

ผลของสารสกัดจากกวาวเครือขาว (*Pueraria candollei*) และ miroestrol ต่อการทำงานของ P-glycoprotein ในลำไส้หนู mice

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บทคัดย่อ

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ว. เกษตรศาสตร์อีสาน 2556; 9(2) : 90-98

Received : 6 January 2013

Accepted : 17 April 2013

บทนำ: กวาวเครือขาว *Pueraria candollei* Wall. ex Benth. var. *mirifica* (family Leguminosae) ได้ถูกนำมาใช้ประโยชน์ในทางแพทย์แผนไทยโดยจำหน่ายในรูปผลิตภัณฑ์สุขภาพอย่างกว้างขวาง โดยเน้นสรรพคุณด้านการชะลอวัย จึงเป็นที่น่าสนใจในการศึกษาผลของสารสกัด และสารสำคัญ miroestrol ที่ได้จากกวาวเครือขาวต่อการทำงานของ P-glycoprotein ซึ่งเป็น efflux transporter ที่สำคัญที่มีผลต่อเภสัชจลนศาสตร์ของยาหลายชนิด เพื่อเป็นข้อมูลการเกิดอันตรกิริยาระหว่างสมุนไพรกับยาในเชิงคลินิก **วัตถุประสงค์การศึกษา:** การศึกษานี้มีวัตถุประสงค์เพื่อทดสอบผลของสารสกัดจากกวาวเครือขาว และ miroestrol ต่อการทำงานของ P-glycoprotein **วัสดุและวิธีการทดลอง:** โดยพิจารณาจากผลของสารสกัดจากกวาวเครือขาว และ miroestrol ต่อการขนส่งสาร Rhodamine 123 ซึ่งเป็น substrate ของ P-glycoprotein ผ่านลำไส้หนู กลับด้าน ในหลอดทดลอง **ผลการศึกษา:** ผลการศึกษาพบว่าสารสกัดทั้งกวาวเครือขาว และ ส่วนสกัด ethyl acetate ของกวาวเครือขาว ไม่มีผลต่อการยับยั้งการทำงานของ P-glycoprotein ส่วน miroestrol ซึ่งเป็นสารสำคัญที่พบในกวาวเครือขาวและมีฤทธิ์ต่อเอสโตรเจนสูง สามารถยับยั้งการทำงานของ P-glycoprotein ในลำไส้หนูได้เล็กน้อย โดยพบว่ามีฤทธิ์ยับยั้ง P-glycoprotein ได้ที่ความเข้มข้นตั้งแต่ 100 μ M ขึ้นไป และเมื่อให้ miroestrol แก่หนูทดลองในขนาด 0.5 mg/kg/วัน ฉีดเข้าใต้ผิวหนัง (subcutaneous) เป็นเวลาติดต่อกัน 7 วัน พบว่าไม่มีการเปลี่ยนแปลงการแสดงออกของ ABCB1A และ ABCB1B mRNA **สรุปผล:** miroestrol และสารสกัดจากกวาวเครือขาวมีผลการต่อการทำงานของ P-glycoprotein น้อยมาก อย่างไรก็ตาม ควรมีการศึกษาเพิ่มเติมถึงผลของการบริโภคกวาวเครือขาวในระยะยาว และฤทธิ์ของสารสำคัญชนิดต่างๆ ที่พบในกวาวเครือขาวต่อการทำงานของ protein transporter ชนิดอื่นๆ ต่อไป

คำสำคัญ: *Pueraria candollei*, miroestrol, P-glycoprotein, efflux protein transporter

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Abstract

Effects of *Pueraria candollei* extracts and miroestrol on intestinal P-glycoprotein function in mice

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IJPS, 2013; 9(2) : 90-98

Introduction: *Pueraria candollei* Wall. ex Benth. var. *mirifica* (family Leguminosae), as known Kwao Kruea Khaw in Thai, has been widely used in Thai traditional medicine and is supplied in commercial health products for rejuvenation. It is interesting to investigate the potential effect of *P. candollei* and its component, miroestrol, on P-glycoprotein function to provide the important drug-herb interaction data for applying in clinical relevance. **Objective:** This present study is investigated the effect of *P. candollei* extract and miroestrol, the potent phytoestrogen found in *P. candollei* on P-glycoprotein, the efflux transporter, function. **Materials and Methods:** Determine effect of substances on P-glycoprotein function by employing the *in vitro* transport of rhodamine 123, a substrate of P-glycoprotein, across everted intestinal sac was studied in mice. The ability of *P. candollei* tincture and the *P. candollei* ethyl acetate extract fraction to inhibit p-glycoprotein function were examined. **Results:** *P. candollei* extract has no effect on P-glycoprotein function. Miroestrol showed negligible inhibitory effect on P-glycoprotein function at the concentration at 100 μ M and higher. For expression of P-glycoprotein, change in expression of ABCB1 and ABCB2 mRNAs in mice administered with miroestrol at 0.5 mg/kg/day subcutaneously for 7 days was not observed. **Conclusion:** Miroestrol, and *P. candollei* extracts have negligible effect on p-glycoprotein function. However, the effect of phytoestrogens contained in the tuberous root of *P. candollei* on P-glycoprotein and other protein transporters after long term of administration should be further investigated.

Keywords: *Pueraria candollei*, miroestrol, P-glycoprotein, efflux protein transporter

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1. Introduction

One of the membrane transporter superfamily having the ATP-binding cassette (ABC) with well-preserved homology of the site where ATP binds is P-glycoprotein (P-gp), about 170 kDa in size, that is well known as the most typical efflux pump in the cell membrane. (Takano *et al.*, 2006). P-glycoprotein involved in the transport of many substances including toxins from the liver, kidney, and gastrointestinal tract. P-glycoprotein and other efflux

transporters limit permeation of toxins and xenobiotics to vital structure, such as the brain, placenta and testis (Leonard *et al.*, 2003). Many *in vitro* and *in vivo* studies have demonstrated that P-glycoprotein plays a significant role in drug absorption and disposition, as its localization appears to have a greater impact on limiting cellular uptake of drugs from intestinal lumen into epithelial cells and from blood circulation into the brain than on enhancing the excretion of drugs out of hepatocytes and renal tu-

bules into the adjacent luminal space (Lin and Yamazaki, 2003). By the major role of this efflux transporter as described, limiting influx into and facilitating efflux from the enterocytes of their substrates, it could be able to serve as a determinant of oral bioavailability and intestinal efflux clearance for certain drugs, and also be related to drug-drug interactions, when multiple drugs that are substrates, inducers, or inhibitors for this transporter are administered together. In a clinical setting, interindividual and intraindividual variations and the modulation of expression and functional activity of p-glycoprotein intestinal efflux transporters are particularly important especially in patients who uses narrow therapeutic index drugs whose pharmacokinetic parameters would be affected.

Pueraria candollei Wall. ex Benth. var. *mirifica* (family Leguminosae), as known Kwao Kruea Khaw in Thai, has long been widely used in traditional medicine and supplied as commercial health products for rejuvenation. (Cherdshewasart and Sriwatcharakul, 2008). Miroestrol, one of the strong phytoestrogens (Chansakaow *et al.*, 2000) among many of phytoestrogens found in tuberous root of *P. candollei* first demonstrated its estrogenic activity by induction of mamrogenic effects in ovariectomized rats (Benson *et al.*, 1961). It has been postulated to produce effects on reproductive organs, such as vaginal cornification and increased uterine weight in ovariectomized rats (Malaivijitnond *et al.*, 2004), and the plant extract also increases the length of the follicular phase and total menstrual cycle and causes ovulation blockage in cynomolgus monkeys (Trisomboon *et al.*, 2005). Estrogenic activity of miroestrol was estimated to have 0.25 times the estrogenic activity of 17 β -estradiol using a vaginal cornification assay (Jones *et al.*, 1961).

Recently, the effects of this plant and its compounds on the drug metabolizing enzymes, CYP2B9 and CYP1A2 were reported (Udomsuk *et al.*, 2010). It is of interest investigate the potential effect of

P. candollei and its component, miroestrol, on P-glycoprotein function to provide important drug-herb interaction data for applying in clinical relevance. The objective of this present study is to investigate the effect of *P. candollei* extract and miroestrol on p-glycoprotein function by employing *in vitro* transport of rhodamine 123 across everted intestinal sac study and the effect on expression of ABCB1 and ABCB2 mRNAs by using semi-quantitative reverse transcription-polymerase chain reaction technique.

2. Materials and Methods

2.1 Materials

Miroestrol was isolated from tuberous roots of *P. candollei* var. *mirifica* as described previously (Chansakaow *et al.*, 2000) and NMR identification was performed and compared with authentic standards of miroestrol from Assoc. Prof. Dr. Chaiyo Chaichantipyuth, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. Rhodamine 123 and verapamil hydrochloride were supplied by Sigma-Aldrich (Germany). TRIZOL® reagent and dNTP mixture were supplied by Invitrogen® (Carlsbad, CA, USA). Random primers and RNase inhibitor were obtained from Takara Bio Inc. (Otsu, Shiga, Japan). Forward and reverse primers of *ABCB1A*, *ABCB1B*, and *GAPDH* genes were synthesized by Bio Basic, Inc. (Markham Ontario, Canada). Primer sequences were as follows; *ABCB1A* forward primer 5'-GCTTACAGCCAGCATTCTCC-3'; *ABCB1A* reverse primer 5'-CCAGCTCACATCCTGTCTCA-3'; *ABCB1B* forward primer 5'-ACTCGGGAGCAGAAGTTTGA-3'; *ABCB1B* reverse primer 5'-GCACCAAAGACAACAACAGCAGA-3'; *GAPDH* forward primer 5'-TCC ACT CAC GGC AAA TTC AAC G-3'; *GAPDH* reverse primer 5'-TAG ACT CCA CGA CAT ACT CAG C-5'. All others chemicals used for the experiments were of the highest available purity from commercial suppliers.

2.2 Preparation of *P. candollei* tincture extract

The fresh tuberous roots were washed, dried in an oven at 45°C until the weight remained constant, and then powdered. The extract was prepared as *P. candollei* tincture. Powdered tuberous root of *P. candollei* (2.5 g) was extracted with 40% (v/v) ethanol (62.5 mL) at room temperature for 7 days. Then, the solvent was evaporated in vacuo and the extract fraction was dissolved in DMSO to obtain *P. candollei* tincture extract fraction.

2.3 Animal Experiments

Experiments with animals, animal handling, and the treatment protocol were approved by the Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (Approval document No. AEKKU42/2552). Mice in this study were obtained from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. At all times, mice were housed on wood chip bedding in stainless-steel cages with water and commercial mouse diet supplied *ad libitum* and acclimated for at least 7 days in housing with a 12-h dark light cycle under controlled temperature (22±2°C) before performing experiments.

2.4 In vitro transport of rhodamine 123 across everted intestinal sac

To examine the effects of *P. candollei* extract and miroestrol on P-gp function, serosal-to-mucosal transport of a substrate across intestinal everted sac was examined, as described previously (Takano *et al.*, 2006; Yumoto *et al.*, 1999). Briefly, male C57BL/6 mice at 6 weeks of age were fasted overnight with free access to water before the experiments. The whole small intestine was flushed with 50 mL of ice-cold saline. The mice were exsanguinated, and the small intestine was isolated and a segment of jejunum was everted, and a 3-cm-long everted ileum sac was prepared. Rhodamine 123, a p-glycoprotein substrate, was prepared at a concentration of 5 µM in pH 7.4 isotonic Dulbecco's PBS containing 25 mM glucose and 0.4%

DMSO. The rhodamine 123 solution (0.3 mL) was introduced into the everted sac (serosal side), and the both ends of the sac were ligated tightly. The sac containing rhodamine 123 was immersed into 3 mL of PBS, prewarmed at 37°C and pre-oxygenated with 5% CO₂/95% O₂. The bubbling of CO₂/O₂ gas was continued throughout the efflux study. In an inhibition study, verapamil, a typical inhibitor for P-gp, was added in the mucosal medium at the final concentration of 300 µM for verapamil. Similarly, the test compounds, miroestrol, *P. candollei* tincture, or *P. candollei* ethyl acetate extract were added in the mucosal medium at various final concentrations. The efflux of rhodamine 123 across everted mice intestine following application to the serosal side was measured by sampling 250 µL of the mucosal medium periodically for 90 min and equal volume of fresh mucosal medium was re-supplied each time. After sampling, 250 µL acetonitrile (ACN) was added into samples, and then centrifuged at 10,000 rpm for 5 min. Concentrations of rhodamine 123 in samples were determined by using a microplate fluorometer (Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 485 nm and an emission wavelength of 538 nm because no metabolism of rhodamine 123 was observed under the present experimental conditions.

2.5 Semi-quantitative reverse transcription-polymerase chain reaction

Male C57BL/6 mice at 6 weeks of age were used in this study. Mice were subcutaneously administered with miroestrol in corn oil at a dose of 0.5 mg/kg/day once a day for 7 days. The control group was subcutaneously administered with corn oil daily for 7 days. The mice were decapitated 24 h after the last treatment. Testes were immediately excised for preparing total RNAs as described elsewhere (Udomsuk *et al.*, 2010).

Mouse ABCB1A, ABCB1B, and GAPDH mRNAs were semi-quantified by RT-PCR. Testicular total RNA was reverse-transcribed using ReverTraAce

reverse transcriptase (Toyobo Co., Ltd.), then cDNA was amplified under the conditions recommended by the supplier of Illustra Hot Start Master Mix (GE Healthcare, UK). The conditions of PCR cycle of ABCB1A and ABCB1B were 95°C 3 min; 39 cycles of 95°C 30 sec, 56°C 30 sec, 72°C 1 min; 72°C 3 min. The condition of GAPDH was 95°C 4 min; 32 cycles of 95°C 30 sec, 56°C 30 sec, 72°C 1 min; 72°C 5 min. After separation of the PCR products by 2% agarose gel electrophoresis, the target cDNA were detected under ultraviolet light in the presence of ethidium bromide and semi-quantified by Syngene gel documentation (Ingenius L, Cambridge, UK) and the GeneTools match program.

2.6 Data analysis

Statistically significant differences were determined by Student's t-test, or one way analysis of variance (ANOVA) with the Tukey's pos hoc or the Scheffe's test for post-hoc analysis. A difference of P value less than 0.05 was considered statistically significant.

3. Results

3.1 Effect of *P. candollei* tincture extract on rhodamine 123 transport across everted mice ileum in vitro To test the effect of *P. candollei* tincture extract on transport of rhodamine 123 across everted ileum, *P. candollei* tincture extract was prepared to a final concentration of 30% of the original tincture preparation in mucosal medium. As shown in Figure 1, an inhibitory effect by 30% *P. candollei* tincture extract in the transport of rhodamine 123 across everted mice ileum from serosal to mucosal surface was observed but there was no statistically significant difference between the treatment group and the control group. The inhibitory potencies of both verapamil and 30% *P. candollei* tincture on rhodamine 123 transport were compared. It was revealed that the inhibitory effect of 30% *P. candollei* tincture was less potent than that of 300 µM verapamil, a typical P-gp inhibitor (22.7% and 72.7% inhibition in comparison with control, respectively).

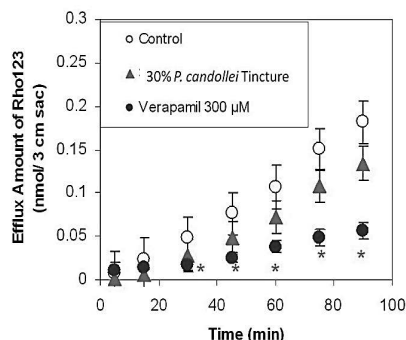


Figure 1. Transport of rhodamine 123 from serosal to mucosal surfaces across the everted ileum in the absence (open circle, control) or presence of *P. candollei* tincture (closed triangle, 30% of the original tincture) and verapamil (closed circle, 300 µM). The concentration of rhodamine 123 in the serosal side was 5 µM. Each value represents mean ± SE (n=6), * P<0.05, significantly different from control.

3.2 Effect of *P. candollei* ethyl acetate extract on rhodamine 123 transport across everted mice ileum in vitro

As shown in Figure 2, it was revealed that the inhibitory effect on P-glycoprotein function of 30% ethyl acetate extract of *P. candollei* seemed to be higher than 30% *P. candollei* tincture extract. The inhibitory potency in the transportation of rhodamine 123 across everted mice ileum were around 31.8% (compared to control), however, it could not be shown to be statistically significant (P>0.05).

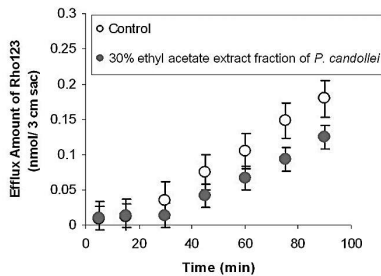


Figure 2. Transport of rhodamine 123 from serosal to mucosal surfaces across the everted ileum in the absence (open circle, control) or presence of *P. candollei* ethyl acetate extract (closed circle, 30% of the original extract fraction). The concentration of rhodamine 123 in the serosal side was 5 μ M. Each value represents mean \pm SE (n=6), * P<0.05, significantly different from control.

3.3 Effect of Miroestrol on rhodamine 123 transport across everted mice ileum in vitro

The effects of miroestrol, the strong phytoestrogen from tuberous roots of *P. candollei*, on p-glycoprotein function were investigated. Efflux of rhodamine 123 transport across ileum were inhibited 31.8, 18.2, and 0% by 500, 100, and 30 μ M of miroestrol, respectively. The inhibitory effects of miroestrol were statistically significant (P<0.05) at the concentration of 100 μ M or higher as shown in Figure 3 (A, B, C).

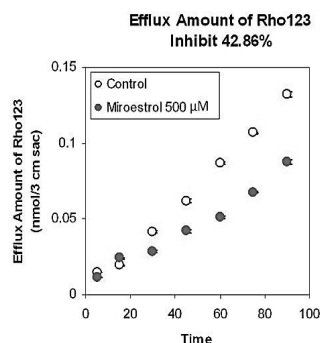


Figure 3A

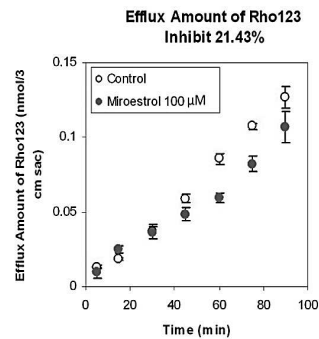


Figure 3 B

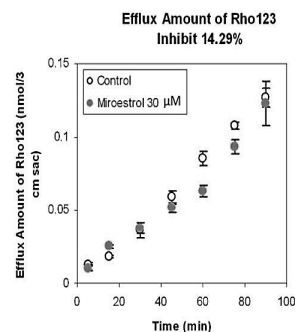


Figure 3C

Figure 3. Transport of rhodamine 123 from serosal to mucosal surfaces across the everted mice ileum in the absence (open circle, control) or presence of miroestrol (closed circle) at concentrations of 30, 100 and 500 μ M as presented in Figure 3A, 3B, and 3C, respectively. The concentration of rhodamine 123 in the serosal side was 5 μ M. Each value represents mean \pm SE (n=6), * P<0.05, significantly different from control.

3.4 Expression of ABCB1 mRNA in mice administered miroestrol

Expression of ABCB1A and ABCB1B mRNA in mice, that were administered 0.5 mg/kg/day of miroestrol by subcutaneously administration for 7 days, was analyzed by semi-quantitative reverse transcription-polymerase chain reaction. When compared to the

control group, only a small upregulated ABCB1A and ABCB1B mRNA expression was observed in the test group, there were no statistical significant changes of ABCB1A and ABCB1B mRNA expression (Figure 4).

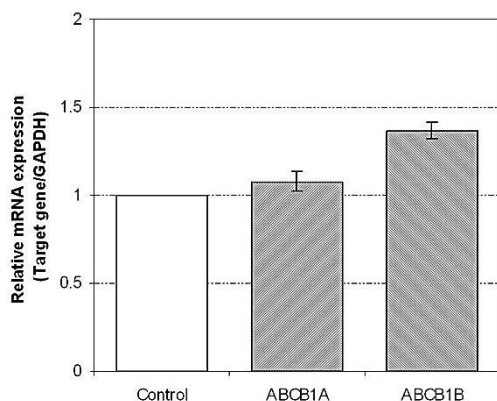


Figure 4. Expression of ABCB1A and ABCB1B mRNAs in mice exposed to miroestrol by semi-quantitative reverse transcription-polymerase chain reaction. The dose of miroestrol was 0.5 mg/kg/day subcutaneously administration for 7 days. Each value represents mean \pm SD, * $P < 0.05$, significantly different from control.

4. Discussion and Conclusion

The tuberous root of *P. candollei*, known as “Kwao Kruer Khaow”, the rejuvenating herb, has often been used in Thai traditional medicine. Because of the wide use of phytoestrogens in many aspects of healthcare, they have recently gained much attention and can decrease the incidence of estrogen related cancer and cardiovascular disease, and even climacteric symptoms (Albertazzi *et al.*, 1999). However, the scientific evidence for clinical usefulness of this plant is still limited. From this reason, the drug-herb interaction should be of concern for safety in concurrent use with other drugs. In this study, the effects of *P. candollei* on P-glycoprotein function, an important efflux protein transporter, was

investigated by employing *in vitro* transport study using rhodamine 123 transport across mice intestine.

From our results in Figure 1., verapamil, a well characterized P-gp inhibitor, significantly inhibited 72.7% transport of rhodamine 123, p-glycoprotein substrate, across everted rat ileum, similar to that was similar to that reported by Yumoto *et al.* (1999). This revealed that the expression of P-glycoprotein in mice intestine has been functionally confirmed.

P. candollei tincture was prepared by immersing the tuberous root powder in 40% ethanol for 7 days. The extract of *P. candollei* tincture inhibited the transport of rhodamine 123 across everted rat ileum by only 22.7% without significant difference from control. It can be assumed that the tincture of *P. candollei* has negligible or no effect on p-glycoprotein function in case of concurrent administration with drug that are substrates of p-glycoprotein, such as digoxin and cyclosporine, etc.

From data shown in Figure 2 and Figure 3, the ethyl acetate extract fraction had slightly more inhibitory effect on p-glycoprotein function, than that of *P. candollei* tincture (31.8% vs 22.7%).

Among phytoestrogens found in *P. candollei* ethyl acetate extract fraction (Chansakaow *et al.*, 2000), genistein and daidzein, have been reported for their ability to inhibit p-glycoprotein-mediated transport (Limtrakul *et al.*, 2005; Branda *et al.* 2006), we next examined the effect of this extract fraction on rhodamine 123 transport across everted mice ileum. Miroestrol, a potent phytoestrogen, also found in highest yield in the ethyl acetate extract fraction, are of interest to examine the effect of miroestrol on p-glycoprotein function. From the experiment we found that the minimum concentration required for miroestrol to produce a significant modulation of p-glycoprotein activity appeared to be, in general, 100 μ M or higher (Figure 3).

In humans, two members of the P-glycoprotein gene family, *ABCB1* and *ABCB3* (*MDR1* and *MDR3*)

exist, while three members of this family, *abcb1a*, *abcb1b*, and *abcb2* (*mdr1a*, *mdr1b* and *mdr2*), are found in mice (Gottesman and Pastan, 1993; Schinke, 1997). The P-glycoprotein encoded by the human *MDR1* and mouse *mdr1a/1b* genes functions as a drug efflux transporter, whereas human *MDR3* P-glycoprotein and mouse *mdr2* P-glycoprotein are believed to be functional in phospholipid transport (van Helvoort *et al.*, 1996; Ruetz and Gros, 1994). To determine the effect of miroestrol on expression of p-glycoprotein in the intestine, we employed the semi-quantitative reverse transcription-polymerase chain reaction of ABCB1A and ABCB1B mRNAs. From our experiment, it was revealed that there was no significant change in expression of either ABCB1A and ABCB1B mRNA after administration of miroestrol at the dose of 0.5 mg/kg/day by subcutaneously administration for 7 days in mice. Competitive inhibition might be suspected to be the mechanism of miroestrol in inhibition of p-glycoprotein function.

As reviewed by Lin and Yamazaki. (2003), the potential risk of P-glycoprotein-mediated drug interactions may be greatly underestimated if only plasma concentration is monitored. From animal studies, it is clear that P-glycoprotein inhibition always has a much greater impact on tissue distribution, particularly with regard to the brain, than on plasma concentrations. Therefore, the potential risk of P-glycoprotein mediated drug interactions should be assessed carefully. According to the transport data from this present study, and considering together with assuming human intestinal volume of 1.65 L/70 kg (Davies *et al.*, 1993), for a single oral dose, it would need an intake around 60 mg of miroestrol to achieve the minimum effective concentration (100 μ M) that can interact with intestinal p-glycoprotein. From our survey (data not shown), six of the various commercial products of *P. candollei* available in Thailand contain around 150-800 mg of *P. candollei* dried powder per capsule contains around 0.2-1 μ g miroestrol per capsule, so the concentration

of miroestrol in the intestine after a single oral dose is estimated to be only 0.34 -1.6 nM.

In conclusion, these findings indicate that miroestrol, the strong phytoestrogen contained in tuberous root of *P. candollei* has negligible effect on p-glycoprotein function and has no effect on expression of ABCB1 and ABCB2 mRNAs in mice. The minimum p-glycoprotein inhibitory effect concentration is 100 μ M, therefore, systemic inhibition of P-glycoprotein by miroestrol may be insignificant after regular supplement of commercial product of *P. candollei*. However, interaction could occur in case of administration of extremely high dose of miroestrol, and especially with intravenous administration. Further investigation concerning the long-term safety effect of using commercial product of *P. candollei* and miroestrol, and also, the effect on other efflux protein transporters should be performed.

Acknowledgements

The support in part by a research grant Khon Kaen University (542104), and Faculty of Pharmaceutical Sciences, Khon Kaen University are gratefully acknowledged.

References

- Albertazzi P, Pansini F, Bottazzi M, Bonaccorsi G, De Aloysio D, Morton MS. Dietary soy supplementation and phytoestrogen levels. *Obstet Gynecol* 1999;94:229-31.
- Benson GK, Cowie AT, Hosking ZD. Mammogenic activity of miroestrol. *J Endocrinol* 1961;21: 354-60.
- Branda W, Schutteb ME, Williamsa G, *et al.* Rietjensb IMCM. Flavonoid-mediated inhibition of intestinal ABC transporters may affect the oral bioavailability of drugs, food-borne toxic compounds and bioactive ingredients. *Biomedicine* 2006; 60:508-19.

- Chansakaow S, Ishikawa T, Seki H, *et al.* Identification of Deoxymiroestrol as the actual rejuvenating principle of “Kwao Keur”, *Pueraria mirifica*. The known miroestrol may be an artifact. *J Nat Prod* 2000;63(2):173-5.
- Cherdshewasart W, Sriwatcharakul S. Metabolic activation promotes estrogenic activity of the phytoestrogen-rich plant. *Maturitas* 2008; 59:128-36.
- Davies B, Morris T. Physiological parameters in laboratory animal and humans. *Pharm Res* 1993;10:10:1093-5.
- Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* 1993; 62: 385-427
- Jones HEH, Waynforth HP, Pope GS. The effect of miroestrol on vaginal cornification, pituitary function and pregnancy in the rat. *J Endocrinol* 1961;22:293-302.
- Leonard GD, Fojo T, Bates SE0. The role of ABC transporters in clinical practice. *The oncologist* 2003; 8:411-24.
- Limtrakul P, Khantamat O, Pintha K. Inhibition of P-glycoprotein function and expression by kaempferol and quercetin. *J Chemother.* 2005 Feb;17(1):86-95.
- Lin JH, Yamazaki M. Role of p-glycoprotein in pharmacokinetics clinical implications. *Clin Pharmacokinet* 2003; 42 (1): 59-98.
- Malaivijitnond S, Kiatthaipipat K, Cherdshewasart W, Watanabe G, Taya K. Different effects of *Pueraria mirifica*, a herb containing phytoestrogens, on LH and FSH secretion in gonadectomized female and male rats. *J Pharm Sci* 2004;96:428-35.
- Ruetz S, Gros P. Phosphatidylcholine translocase: a physiological role for the *mdr 2* gene. *Cell* 1994; 77: 1071-81
- Schinkel AH. The physiological function of drug-transporting P- glycoproteins. *Cancer Biol* 1997; 8: 161-70
- Takano M, Yumoto R, Murakami T. Expression and function of efflux drug transporters in the intestine. *Pharmacol Ther* 2006;109:137–61.
- Trisomboon H, Malaivijitnond S, Watanabe G, Taya K. Ovulation block by *Pueraria mirifica*: a study of its endocrinological effect in female monkeys. *Endocrine* 2005;26:33-40.
- Udomsuk L, Churikawit K, Putalun W, Jarukamjorn K. Impact of *Pueraria candollei* root cultures on cytochrome P450 2B9 enzyme and lipid peroxidation in mice. *J Health Sci* 2010;56(2):182-7.
- van Helvoort A, Smith AJ, Sprong H, *et al.*. MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. *Cell* 1996; 87: 507-17
- Yumoto R, Murakami T, Yuko Nakamoto, Hasegawa R, Nagai J, Mikihiisa Takano. 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. *JPET* 1999; 289:149 –55.