

Interaction of Compounds Isolated from *Boesenbergia rotunda* with Human Renal Organic Anion and Cation Transporters

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Abstract

Boesenbergia rotunda extract (BPE) is used as a dietary supplement and therapeutic purposes, such as antioxidant activity, antibacterial, anticancer, and antiobesity. Therefore, co-use of this herb and therapeutic drug possibly causes herb-drug interaction and leads to altering drug efficiency and toxicity. Organic anion transporters (OAT1 and OAT3) and organic cation transporters (OCT2) play an importance role in renal excretion of anionic and cationic drugs. Inhibition of these transporters influences the plasma level of the drugs. Thus, the study was set up to determine the interaction of the extract and pure compounds isolated from *B. rotunda* with OAT1, OAT3 and OCT2 in human renal proximal tubular cells (RPTEC/TERT1) by measuring the uptake of ^3H -PAH, ^3H -ES and ^3H -MPP⁺ which is a prototypical substrate of OAT1, OAT3 and OCT2, respectively. The results showed that BPE did not inhibit OAT1-mediated ^3H -PAH uptake and OAT3-mediated ^3H -ES uptake. BPE inhibited OCT2-mediated ^3H -MPP⁺ uptake with a half maximal inhibitory concentration (IC₅₀) of 44.17 ± 0.09 $\mu\text{g}/\text{ml}$. The effect of major compounds isolated from *B. rotunda*, panduratin A, pinocembrin and pinostrobin, on OCT2 function was determined. Pinocembrin and pinostrobin but not panduratin A inhibited OCT2-mediated ^3H -MPP⁺ uptake with an IC₅₀ of 33.50 ± 0.16 μM . These data suggest that the use of *B. rotunda* as dietary supplement or for therapeutic purposes may cause herb-drug interaction and subsequently alter renal cationic drug clearance.

J Physiol Biomed Sci. 2017; 30(2): 52-6

Keywords: *Boesenbergia rotunda*, drug interaction, organic anion transporters, organic cation transporters, renal basolateral transporter.

Introduction

Use of herbal medicinal products has been rapidly increased over decades due to a hope to cure and prevent diseases. However, co-use of herbs and drugs might cause drug interactions and alter the drug's pharmacokinetics leading to unexpected adverse effects.¹ The kidney is a major organ responsible for drug excretion and determines the pharmacokinetics of drugs. The excretion of drugs by the kidney is mediated by several membrane transporters.² The major transporters involved in tubular secretion of anionic drugs are organic anion transporters 1 and 3 (OAT1 and OAT3). Whereas, the major transporter engaged in secretion of cationic drugs is organic cation transporters 2 (OCT2). The substrates of OAT1 and OAT3 are small negatively charged substance such as NSAIDs, penicillin;³ small cationics such as cisplatin, metformin, are substrate

of OCT2.⁴ It has been reported that co-administration of dolutegravir (strong OCT2 inhibitor) with metformin (OCT2 substrate) increases plasma concentration of metformin, suggesting that dolutegravir enhances metformin activity together with reducing renal accumulation and nephrotoxicity of metformin.⁵ Moreover, there is evidence showing that herbs inhibit several drug transporters including OCT1, OCT2, and P-glycoprotein (P-gp). Green tea extract inhibits OCT2, OCT1, and P-gp,⁶⁻⁹ leading to increased plasma concentration of diltazem, verapamil, tamoxifen, simvastatin, and nifedipine.^{10,11} *Ginkgo biloba* extract and grapefruits inhibit P-gp affecting plasma concentration of some drugs, such as amiodarone, atorvastatin, buspirone, ciclosporin, felodipine, tadalafil, and nimodipine.^{12,13}

Boesenbergia rotunda, rhizome plants belonging to the Zingiberaceae family, is a traditional herb in Thailand. It is used as a dietary supplement for therapeutic purposes, such as stomach discomfort, rheumatism, muscular pain, diabetes, allergy, asthma, and diarrhea.^{13,14} Bioactive compounds of *B. rotunda* are boesenbergin, cardamonin, pinostrobin, pinocembrin, panduratin A, alpinetin, and 4-hydroxypanduratin.¹⁵ These compounds have several biological activities such as antioxidant activity, antibacterial, anti-inflammatory, and anticancer.¹⁶ Based on previous evidence, it is possible that the biological active compounds of *B. rotunda* may have an interaction with drug transporter as well. Since the

Parts of this work were presented at the 45th Annual Scientific Meeting, Physiological Society of Thailand, December 6-8, 2017, Khon Kaen, Thailand.

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Received 29 September 2017

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Available online at www.j-pbs.org

data concerning the effect of *B. rotunda* on drug transporter expressed in kidney are not available, this study therefore aims to investigate whether biological active compounds of *B. rotunda* interact with the renal transporters in an *in vitro* model. The study will provide the information concerning whether *B. rotunda* can cause herb-drug interaction.

Materials and Methods

Boesenbergia rotunda extract

B. rotunda ethanol extract (BPE) and pure compounds (pinostrobin, pinocembrin and panduratin A) were kindly provided by Dr. Patoomratana Tuchinda, Department of Chemistry, Faculty of Science, Mahidol University (Bangkok, Thailand).

Chemicals

³H-1-methyl-4-phenylpyridinium (³H-MPP⁺; 80 μCi/mol), ³H-para-aminohippurate (³H-PAH; 2.53 Ci/mmol), and ³H-estrone sulfate (³H-ES; 74 μCi/mmol) are purchased from PerkinElmer (MA, USA). Tetrapentyl-ammonium (TPeA), transferrin, selenium, and epidermal growth factor were purchased from Sigma-Aldrich (MO, USA). All other chemicals are analytical grade and purchased from commercial sources.

Cell culture

Human renal proximal tubular (RPTEC/TERT1) cells were derived from normal human kidney which was purchased from American Type Culture Collection (ATCC). This cell line was cultured in Dulbecco's modified Eagle's medium/F-12 medium supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin (Invitrogen), and growth factors according to the ATCC protocol. Cells were grown in a humidified incubator under conditions of 5%CO₂/95%O₂ at 37°C.

Cell viability assay

Cell viability was determined using an MTT assay. Briefly, RPTEC/TERT1 cells were seeded on 96-well

plates at a density of 10,000 cells/well. After growing the cells for 10-14 days in a humidified 5%CO₂ incubator, test compounds at various concentrations were added into the culture medium with the final volume of 200 μl per well for 24 hours. The cells were stained for 1 hour at 37 °C with 0.5 mg/ml MTT reagents. Then, 50 μl of DMSO was added before the measurement of an absorbance at 570 nm by a spectrophotometer.

Uptake studies

RPTEC/TERT1 cells were seeded in 24-well plates and maintained in culture at 37 °C under a humidified 95% air atmosphere, containing 5%CO₂. After confluent, cells were rinsed twice with warm Waymouth buffer (WB) pH 7.40 (WB: 135 mM NaCl, 5 mM KCl, 13mM HEPES, 2.5mM CaCl₂.2HO, 1.2 mM MgCl₂.0.8 mM MgSO₄.7H₂O, and 28 mM D-glucose) and incubated with WB for a further 15 minutes. Cells were incubated with WB containing ³H-MPP⁺ with or without compound for 10 minutes, while ³H-PAH or ³H-ES with or without compound for 30 minutes, for measuring OCT2, OAT1, and OAT3 activity, respectively. Uptakes were stopped by removing the transport buffer and rinsing the cells with three successive washed of 1 ml ice-cold WB. Cells were solubilized with 200 μl of 0.4 N NaOH in 10% SDS overnight, followed by neutralizing with 100 μl of 1 N HCl. Cell lysate was transferred into scintillation vials to measure accumulated radioactivity with liquid scintillation β counter (1214 Rackbeta, Wallac). The uptake of ³H-MPP⁺ or ³H-PAH or ³H-ES were calculated and expressed as fmol/min/cm² and then expressed as a mean percentage of the control.

Statistical analysis

Results were presented as mean ± SD. Statistical differences between control and treatment groups were determined using one-way ANOVA followed by Tukey's test, with *P* < 0.05 considered to be statistically significant.

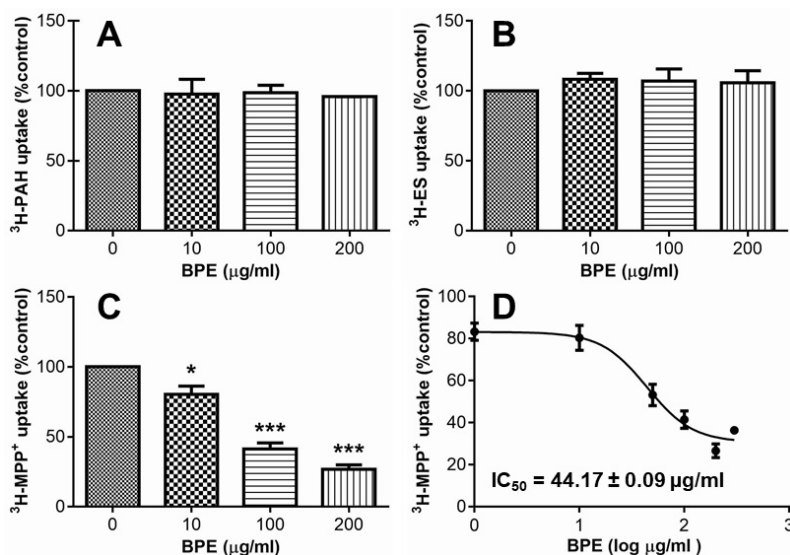


Figure 1 Effect of BPE on OAT1-, OAT3-, and OCT2-mediated uptake of ³H-PAH, ³H-ES, ³H-MPP⁺, respectively, in RPTEC/TERT 1 cells. The uptake measurements were carried out in the presence of BPE at various concentrations for 30 minutes of ³H-PAH (A) and ³H-ES (B), or 10 minutes of ³H-MPP⁺ (C and D). The uptake is expressed as a mean percentage of control (mean ± SD) from three separate experiments. The significance of difference from control was determined by one-way ANOVA followed by Tukey's test (****P* < 0.0001, **P* < 0.05).

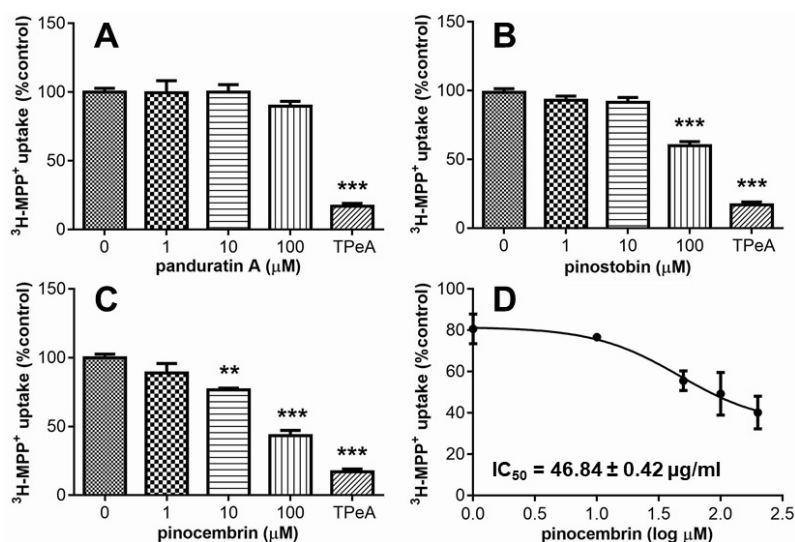


Figure 2 Inhibitory potential of panduratin A (A), pinostrobin (B), and pinocembrin (C and D) on $^3\text{H-MPP}^+$ uptake in RPTEC/TERT1 cells. The cells were incubated for 10 min in uptake buffer contains $^3\text{H-MPP}^+$ in the presence or absence of panduratin A, pinostrobin, pinocembrin, and tetrapentylammonium (200 μM TPeA; OCT2 inhibitor). Each point represents the mean \pm SD of one separate experiment. The significance of difference from control was determined by one-way ANOVA followed by Tukey's test ($***P < 0.0001$, $**P < 0.01$).

Results

Interactions of BPE with human renal organic anion and cation transporters

To determine the potential interactions of BPE with OAT1, OAT3, and OCT2; the effects of BPE on OAT1-mediated $^3\text{H-PAH}$ uptake, OAT3-mediated $^3\text{H-ES}$ uptake, and OCT2-mediated $^3\text{H-MPP}^+$ uptake were determined in RPTEC/TERT1 cells. The results showed that BPE did not significantly alter OAT1-mediated $^3\text{H-PAH}$ uptake and OAT3-mediated $^3\text{H-ES}$ uptake (Figure 1A and B), indicating BPE did not interact with OAT1 and OAT3. In contrast to OAT, BPE decreased $^3\text{H-MPP}^+$ uptake in dose-dependent manners (Figure 1C) with an IC_{50} of $44.17 \pm 0.09 \mu\text{g/ml}$ (Figure 1D). The results suggested that BPE interacted with OCT2.

Inhibitory effect of panduratin A, pinocembrin, and pinostrobin on OCT2 function

The major compounds in *B. rotunda* fresh rhizome ethanol extract consist of panduratin A, pinocembrin, and pinostrobin. Next, the effects of these major compounds on OCT2-mediated $^3\text{H-MPP}^+$ uptake were investigated in RPTEC/TERT1 cells. Panduratin A did not significantly inhibit OCT2-mediated $^3\text{H-MPP}^+$ uptake (Figure 2A), whereas pinostrobin and pinocembrin significantly decreased OCT2-mediated $^3\text{H-MPP}^+$ uptake (Figure 2B and 2C). The IC_{50} of pinocembrin inhibiting OCT2 was $46.84 \pm 0.42 \mu\text{M}$ (Figure 2D). These results suggested that pinostrobin and pinocembrin inhibited OCT2 function in RPTEC/TERT1 cells.

Effects of BPE and major compounds found in BPE on cell viability

To determine whether BPE, pinocembrin, pinostrobin, and panduratin A caused cytotoxicity, we examined cell viability using MTT assay, following exposure of the cells with these compounds. BPE, pinocembrin, pinostrobin, and panduratin A did not affect the viability of

RPTEC/TERT1 cells (Figure 3), suggesting that BPE, pinocembrin and pinostrobin inhibition of OCT2-mediated $^3\text{H-MPP}^+$ uptake, was not a result of cytotoxicity.

Discussion

Natural products have become extensively used for both health prevention and therapeutic purposes.¹⁷ However, the adverse drug reactions resulting from the interactions between herbs and drugs widely remain a public health problem.¹⁸ In Thailand, *B. rotunda* is used as a dietary supplement and for therapeutic purposes, such as anticancer, antibacterial, and antiobesity.¹⁶ It is possible that co-use of *B. rotunda* products and therapeutic drugs may have some interactions with renal transporters, which play crucial roles in the excretion of drugs.

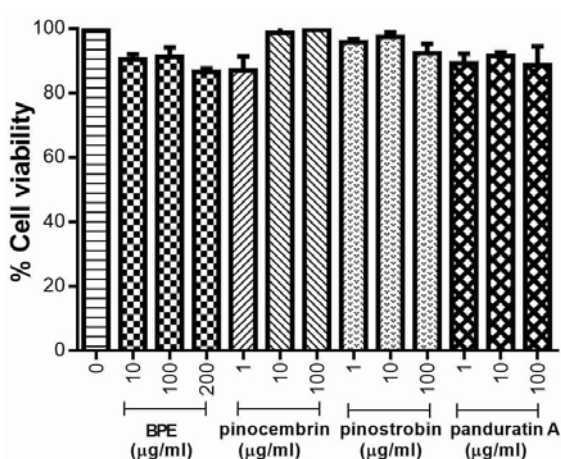


Figure 3 Toxicity testing of BPE, pinocembrin, pinostrobin, and panduratin A on cell viability. Ten days after seeding of RPTEC/TERT1 cells in 96-well plate, the cells were incubated in the presence or absence of BPE, pinocembrin, pinostrobin, panduratin A for 24 hours at 37°C . The viability of RPTEC/TERT1 cells was determined by MTT assay. Data represents the mean \pm SD. The significance of difference from control was determined by one-way ANOVA followed by Tukey's test.

In this study, the interactions of BPE with OAT1, OAT3, and OCT2 in human renal proximal tubule cell line (RPTEC/TERT1) were explored. RPTEC/TERT1 cells is a model system for xenobiotic transport studies which expresses OCT2, OAT1, and OAT3.¹⁹ Using stable RPTEC/TERT1 cells expressing OAT1, OAT3, and OCT2 allowed for an examination of direct interactions between BPE with transporters specifically. The results showed that BPE inhibited ³H-MPP⁺ uptake without producing an effect on OAT1 and OAT3 in RPTEC/TERT1 cells, suggesting that BPE interacted directly with renal human OCT2, and interfered with the systemic profiles of therapeutic cationic drugs. We next determined the effect of major compounds found in BPE, pinostrobin, pinocembrin, and panduratin A, with OCT2 function. The results demonstrated that pinocembrin and pinostrobin, but not panduratin A, inhibited OCT2-mediated ³H-MPP⁺ uptake. The IC₅₀ of pinocembrin was 46.84 ± 0.42 μM, whereas the IC₅₀ of pinostrobin was not calculated because of its low solubility. These data suggest that the inhibitory effect of BPE on OCT2 was at least caused by pinocembrin. In addition, the inhibition of transporters function during the experiment could be a result of cell death, so we investigated the cytotoxicity of BPE and major compounds found in BPE in RPTEC/TERT1 cells. Our data showed that BPE and major compounds, pinostrobin, panduratin A, and pinocembrin, did not have cytotoxic effects on RPTEC/TERT1 cells. These data suggest that the inhibitory effect of BPE and its major compounds on OCT2 were not caused by compounds-induced toxicity. The results of the present study provide the information that BPE inhibited renal organic cation transporter 2 in renal proximal tubular cells.

In conclusion, we provide the first evidence showing BPE and its pure compound, pinocembrin, inhibit OCT2 transporter function but not OAT1 and OAT3 in renal proximal tubular cells. Thus, users of BPE supplement should be aware of the potential effect of BPE that can lead to altered organic cation clearance.

Acknowledgments

This study was supported by a grant from Mahidol University.

Conflict of Interest

None to declare.

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