การยับยั้งการทำงานของตัวขนส่งสารอินทรีย์ประจุบวกชนิด 2 โดยสารจากพืชกลึงกล่อม

Uptake Inhibition of Renal Organic Cation Transporter 2 by Compounds from *Polyalthia suberosa*

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บทคัดย่อ

ตัวขนส่งสารอินทรีย์ประจุบวกชนิด 2 (Oct2) มีความสำคัญต่อการกำจัดยาที่มีประจุบวกทางไต การยับยั้งการทำงานของตัวขนส่งนี้จึงมีผลต่อระดับยาประจุบวกในเลือด การวิจัยนี้มีจุดประสงค์เพื่อศึกษาสาร 7 ชนิดที่สกัดจากพืชกลึงกล่อมว่ามีผลหรือไม่ต่อการทำงานของตัวขนส่งสารอินทรีย์ประจุบวกชนิด Oct2 ซึ่งทำหน้าที่หลักในการขับยาที่มีประจุบวกออกจากร่างกาย จากการศึกษาการทำงานของ Oct2 ในเซลล์ CHO-K1ที่มีการแสดงออกเฉพาะตัวขนส่งสารอินทรีย์ประจุบวก Oct2 พบว่า สาร PT-400-SS, PT-402-SS และ PT-405-SS ยับยั้งการขนส่งสาร [^{3}H]-MPP^+ ซึ่งเป็นดัชนีบ่งบอกการทำงานของตัวขนส่ง Oct2 ขณะที่สารอื่นที่พบในกลึงกล่อมไม่มีผลต่อการยับยั้งทำงานของตัวขนส่ง Oct2 การยับยั้งการทำงานของตัวขนส่ง Oct2 ของสารทั้ง 3 ชนิด ไม่ได้เกิดจากความเป็นพิษต่อเซลล์ ผลการศึกษาพบว่าสาร PT-400-SS มีความแรงในการยับยั้งการทำงานของตัวขนส่ง Oct2 สูงสุดโดยค่า IC_{50}ของสาร PT-400-SS, PT-402-SS และ PT-405-SS ในการยับยั้งตัวขนส่ง Oct2 มีค่า 13.8±2.3 µM, 59.8±7.1 µM และ 23.4±4.5 µM ตามลำดับ ข้อมูลที่ได้จากการวิจัยนี้บ่งบอกว่าสารทั้ง 3 ชนิดนี้อาจมีผลเปลี่ยนแปลงการทำงานของตัวขนส่ง Oct2 และส่งผลต่อระดับในเลือดของยาประจุบวกได้

คำสำคัญ: *ตัวขนส่งสารอินทรีย์ประจุบวกชนิด 2, ไต, Polyalthia suberosa*

Abstract

Organic cation transporter 2 (Oct2) plays an important role in renal excretion of cationic drugs. Inhibition of this transporter influences the plasma level of the drugs. The objective of this study is to determine compounds which are isolated from *Polyalthia suberosa* (Klueng Klom) and influence the transport function of Oct2. The

question was addressed using Chinese hamster ovarian cell (CHO-K1), stably transfected with rabbit Oct2 (rbOct2-CHO-K1). The results showed that three compounds of the plant including PT-400-SS, PT-402-SS, and PT-405-SS inhibited Oct2-mediated [^{3}H]-MPP^{+} uptake whereas others produced no effects. The half maximal inhibitory concentration (IC_{50}) of PT-400-SS, PT-402-SS, and PT-405-SS were 13.8±2.3 µM, 59.8±7.1 µM and 23.4±4.5 µM, respectively. These data suggest that these compounds may alter renal cationic drug clearances, which affect plasma concentration of cationic drugs.

Keywords: *Organic cation transporters2, kidney, Polyalthia suberosa*

1. Introduction

Renal organic cation transporter 2 (Oct2) plays a crucial role in tubular secretion of a wide range of endogenous organic cations and many therapeutic cationic drugs (Wright and Dantzler, 2004; Wright, 2005; Motohashi et al, 2002) Therefore, this transporter is responsible for elimination of drugs from systemic circulation, and thus it is an important determinant of drugs efficacy and toxicity. The process of cationic drug secretion in kidney requires the uptake of cationic drug from the blood circulation into the renal proximal tubular epithelial cells via Oct2, which is driven by an inside-negative membrane potential (Okuda Urakami, Saito & Inui, 1999). The transport of cationic drug across the apical membrane into the tubular fluid is mediated largely by electroneutral H^{+}/organic cation exchanger, multidrug and toxin extrusion 1 (Mate1), Mate2 and/or Ocnt1 (Otsuka et al, 2005).

Polyalthia suberosa (Roxb.) Thwaites, family Annonaceae, or Klueng Klom is a shrubby tree found in Southeast Asia. The compounds identified are a coumarin, triterpenes, sterols, alkaloids and a nitrogen heterocyclic compound (Tuchinda, Pohmkotr, Munyoo & Reutrakul, 2000). The compounds from various parts of *P. suberosa* including leaves, stems and bark have been isolated and identified as shown in figure 1. Interestingly, investigation of the stems and leaves of the south China species has resulted in the discovery of a new triterpene, suberosol, as an anti-HIV (Li et al, 1993) and N-trans-Feruloyltyramine has been suggested to be a potent anti-oxidant and prevents against amyloid peptide-induced neurotoxicity in rat cultured cortical neurons (Thangnipon et al, 2012). The pharmacological effects of compounds from traditional plants were reported and will be used as alternative medicine. Clinical studies and case reports have identified a number of drug interactions potentiated by the concurrent use of herbal medicines with prescription drugs (Hu et al, 2005; Tzzo & Ernst, 2001). Since Oct2 plays a crucial role in elimination of cationic drug, therefore, alterations in transport activity of Oct2 can affect cationic drugs secretion, which thereby affects pharmacokinetics and therapeutic efficacies of drugs. The Study to determine whether the compounds from Klueng Klom influence the transport activity of Oct2.

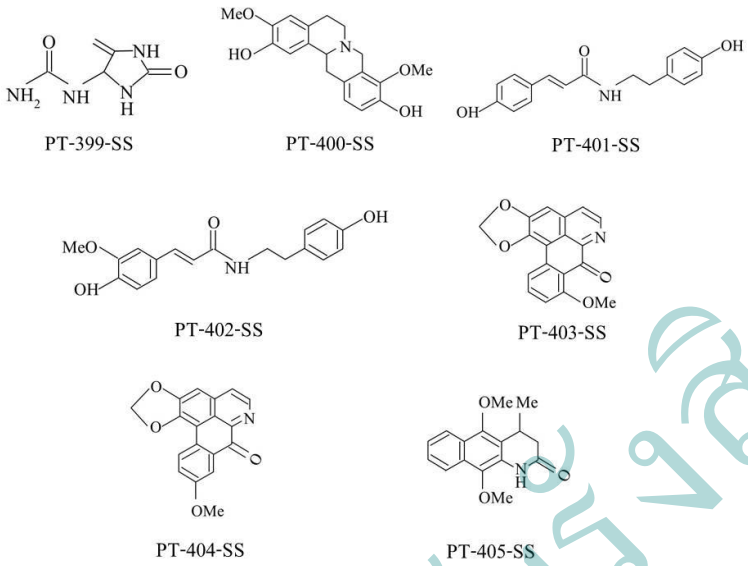

Figure 1 Compounds from *Polyalthia suberosa* (Klueng Klom)

2. Objective

The present study aims to investigate the effect of compounds isolated from Klueng Klom on Oct2 transport function.

3. Material and Method

3.1 Chemicals

Radiolabelled methyl 4-phenylpyridinium acetate, [^{3}H]-MPP$^+$ was obtained from American Radiolabeled Chemical Inc. Compounds from Klueng Klom were obtained from Department of Chemistry, Faculty of Science, Mahidol University. Other chemicals were obtained from various sources with highest purity available.

3.2 Cell culture

Chinese hamster ovarian (CHO-K1) stably transfected with rabbit Oct2 (rbOct2-CHO-K1) was used to study the inhibition of compounds with Oct2. rbOct2-CHO-K1 cells were cultured in F12 HAM medium supplemented with 10% fetal bovine serum, 100 units/ml of penicillin and 100 µg/ml of streptomycin, and were humidified incubator under 5% CO_2/95% air at 37°C.

Inhibition study

The inhibition uptake study was performed as previously described (Soodvilai, Nantavishit, Muanprasat & Chatsudthipong, 2011). The rbOct2-CHO-K1 cells were seeded in 24-well plate and maintained in culture at 37ºC under a humidified 5% CO_2 95% air atmosphere until become confluence. Then, they were washed 3 times with Waymouth's Buffer (WB) [WB; (in mM) 135 NaCl, 13 HEPES, 28 D-glucose, 5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 0.8 $MgSO_4$, pH adjusted to 7.4 with NaOH] and then incubated for 15-30 min in WB. Cells were incubated with WB

containing [^{3}H]-MPP$^+$ (10 nM), a prototypic substrate of Oct2, at 37ºC for 1 min uptake in control group and combination of [^{3}H]-MPP$^+$ and 100 µM of the compound for 1 min in treated groups. The uptake was stopped by removing the transport buffer and washed 3 times with the addition of ice-cold WB. Then, the cells were solubilized overnight by adding with 10% SDS in 0.4 N NaOH, neutralized with 0.4N HCl. The accumulated radioactivity was determined by liquid scintillation counting. The transport of [^{3}H]-MPP$^+$ was calculated as mole/min/cm^2 of the confluent monolayer surface.

3.3 Measurement of inhibitory potency

The half maximal inhibitory concentration (IC_{50}) of compounds for Oct2-mediated MPP$^+$ uptake was determined by incubating the cells with WB containing radioactive medium (10 nM of [^{3}H]-MPP$^+$) plus varying concentrations of the compound for 1 min. The accumulation of [^{3}H]-MPP$^+$ in the cells was calculated as mole/min/cm^2 of the confluent monolayer surface and expressed as percent of control (no compound). The IC_{50} values were estimated using nonlinear regression analysis with GraphPad Prism.

3.4 Cell viability assay

Cell viability was evaluated by using MTT assay. In brief, rbOct2-CHO-K1 cells were seeded in 96-well plate until become confluence in a humidity 5% CO_2 incubator and subsequently incubated with MTT reagent (5 mg/ml) at 37ºC for 4 h. At the end of incubation period, the medium was replaced with 150 µl/well DMSO followed by measurement of absorbance at 540 nm. by a spectrophotometer. Data were calculated as percent viability compared to that of vehicle treatment.

3.5 Statistical analysis

The data were expressed as mean±S.E. and analyzed using one-way analysis of variance (ANOVA) followed by Newman–Keuls post-test for all other studies. The significance of statistical tests was concerned with P value < 0.05.

4. Results

Effect of compounds from Klueng Klom on rbOct2-mediated [^{3}H]-MPP$^+$ uptake.

We first investigated the effect of the compounds isolated from Klueng Klom on Oct2-mediated [^{3}H]-MPP$^+$ uptake in the Oct2 expressing CHO-K1 cells. Cells were incubated with transport buffer containing [^{3}H]-MPP$^+$ alone (control) or in the presence of 100 µM of the compounds for 1 min. As shown in figure 2, incubating the cells to PT-400-SS, PT-402-SS, and PT-405-SS led to significant inhibition of [^{3}H]-MPP$^+$ uptake compared to control. In contrast, PT-399-SS, PT-401-SS, PT-403-SS, and PT-403-SS produced no effect.

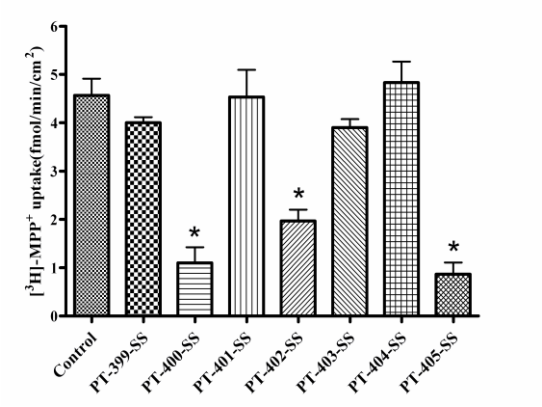

Figure 2 Inhibitory effect compounds from Klueng Klom on [^{3}H]-MPP$^+$ uptake in the Oct2 expressing CHO-K1 cells. The cells were incubated with [^{3}H]-MPP$^+$ alone or in the presence of 100 µM compounds for 1 min. Each data point represents the mean±S.E. of uptake measured from 3 independent experiments, expressed as fmol/min/cm^2. *P < 0.05 compared to control.

Inhibitory potency of compounds on rbOCT2-mediated [^{3}H]-MPP$^+$ uptake

The potency of the compounds (PT-400-SS, PT-402-SS, and PT-405-SS) to inhibit Oct2-mediated [^{3}H]-MPP$^+$ uptake was examined in rbOct2-CHO-K1 cells. As shown in figure 3, compound PT-400-SS, PT-402-SS, and PT-405-SS inhibited rbOct2-mediated [^{3}H]-MPP$^+$ uptake in a dose-dependent manner. The IC_{50} of PT-400-SS, PT-402-SS, and PT-405-SS for rbOct2 were 13.8±2.3 µM, 59.8±7.1 µM and 23.4±4.5 µM, respectively.

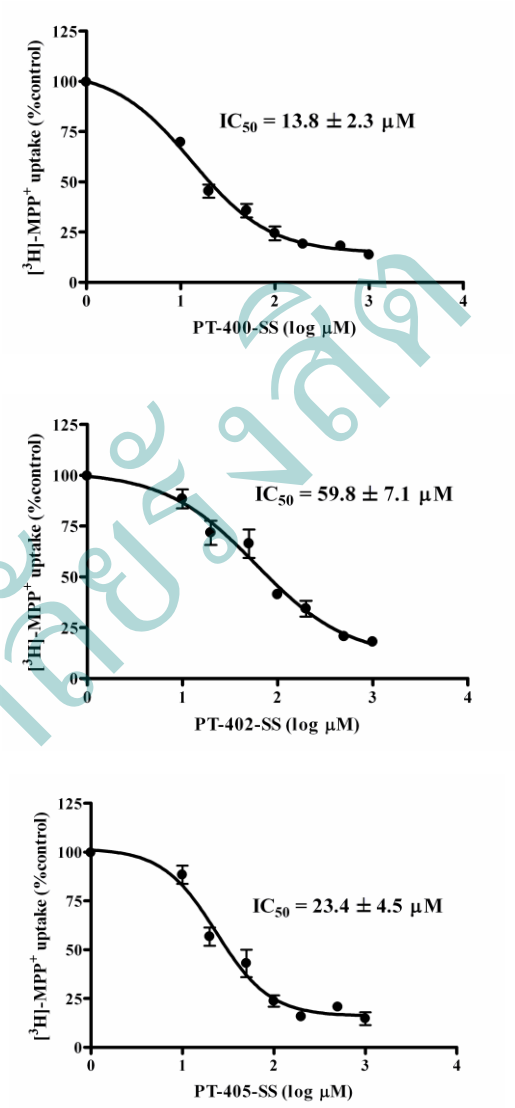

Figure 3 IC_{50} of the compounds on rbOct2-mediated [^{3}H]-MPP$^+$ uptakes in CHO-K1 cells. CHO-K1 cells singly expressed rbOct2 were incubated with [^{3}H]-MPP$^+$ alone, or in the presence of various concentrations of PT-400-SS, PT-402-SS, and PT-405-SS for 1 min and then radioactivity accumulation was determined. The IC_{50} values were calculated from uptake measured in triplicate from 3 independent experiments and expressed as percent of control uptake.

Acute toxicity of PT-400-SS, PT-402-SS, and PT-405-SS in rbOct2-CHO-K1 cells

To determine whether the inhibitory effects of PT-400-SS, PT-402-SS, and PT-405-SS were resulted from their toxicity, the rbOct2-CHO-K1 cells were incubated with PT-400-SS, PT-402-SS, and PT-405-SS at 100 µM for 30 min followed by measurement of cell viability using MTT assay. The results showed that there was no cytotoxicity effect of PT-400-SS, PT-402-SS, and PT-405-SS on the rbOct2-CHO-K1 cells (figure 4). These data indicated that the inhibitory effects of PT-400-SS, PT-402-SS, and PT-405-SS on Oct2-mediated [^{3}H]-MPP$^+$ uptake were not the result of toxicity.

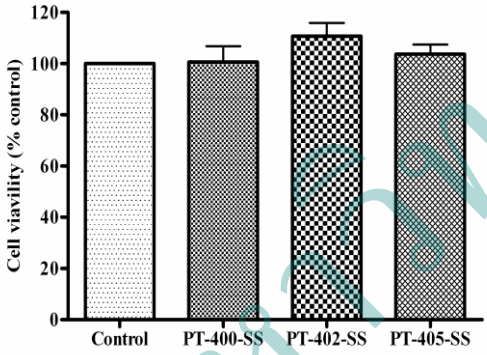

Figure 4 Effect of compounds on cell viability of rbOct2-CHO-K1 cells. Cells were incubated with vehicle 100 µM compounds for 30 min. Cell viability was determined using MTT assay. Data are presented as percent viability compared to control. Each data point represents mean±S.E. from 3 independent experiments.

5. Discussion

Among OCT family, the Oct2 localized at basolateral membrane of renal proximal tubular cell has the highest expression levels in the kidney (Motohashi et al, 2002). Therefore, alterations in functions and/or expression levels of Oct2 transporter can affect tubular secretion of organic cationic drugs, which thereby affects cationic drug pharmacokinetics and therapeutic efficacies. The present study investigated the effect of seven compounds isolated from Klueng Klom with on transport function of renal Oct2. We found that PT-400-SS, PT-402-SS, and PT-405-SS significantly inhibited transport function of Oct2. In addition, PT-400-SS showed higher inhibitory potency than others as shown by the IC_{50} values. The structure function relationship data are required to explain the different degree of the inhibitions. The inhibitory potency of these compounds were higher than that of tetaethylammonium (TEA) and cimetidine that known as the prototypic substrate of Oct2 (Harper and Wright, 2013).

Since the cytotoxicity effects of PT-400-SS, PT-402-SS, and PT-405-SS were not found, therefore, the inhibitory effects of these compounds were not mediated by the compounds-induced toxicity. The inhibition of the compounds may have occurred as follow; binding with the compounds immobilized the transporters thus inhibiting the transport activity with itself not entering the cells or; the compounds were competitively transported into the cell with [^{3}H]-MPP$^+$ (Soodvilai, Muanprasat, Chatsudthipong & Soodvilai, 2013). This notion needs to be further investigated. The Oct2 is a major determinant of cationic drug pharmacokinetics (Wright and Dantzler, 2004) inhibition of Oct activity by PT-400-SS, PT-402-SS, and PT-405-SS could decrease body clearance of

therapeutic cationic drugs lead to increase the plasma level of the cationic drugs that are eliminated by Oct2.

6. Conclusion

The present study aims to investigate the potential influence of compounds from Klueng Klom on transport function of Oct2. The effect of between compounds on Oct2 was determined using inhibition study in Oct2 expressing CHO-K1 cells. Among seven compounds isolated from the plant, PT-400-SS, PT-402-SS, and PT-405-SS inhibited transport activity of Oct2. This study provides the information that these compounds may influence the pharmacokinetic profile of drugs. The impact of these compounds on pharmacokinetic data in vivo study is important to be obtained in the future.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

7. Acknowledgements

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