Prentice-Hall Inc. 5, 68, 99, 300, 1010.
- Sharma, A., K. Lal and S.S. Handa, 1992. Standardization of Indian crude drug Kalmegh by high pressure liquid chromatographic determination of andrographolide. *Phytochem. Anal.*, 3: 129.
- Singh, Y.N., 2005. Potential for interaction of kava and St. John's wort with drugs. *Ethnopharmacology.*, 100: 108-113.
- Spasov, A.A., O.V. Ostrovskij, M.V. Chernikov and G. Wikman, 2004. Comparative controlled study of *Andrographis paniculata* fixed combination, Kan Jang and an Echinacea preparation as adjuvant, in the treatment of uncomplicated respiratory disease in children. *Phytother. Res.*, 18: 47-53.
- Sriram, R., R.A. Kumar, D.S. Deevi, C. Satyanarayana and R. Rajagopalan, 2003. Andrographolide, a potential cancer therapeutic agent isolated from *Andrographis paniculata*. *J. Exp. Ther. Onc.*, 3: 147.
- Suzuki, H. and Y. Sugiyama, 2002. Single nucleotide polymorphisms in multidrug resistance associated protein 2 (MRP2/ABCC2): Its impact on drug disposition. *Adv. Drug Deliv. Rev.*, 54: 1311-1331.

- Takano, M., R. Yumoto and T. Murakami, 2006. Expression and function of efflux drug transporters in the intestine. *Pharmacol. Therap.*, 109: 137-161.
- Terao, T., E. Hisanaga, Y. Sai, I. Tamai and A. Tsuji, 1996. Active secretion of drugs from the small intestinal epithelium in rats by P-glycoprotein functioning as an absorption barrier. *J. Pharm Pharmacol.*, 48: 1083-1089.
- Vaclavikova, R., A. Boumendjel, M. Ehrlichova, J. Kovar and I. Gut, 2006. Modulation of paclitaxel transport by flavonoid derivatives in human breast cancer cells. Is there a correlation between binding affinity to NBD of P-gp and modulation of transport? *Bioorg. Med. Chem.*, 14: 4519-4525.
- Wang, Y., J. Cao and S. Zeng, 2005. Involvement of P-glycoprotein in regulating cellular levels of Ginkgo flavonols: Quercetin, kaempferol and isorhamnetin. *J. Pharm. Pharmacol.*, 57: 751-758.
- Yumoto, R., T. Murakami, Y. Nakamoto, R. Hasegawa, J. Nagai and M. Takano, 1999. Transport of rhodamine 123, a P-glycoprotein substrate, across rat intestine and Caco-2 cell monolayers in the presence of cytochrome P-450 3A-related compounds. *J. Pharmacol. Exp. Ther.*, 289: 149-155.