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BRIEF ARTICLE

Inhibitory effects of apigenin and kaempferol on the essential solute carrier transporters

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Abstract

AIM: To evaluate the inhibitory effects of apigenin and kaempferol on the uptake of several important solute carrier (SLC) transporters.

METHODS: Various SLC transporters including the essential human organic anion transporter 1 (OAT1), OAT2, OAT3 and OAT4 as well as the important organic cation transporter 1 (OCTN1) and OCTN2, were over-expressed in human embryonic kidney (HEK)-293 cells, a well-established cell model of transporter studies. Transport uptake assay was performed 24 h after the transfection. The transport activity was assessed with the uptake of previously determined transporter model substrates and the inhibitory effect of apigenin and kaempferol was evaluated with the substrate uptake in the presence of 10 μmol/L of each compound. Uptake measurements with varying concentrations of inhibitors (ranged from 0.0001 to 50 μmol/L) were performed to further characterize the inhibitory potency of apigenin and kaempferol. The IC_{50} value (the concentration that inhibits 50% of the transporter function) of each compound was then calculated by the nonlinear regression model of Graphpad Prism 6.0 software.

RESULTS: Our data indicated that apigenin could potently inhibit the uptake of estrone-3-sulfate (ES) mediated by the HEK-293 cells expressing OAT2, OAT3 and OAT4 as well as the L-ergothioneine uptake *via* OCTN1-expressing HEK-293 cells. Among these transporters, the most prominent inhibition of apigenin was observed in the case of OAT3. Kaempferol showed significant inhibitory effects on the uptake of ES mediated through OAT2 and OAT3. Impaired L-ergothioneine uptake due to the presence of kaempferol was also observed in OCTN1-expressing HEK-293 cells. Similar to apigenin, kaempferol showed the most potent inhibitory effect on OAT3 as well. To further assess the inhibitory potencies of these two compounds on the uptake of ES mediated by OAT3-expressing HEK-293 cells, their IC_{50} values were then determined. Both chemicals showed pronounced inhibitory potencies on OAT3 with the IC_{50} values of 1.7 ± 0.1 and 1.0 ± 0.1 μmol/L (P < 0.01) for apigenin and kaempferol, respectively.

CONCLUSION: Both apigenin and kaempferol are potent inhibitors of OAT3; precautions will be necessary when co-administrating them with drugs that are substrates of OAT3.

Key words: Apigenin; Kaempferol; Organic anion transporters; Organic cation transporters; Pharmacokinetics; Drug-drug/herb interactions

Core tip: Our study showed that both apigenin and kaempferol could significantly inhibit various solute car-

rier transporters, in particular organic anion transporter 3, at their clinical doses. Inhibition on these transporters can greatly impact on pharmacokinetic performance of drugs that are substrates of these transporters by altering their absorption, distribution and excretion. Precautions should be implemented when co-administering apigenin and kaempferol with drugs that are substrates of these specific solute carrier transporters, in order to maximise desired therapeutic outcomes and avoid unexpected toxicities. This overall enhances our understanding of drug-drug/herb interactions related to apigenin and kaempferol and provides critical information to improve future multi-drug therapies.

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INTRODUCTION

Herbal preparations, such as ginkgo biloba, are currently used with increasing popularity for their broad range of pharmacological effects. Apigenin and kaempferol are flavonoids found in ginkgo biloba as well as in a wide range of vegetables and fruits like tea, beans, broccoli and apples[1,2]. Apigenin and kaempferol have been considered to be clinically important for their anti-oxidative and neuroprotective effects[3,4] as well as chemoprotective properties[2,5]. However, when co-administered with other agents, they could largely impact on the pharmacokinetic performance of other drugs, mainly due to drug-drug/herb interactions[4,6]. The common pathways for such interactions are through competing for specific metabolising enzymes[7-10] or the influx/efflux transporters in charge of the cellular entry/exit of various drugs in body[11-13]. Impaired biotransformation and/or cellular entry/exit can lead to unsatisfactory clinical outcomes and/or increased risk of side effects.

The solute carrier (*SLC*) gene super family is responsible for transporting both endogenous and exogenous substrates across the cell membrane[14,15]. *SLCO* genes coding the organic anion transporting polypeptides (OATPs) as well as *SLC22A* genes coding the organic anion transporters (OATs) and organic cation transporters (OCTs/OCTNs) are the two most important SLC subfamilies involved with drug transport[16]. These organic ion transporters mediate the influx of a wide range of commonly prescribed therapeutic drugs including antibiotics, cholesterol-lowering medications and anti-virals[16,17]. They are expressed in various tissues including intestines, liver and kidney, which are the key organs involved with drug performance in body. Any alteration in the function of these transporters may consequently alter the efficacy and/or inducing toxicity of these agents[18,19].

Up till now, apigenin and kaempferol have been found to be inhibitors of a few SLC transporters, in particular OATP1A2, 1B1, 1B3, 2B1 and OCT2[20-22], which effect may impact on the pharmacokinetics or pharmacodynamics of co-administered drugs[20]. However, no data has yet been shown to demonstrate the relationship of apigenin and kaempferol with the OATs and OCTNs.

Apigenin and kaempferol are structurally similar to naringin, an active constituent found in grapefruit (Figure 1). It has reported that naringin could interact with multiple drugs through competing for OATPs[23]. In addition, naringin is an potent inhibitor of OAT3[24]. Therefore, it is reasonable to postulate that, apigenin and kaempferol may also exhibit inhibitory effects on OATs as well as OCTNs. In this study, we investigated the potential relationship of apigenin and kaempferol with a number of important OATs and OCTNs on their uptake of specific substrates, which could assist our understanding of the potential drug-drug/herb interactions between these flavonoids and other co-administered drugs.

MATERIALS AND METHODS

Chemicals

[^{3}H]-estrone-3-sulfate (ES) (57.3 Ci/mmol) was purchased from PerkinElmer (Melbourne, VIC, Australia). [^{3}H]-4-Aminohippuric acid (PAH, 60 Ci/mmol), [^{3}H]-*L*-ergothioneine (1.7 Ci/mmol) and [^{14}C]-*L*-carnitine (56 mCi/mmol) were purchased from BioScientific Pty. Ltd., Gymea, NSW, Australia. Culture media were purchased from Invitrogen (Mount Waverley, VIC, Australia). Apigenin, kaempferol and all the other chemicals were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).

As described previously[25], the plasmids containing the full-length human OCTN1 (reference sequence: NM-003059) and OCTN2 (reference sequence: NM_003060) cDNAs were obtained from Gene-Ethics (Asia) Pte Ltd., Singapore. The plasmids cloned with the ORFs of human OAT1 (reference sequence: NM-004790.4), OAT2 (reference sequence: NM-006672.2) and OAT3 (reference sequence: NM-004254.2) were purchased from Australian Biosearch, Balcatta, Western Australia. The plasmids of human OAT4 was cloned in house as described previously[26].

Expression of SLC transporters in human embryonic kidney-293 cells

Human embryonic kidney (HEK)-293 cells were cultured in Dulbecco's modified Eagle's medium containing 10% heat-deactivated fetal calf serum at 37 ℃ and 5% CO_2. Cells were transfected with plasmid DNA using Lipofectamine 2000 Reagent (Invitrogen, Mount Waverley, VIC, Australia) following the manufacturer's instructions. Twenty-four hours after transfection, transport activities were measured.

Transport assays

Uptake of radio-labelled typical substrate of each SLC transporter was initiated in phosphate-buffered saline (PBS), pH 7, containing 5 mmol/L glucose, 1mM $CaCl_2$

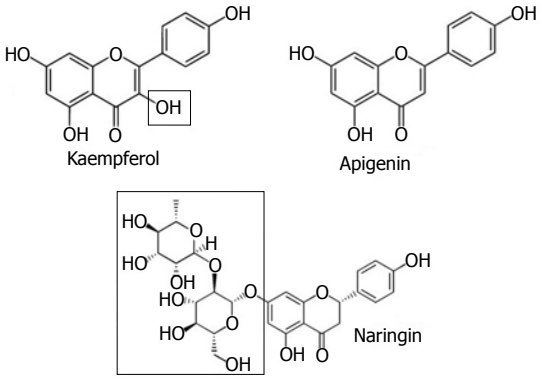

Figure 1 Chemical structures of apigenin, kaempferol and naringin.

and $MgCl_2$. Substrate concentrations and time used in the study were compatible with the previously determined time- and concentration-dependence studies of these transporters: 0.3 μmol/L [^{3}H]-ES for OAT3, OAT4[26,27]; 0.5 μmol/L [^{3}H]-ES for OAT2 (pH 5.5)[28]; 1 μmol/L [^{3}H]-PAH for OAT1[29]; 1 μmol/L [^{3}H]-*L*-ergothioneine for OCTN1[30]; 5 μmol/L [^{14}C]-*L*-carnitine for OCTN2[31], respectively. The uptake was terminated at 8 min intervals by rapidly washing the cells in ice-cold PBS. As our experiments indicated, the initial rates of transporter-mediated substrate uptake in HEK293 cells were linear over at least 8 min (data not shown). The cells were then solubilized in 0.2 mol/L NaOH and neutralized with 0.2 mol/L HCl, and the intracellular accumulation of activity was determined through liquid scintillation counting. Uptake count was standardized to the amount of protein in each well and all uptake data were subtracted with the background counts of vector transfected cells. Data are presented as mean ± SE (n = 3).

A range of uptake measurements with varying concentrations (ranged from 0.0001 to 50 μmol/L) were performed to assess the inhibitory effect of each compound. The IC_{50} value (the concentration that inhibits 50% of the transporter) of each compound was then calculated by nonlinear regression models in GraphPad Prism 6.0 (GraphPad Inc, LaJolla, CA, United States). Each experiment was repeated for three times with triplicate wells for each data group in every experiment.

Statistical analysis

The Student's *t* test was used for differences between two data sets. The criterion of significance was taken to be $P < 0.01$ and $P < 0.001$.

RESULTS

Inhibitory effects of apigenin and kaempferol on the uptake of the specific substrates of OATs and OCTNs

HEK293 cells are well established in over-expressing genes, which was also widely used in a number of transporter studies[25,31-34]. The uptake of the specific substrate for human OAT1, OAT2, OAT3, OAT4, OCTN1 and OCTN2 with the presence of apigenin at 10 μmol/L was measured to examine the potential inhibitory effect of this compound. As shown in Figure 2A, apigenin led to a significant inhibition of the uptake of ES in OAT2, OAT3 and OAT4. The inhibition of L-ergothioneine uptake *via* OCTN1 was also observed (Figure 2A). Among these transporters, OAT3 showed the most prominent inhibition (greater than 80%).

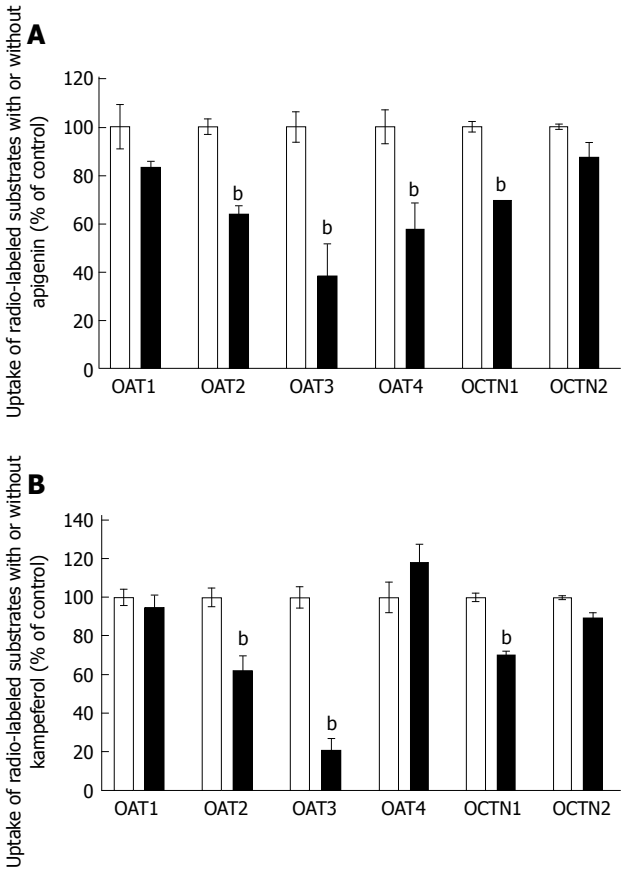

Figure 2 Inhibitory effects of apigenin and kaemperol on the uptake of the specific substrates of organic anion transporters and organic cation transporters. A: Uptake of each radio-labelled substrate was measured in the absence (white bars) and presence of 10 μmol/L apigenin (black bars); B: Uptake of each radio-labelled substrate was measured in the absence (white bars) and presence of 10 μmol/L kaemperol (black bars). The specific substrates used in this experiment were 1 μmol/L [^{3}H]-PAH for OAT1; 500 nmol/L [^{3}H]-ES for OAT2 (pH 5.5); 300 nmol/L [^{3}H]-ES for OAT3 and OAT4; 1 μmol/L [^{3}H]-L-ergothioneine for OCTN1; 5 μmol/L [^{14}C]-L-carnitine for OCTN2. The transporter-mediated uptake of each substrate was calculated by subtracting the uptake of vector control and expressed as a percentage of the uptake to the control (without apigenin or kaemperol). Values were mean ± SE (n = 3) of triplicate repeats in three independent experiments. $^bP < 0.01$, *vs* absence group. OAT: Organic anion transporter; ES: Estrone-3-sulfate; OCTN: Organic cation transporter.

The inhibitory effect of kaempferol was also evaluated on the OATs and OCTNs mentioned above (Figure 2B). The uptake measurement was performed with the presence of kaempferol (10 μmol/L) and two of the transporters showed significant reductions in the uptake of their specific substrate, in particular OAT2 and OAT3. An inhibition on L-ergothioneine uptake was also observed for OCTN1-expressing cells. Interestingly, similar to apigenin, kaempferol also showed the most potent inhibitory effect on OAT3.

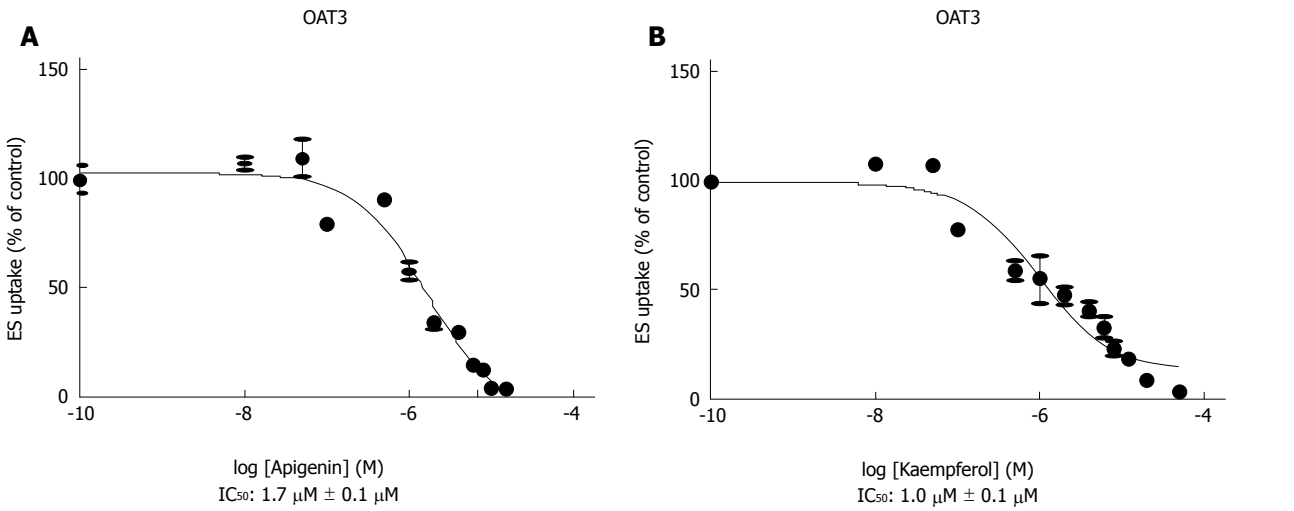

Figure 3 Inhibitory potency of apigenin and kaempferol on the uptake of estrone-3-sulfate mediated by organic anion transporter 3. A: IC_{50} of apigenin inhibition on OAT3; B: IC_{50} of kaempferol inhibition on OAT3. The OAT3 mediated uptake of [^{3}H]-estrone-3-sulfate with the presence of a range of varying concentrations of apigenin or kaempferol (0.0001 to 50 μmol/L) were measured. The transporter-mediated uptake was calculated by subtracting the uptake of vector control and expressed as a percentage of the uptake for the control (without presence of apigenin or kaemperol). Values were mean ± SE (n = 3) of triplicate repeats in three independent experiments. The IC_{50} value of each compound was then calculated by nonlinear regression models in GraphPad Prism 6.0. OAT3: Organic anion transporter 3.

Table 1 Tissue localization and known substrates of organic anion transporter 3

Tissue localization[52]	Endogenous substrates[52]		Exogenous substrates/Inhibitors[44,52]	
Renal proximal tubule cells	Second messengers	cAMP, cGMP	ACE Inhibitors	Captopril, quinapril, enalapril
	Bile salts	Cholate, taurocholate	Angiotensin Ⅱ receptor blockers	Candesartan, losartan, olmesartan, pratosartan, telmisartan, valsartan
	Hormones	Cortisol, DHEA and ES	Antibiotics	Benzylpenicillin, cefadroxil, cefamandol, cefazolin, cefoperazone, cefotaxim, ceftriaxone, cephalexin, cephaloridine, cephalotin, tetracycline
	Prosta-glandins	E_2 and $F_{2\alpha}$	Antivirals	Acyclovir, ganciclovir, valacyclovir, zidovudine
			H2 antagonists	cimetidine, famotidine, ranitidine
			Antiepileptic	Valproate
			Cytostatics	Azathiopurine, methotrexate
			Diuretics	Acetazolamide, bumetanide, chlorothiazide, cyclothiazide, ethacrynate, furosemide, hydrochlorothiazide, methazolamide,trichlormethiazide
			NSAIDs	Acetylsalicylate, diclofenac, flufenamate, ibuprofen, indomethacin, ketoprofen, loxoprofen, mefenamate, naproxen, phenacetin, phenylbutazone, piroxicam, salicylate, sulindac
			Statins	Atorvastatin, fluvastatin, pravastatin, rosuvastatin, simvastatin
			Uricosuric	Probenecid

cAMP: Cyclic adenosine monophosphate; cGMP: Cyclic guanosine monophosphate; DHEA: Dehydroepiandrosterone; ACE: Angiotensin-converting-enzyme inhibitor; ES: Estrone-3-sulfate; NSAIDs: Non-steroidal anti-inflammatory drugs.

Inhibitory potency of apigenin and kaempferol on OAT3

To further assess the inhibitory potency of these two compounds on the uptake of ES *via* OAT3, the IC_{50} values were determined as per described in the methods. The IC_{50} values for all other transporters were considered to be not clinically significant, since the inhibition on their substrate uptake was less than 50% with the presence of apigenin and kaempferol at the concentration of 10 μmol/L. As shown in Figure 3, both chemicals showed similar potency with the IC_{50} values of 1.7 and 1.1 μmol/L for apigenin and kaempferol, respectively.

DISCUSSION

Apigenin and kaempferol are two active flavonoids found in many commonly used herbal preparations such as ginkgo biloba, which is mostly used for its neuroprotective effect to improve memory and concentration problems. They are also enriched in a wide range of daily diet products[35-37]. More importantly, both apigenin and kaempferol have gained popularity in recent years due to their potential chemoprotective effects with low intrinsic toxicity[38-43]. Due to their increased use in the community, potential drug-drug/herb interactions be-

tween these compounds and other therapeutic drugs might occur through competing for metabolising enzymes and/or transporters, which may reduce efficacy and increase toxicity in these combination therapies. As shown in previous literature and the current study (Figure 2A), apigenin has significantly reduced the specific substrate uptake of OAT2, OAT3, OAT4, OCTN1 as well as OATPs and OCTs[20-22]. According to the tissue localisation and physiological roles of these transporters, co-administration of apigenin with drugs that are substrates of these transporters, could likely lead to an impaired pharmacokinetic performance of the specific drugs, which consequently could result in unsatisfactory clinical outcomes and/or elevated toxicities.

Similarly, inhibitory effect was also demonstrated with kaempferol on various SLC transporters by literature and this study (Figure 2B). Previous studies reported that kaempferol is a potent inhibitor of OATP1B1 and OATP2B1[20,21], which transporters are highly liver specific. Additionally, in a previous study conducted by Mandery *et al*[20,21], similar inhibition was also observed in OATP1A2 and OATP1B3 with a different substrate. In the current study, we have demonstrated a significant inhibitory effect of kaempferol on OAT2, OAT3 and OCTN1 for the first time (Figure 2B). Because these transporters are highly expressed in the kidney and liver, co-administration of kaempferol with drugs that are substrates of these transporters could possibly result in poor metabolism and elimination of drugs as well as largely impact on the pharmacological response of such agents.

Furthermore, in our study, both apigenin and kaempferol were observed to be potent inhibitors of human OAT3 with IC_{50} values of 1.7 and 1.1 μmol/L, respectively (Figure 3). OAT3 is mainly expressed in the kidney. At the basolateral membrane of the renal proximal tubules, it is responsible for taking up organic anions from the blood into the proximal tubule cells to assist the secretion of substances like steroid hormones and prostaglandins[44]. In addition, OAT3 has also been found to transport a range of different exogenous drugs including cholesterol lowering medications, antibiotics, antihistamines, antimetabolites and anti-retrovirals[19] (Table 1). Previous studies confirmed that inhibition of OAT3 could lead to accumulation of specific drugs due to its role in the excretion and clearance of exogenous drugs[45-48]. In the study conducted by Yasui-Furukori *et al*[45], it was also found that co-administration of probenecid (a potent inhibitor of OAT3) caused a 50% increase in the AUC and a 70% reduction in the clearance of fexofenadine (a substrate of OAT3) in healthy patients. A similar drug-drug interaction was also found between gemfibrozil (a known inhibitor of OAT3) and pravastatin (a substrate of OAT3), where co-administration of both drugs resulted in a 43% reduction in the renal clearance of pravastatin in healthy patients[47,48]. More importantly, previous *in vivo* studies have found that with 100 mg oral administration of kaempferol, 0.6 mmol/L of maximum concentration was reached in rats, which was significantly higher than the IC_{50} value found in this study[49]. Similarly this was also observed for apigenin where 17 mg oral administration (recommended daily dosage range from 50-150 mg) of apigenin reached a Cmax of 1.27 μmol/L[50]. Based on the information gathered previously and in the current study, it is very likely that both apigenin and kaempferol can inhibit OAT3 transport function under their standard clinical doses. This inhibition can significantly reduce the uptake of co-administered drugs into the renal tubular cells, which may lead to an impaired renal clearance of such agents. Thus drug-drug/herb interactions involved with apigenin and kaempferol, could be clinically significant, especially for drugs with narrow therapeutic window such as methotrexate, a known substrate of OAT3[51].

In summary, the current study assessed the inhibitory effects of two bioactive flavonoids, apigenin and kaempferol, on a number of essential OATs and OCTNs. Our results indicated that both apigenin and kaempferol can largely influence the transport function of several important organic ion transporters including OAT2, OAT4, OCTN1, and particularly OAT3. As these compounds are widely used in our daily life, precautions should be taken when co-administering such compounds together with other drugs that are substrates of these transporters, so as to achieve desired therapeutic outcomes as well as minimise unexpected toxicities.

COMMENTS

Background

Apigenin and kaempferol are both commonly used flavonoids in the community for their potential pharmacological effects. They are both commercially available and are found in our everyday diet. Previous studies have found that both of these compounds may interact with clinically important drugs through competing for transporters or metabolism enzymes. Solute carrier (SLC) transporters [includes anion transporting polypeptides (OATPs), organic anion transporters (OATs), organic cation transporters (OCTs/OCTNs) and OCTNs] are important transporters that mediate the cellular entry of many drugs in different tissues including the intestines, liver and kidney. Up till now, no studies have yet been conducted to explore the effect of apigenin and kaempferol on OATs and OCTNs.

Research frontiers

As herbal preparations have gained their popularity in the community in the recent years, their pharmacological effect and potential drug-drug/herb interactions became the hot topics in this area. As one of the key mediator of such drug-drug/herb interactions, SLC transporters have also been extensively investigated in regards to their transport mechanism and structural function. Most importantly, the interaction between flavonoids and SLCs becomes the center of focus due to its great influence on the overall therapeutic outcome.

Innovations and breakthroughs

Their study was the first to report that apigenin and kaempferol can both inhibit OATs and OCTNs, in particular OAT3 at their clinical doses. Due to the physiological role and tissue localization of these transporters, such information significantly contribute to better understanding of unsatisfied pharmacological effects and/or unexpected adverse events when co-administering apigenin and kaempferol with other drugs that are substrates of these transporters.

Applications

This study enhanced our knowledge of the inhibitory effects of apigenin and kaempferol on SLC transporters, which allows the advancement of optimizing combination therapy in clinical settings. Dosages should be adjusted accord-

ingly to minimize the chance of toxicity.

Terminology

Drug-drug/herb interaction: a drug affects the activity of another drug when both are administered together. This can also apply to the interactions between drugs and herbal preparations.

Peer review

This interesting study explored the inhibitory effects of apigenin and kaempferol on several SLC transporters. The study design was well thought and the results were properly analysed. The potent inhibitory effect of both compounds on OAT3 is with great clinical significance, since this transporter is responsible for the elimination of a wide range of front-line agents. Precautions should be prioritized to optimize the concurrent therapies involving apigenin and kaempferol.

REFERENCES

1 **Jiang L**, Fang G, Zhang Y, Cao G, Wang S. Analysis of flavonoids in propolis and Ginkgo biloba by micellar electrokinetic capillary chromatography. *J Agric Food Chem* 2008; **56**: 11571-11577 [PMID: 19053353 DOI: 10.1021/jf802567r]

2 **Calderón-Montaño JM**, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M. A review on the dietary flavonoid kaempferol. *Mini Rev Med Chem* 2011; **11**: 298-344 [PMID: 21428901 DOI: 10.2174/138955711795305335]

3 **Tang D**, Yang D, Tang A, Gao Y, Jiang X, Mou J, Yin X. Simultaneous chemical fingerprint and quantitative analysis of Ginkgo biloba extract by HPLC-DAD. *Anal Bioanal Chem* 2010; **396**: 3087-3095 [PMID: 20191266 DOI: 10.1007/s00216-010-3536-8]

4 **Hermann R**, von Richter O. Clinical evidence of herbal drugs as perpetrators of pharmacokinetic drug interactions. *Planta Med* 2012; **78**: 1458-1477 [PMID: 22855269 DOI: 10.1055/s-0032-1315117]

5 **Shukla S**, Gupta S. Apigenin: a promising molecule for cancer prevention. *Pharm Res* 2010; **27**: 962-978 [PMID: 20306120 DOI: 10.1007/s11095-010-0089-7]

6 **Chen Y**, Jia X, Chen J, Wang J, Hu M. The pharmacokinetics of raloxifene and its interaction with apigenin in rat. *Molecules* 2010; **15**: 8478-8487 [PMID: 21088662 DOI: 10.3390/molecules15118478]

7 **Si D**, Wang Y, Zhou YH, Guo Y, Wang J, Zhou H, Li ZS, Fawcett JP. Mechanism of CYP2C9 inhibition by flavones and flavonols. *Drug Metab Dispos* 2009; **37**: 629-634 [PMID: 19074529 DOI: 10.1124/dmd.108.023416]

8 **Kimura Y**, Ito H, Ohnishi R, Hatano T. Inhibitory effects of polyphenols on human cytochrome P450 3A4 and 2C9 activity. *Food Chem Toxicol* 2010; **48**: 429-435 [PMID: 19883715 DOI: 10.1016/j.fct.2009.10.041S0278-6915(09)00502-X]

9 **Lynch T**, Price A. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. *Am Fam Physician* 2007; **76**: 391-396 [PMID: 17708140]

10 **Rettie AE**, Jones JP. Clinical and toxicological relevance of CYP2C9: drug-drug interactions and pharmacogenetics. *Annu Rev Pharmacol Toxicol* 2005; **45**: 477-494 [PMID: 15822186 DOI: 10.1146/annurev.pharmtox.45.120403.095821]

11 **Svehlíková V**, Wang S, Jakubíková J, Williamson G, Mithen R, Bao Y. Interactions between sulforaphane and apigenin in the induction of UGT1A1 and GSTA1 in CaCo-2 cells. *Carcinogenesis* 2004; **25**: 1629-1637 [PMID: 15090468 DOI: 10.1093/carcin/bgh169]

12 **Lu X**, Sun D, Chen Z, Ye J, Wang R, Li L, Zeng S, Jiang H. Evaluation of hepatic clearance and drug-drug interactions of luteolin and apigenin by using primary cultured rat hepatocytes. *Pharmazie* 2011; **66**: 600-605 [PMID: 21901983]

13 **Wang Y**, Cao J, Zeng S. Involvement of P-glycoprotein in regulating cellular levels of Ginkgo flavonols: quercetin, kaempferol, and isorhamnetin. *J Pharm Pharmacol* 2005; **57**: 751-758 [PMID: 15969930 DOI: 10.1211/0022357056299]

14 **He L**, Vasiliou K, Nebert DW. Analysis and update of the human solute carrier (SLC) gene superfamily. *Hum Genomics* 2009; **3**: 195-206 [PMID: 19164095 DOI: 10.1186/1479-7364-3-2-195]

15 **DeGorter MK**, Xia CQ, Yang JJ, Kim RB. Drug transporters in drug efficacy and toxicity. *Annu Rev Pharmacol Toxicol* 2012; **52**: 249-273 [PMID: 21942630 DOI: 10.1146/annurev-pharmtox-010611-134529]

16 **Roth M**, Obaidat A, Hagenbuch B. OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. *Br J Pharmacol* 2012; **165**: 1260-1287 [PMID: 22013971 DOI: 10.1111/j.1476-5381.2011.01724.x]

17 **Koepsell H**, Endou H. The SLC22 drug transporter family. *Pflugers Arch* 2004; **447**: 666-676 [PMID: 12883891 DOI: 10.1007/s00424-003-1089-9]

18 **Ciarimboli G**. Role of organic cation transporters in drug-induced toxicity. *Expert Opin Drug Metab Toxicol* 2011; **7**: 159-174 [PMID: 21241199 DOI: 10.1517/17425255.2011.547474]

19 **VanWert AL**, Gionfriddo MR, Sweet DH. Organic anion transporters: discovery, pharmacology, regulation and roles in pathophysiology. *Biopharm Drug Dispos* 2010; **31**: 1-71 [PMID: 19953504 DOI: 10.1002/bdd.693]

20 **Mandery K**, Bujok K, Schmidt I, Keiser M, Siegmund W, Balk B, König J, Fromm MF, Glaeser H. Influence of the flavonoids apigenin, kaempferol, and quercetin on the function of organic anion transporting polypeptides 1A2 and 2B1. *Biochem Pharmacol* 2010; **80**: 1746-1753 [PMID: 20797388 DOI: 10.1016/j.bcp.2010.08.008S0006-2952(10)00617-9]

21 **Mandery K**, Balk B, Bujok K, Schmidt I, Fromm MF, Glaeser H. Inhibition of hepatic uptake transporters by flavonoids. *Eur J Pharm Sci* 2012; **46**: 79-85 [PMID: 22394605 DOI: 10.1016/j.ejps.2012.02.014S0928-0987(12)00073-5]

22 **Ofer M**, Wolffram S, Koggel A, Spahn-Langguth H, Langguth P. Modulation of drug transport by selected flavonoids: Involvement of P-gp and OCT? *Eur J Pharm Sci* 2005; **25**: 263-271 [PMID: 15911222 DOI: 10.1016/j.ejps.2005.03.001]

23 **Diaconu CH**, Cuciureanu M, Vlase L, Cuciureanu R. Food-drug interactions: grapefruit juice. *Rev Med Chir Soc Med Nat Iasi* 2011; **115**: 245-250 [PMID: 21682192]

24 **Hong SS**, Seo K, Lim SC, Han HK. Interaction characteristics of flavonoids with human organic anion transporter 1 (hOAT1) and 3 (hOAT3). *Pharmacol Res* 2007; **56**: 468-473 [PMID: 17920288 DOI: 10.1016/j.phrs.2007.08.007S1043-6618(07)00159-4]

25 **Xu F**, Li Z, Zheng J, Gee Cheung FS, Chan T, Zhu L, Zhuge H, Zhou F. The inhibitory effects of the bioactive components isolated from scutellaria baicalensis on the cellular uptake mediated by the essential solute carrier transporters. *J Pharm Sci* 2013; **102**: 4205-4211 [PMID: 24018852 DOI: 10.1002/jps.23727]

26 **Zhou F**, Zhu L, Cui PH, Church WB, Murray M. Functional characterization of nonsynonymous single nucleotide polymorphisms in the human organic anion transporter 4 (hOAT4). *Br J Pharmacol* 2010; **159**: 419-427 [PMID: 20015291 DOI: 10.1111/j.1476-5381.2009.00545.xBPH545]

27 **Ueo H**, Motohashi H, Katsura T, Inui K. Human organic anion transporter hOAT3 is a potent transporter of cephalosporin antibiotics, in comparison with hOAT1. *Biochem Pharmacol* 2005; **70**: 1104-1113 [PMID: 16098483 DOI: 10.1016/j.bcp.2005.06.024S0006-2952(05)00453-3]

28 **Kobayashi Y**, Ohshiro N, Sakai R, Ohbayashi M, Kohyama N, Yamamoto T. Transport mechanism and substrate specificity of human organic anion transporter 2 (hOat2 [SLC22A7]). *J Pharm Pharmacol* 2005; **57**: 573-578 [PMID: 15901346 DOI: 10.1211/0022357055966]

29 **Race JE**, Grassl SM, Williams WJ, Holtzman EJ. Molecular cloning and characterization of two novel human renal organic anion transporters (hOAT1 and hOAT3). *Biochem Biophys Res Commun* 1999; **255**: 508-514 [PMID: 10049739 DOI: 10.1006/bbrc.1998.9978S0006-291X(98)99978-8]

30 **Gründemann D**, Harlfinger S, Golz S, Geerts A, Lazar A, Berkels R, Jung N, Rubbert A, Schömig E. Discovery of the ergothioneine transporter. *Proc Natl Acad Sci USA* 2005; **102**:

5256-5261 [PMID: 15795384 DOI: 10.1073/pnas.04086241020408624102]
31 **Toh DS**, Murray M, Pern Tan K, Mulay V, Grewal T, Lee EJ, Zhou F. Functional analysis of pharmacogenetic variants of human organic cation/carnitine transporter 2 (hOCTN2) identified in Singaporean populations. *Biochem Pharmacol* 2011; **82**: 1692-1699 [PMID: 21864509 DOI: 10.1016/j.bcp.2011.08.008S0006-2952(11)00657-5]
32 **Zhou F**, Zheng J, Zhu L, Jodal A, Cui PH, Wong M, Gurney H, Church WB, Murray M. Functional Analysis of Novel Polymorphisms in the Human SLCO1A2 Gene that Encodes the Transporter OATP1A2. *AAPS J* 2013; **15**: 1099-1108 [PMID: 23918469 DOI: 10.1208/s12248-013-9515-1]
33 **Toh DS**, Cheung FS, Murray M, Pern TK, Lee EJ, Zhou F. Functional Analysis of Novel Variants in the Organic Cation/Ergothioneine Transporter 1 Identified in Singapore Populations. *Mol Pharm* 2013; Epub ahead of print [PMID: 23663222 DOI: 10.1021/mp400193r]
34 **Yee SW**, Nguyen AN, Brown C, Savic RM, Zhang Y, Castro RA, Cropp CD, Choi JH, Singh D, Tahara H, Stocker SL, Huang Y, Brett CM, Giacomini KM. Reduced renal clearance of cefotaxime in asians with a low-frequency polymorphism of OAT3 (SLC22A8). *J Pharm Sci* 2013; **102**: 3451-3457 [PMID: 23649425 DOI: 10.1002/jps.23581]
35 **Cao Y**, Chu Q, Fang Y, Ye J. Analysis of flavonoids in Ginkgo biloba L. and its phytopharmaceuticals by capillary electrophoresis with electrochemical detection. *Anal Bioanal Chem* 2002; **374**: 294-299 [PMID: 12324852 DOI: 10.1007/s00216-002-1436-2]
36 **Kang JW**, Kim JH, Song K, Kim SH, Yoon JH, Kim KS. Kaempferol and quercetin, components of Ginkgo biloba extract (EGb 761), induce caspase-3-dependent apoptosis in oral cavity cancer cells. *Phytother Res* 2010; **24** Suppl 1: S77-S82 [PMID: 19585476 DOI: 10.1002/ptr.2913]
37 **Birks J**, Grimley Evans J. Ginkgo biloba for cognitive impairment and dementia. *Cochrane Database Syst Rev* 2007; (**2**): CD003120 [PMID: 17443523 DOI: 10.1002/14651858.CD003120.pub2]
38 **O'Prey J**, Brown J, Fleming J, Harrison PR. Effects of dietary flavonoids on major signal transduction pathways in human epithelial cells. *Biochem Pharmacol* 2003; **66**: 2075-2088 [PMID: 14609732 DOI: 10.1016/j.bcp.2003.07.007]
39 **Oh SM**, Kim YP, Chung KH. Biphasic effects of kaempferol on the estrogenicity in human breast cancer cells. *Arch Pharm Res* 2006; **29**: 354-362 [PMID: 16756079 DOI: 10.1007/BF02968584]
40 **Soares VC**, Varanda EA, Raddi MS. In vitro basal and metabolism-mediated cytotoxicity of flavonoids. *Food Chem Toxicol* 2006; **44**: 835-838 [PMID: 16376008 DOI: 10.1016/j.fct.2005.11.006S0278-6915(05)00337-6]
41 **Yang CS**, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu Rev Nutr* 2001; **21**: 381-406 [PMID: 11375442 DOI: 10.1146/annurev.nutr.21.1.381]
42 **Zhang Y**, Chen AY, Li M, Chen C, Yao Q. Ginkgo biloba extract kaempferol inhibits cell proliferation and induces apoptosis in pancreatic cancer cells. *J Surg Res* 2008; **148**: 17-23 [PMID: 18570926 DOI: 10.1016/j.jss.2008.02.036S0022-4804(08)00140-6]
43 **Li W**, Du B, Wang T, Wang S, Zhang J. Kaempferol induces apoptosis in human HCT116 colon cancer cells via the Ataxia-Telangiectasia Mutated-p53 pathway with the involvement of p53 Upregulated Modulator of Apoptosis. *Chem Biol Interact* 2009; **177**: 121-127 [PMID: 19028473 DOI: 10.1016/j.cbi.2008.10.048S0009-2797(08)00576-0]
44 **Rizwan AN**, Burckhardt G. Organic anion transporters of the SLC22 family: biopharmaceutical, physiological, and pathological roles. *Pharm Res* 2007; **24**: 450-470 [PMID: 17245646 DOI: 10.1007/s11095-006-9181-4]
45 **Yasui-Furukori N**, Uno T, Sugawara K, Tateishi T. Different effects of three transporting inhibitors, verapamil, cimetidine, and probenecid, on fexofenadine pharmacokinetics. *Clin Pharmacol Ther* 2005; **77**: 17-23 [PMID: 15637527 DOI: 10.1016/j.clpt.2004.08.026]
46 **Tahara H**, Kusuhara H, Maeda K, Koepsell H, Fuse E, Sugiyama Y. Inhibition of oat3-mediated renal uptake as a mechanism for drug-drug interaction between fexofenadine and probenecid. *Drug Metab Dispos* 2006; **34**: 743-747 [PMID: 16455804 DOI: 10.1124/dmd.105.008375]
47 **Kyrklund C**, Backman JT, Neuvonen M, Neuvonen PJ. Gemfibrozil increases plasma pravastatin concentrations and reduces pravastatin renal clearance. *Clin Pharmacol Ther* 2003; **73**: 538-544 [PMID: 12811363 DOI: 10.1016/S0009-9236(03)00052-3S0009923603000523]
48 **Nakagomi-Hagihara R**, Nakai D, Tokui T. Inhibition of human organic anion transporter 3 mediated pravastatin transport by gemfibrozil and the metabolites in humans. *Xenobiotica* 2007; **37**: 416-426 [PMID: 17455113 DOI: 10.1080/00498250601188808777020814]
49 **Barve A**, Chen C, Hebbar V, Desiderio J, Saw CL, Kong AN. Metabolism, oral bioavailability and pharmacokinetics of chemopreventive kaempferol in rats. *Biopharm Drug Dispos* 2009; **30**: 356-365 [PMID: 19722166 DOI: 10.1002/bdd.677]
50 **Meyer H**, Bolarinwa A, Wolfram G, Linseisen J. Bioavailability of apigenin from apiin-rich parsley in humans. *Ann Nutr Metab* 2006; **50**: 167-172 [PMID: 16407641 DOI: 10.1159/000090736]
51 **Maeda A**, Tsuruoka S, Kanai Y, Endou H, Saito K, Miyamoto E, Fujimura A. Evaluation of the interaction between nonsteroidal anti-inflammatory drugs and methotrexate using human organic anion transporter 3-transfected cells. *Eur J Pharmacol* 2008; **596**: 166-172 [PMID: 18789319 DOI: 10.1016/j.ejphar.2008.08.023S0014-2999(08)00898-4]
52 **Burckhardt G**. Drug transport by Organic Anion Transporters (OATs). *Pharmacol Ther* 2012; **136**: 106-130 [PMID: 22841915 DOI: 10.1016/j.pharmthera.2012.07.010S0163-7258(12)00159-3]

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