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Effect of St John's wort and ginseng on the pharmacokinetics and pharmacodynamics of warfarin in healthy subjects

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Aim
The aim of this study was to investigate the effect of St John's wort and ginseng on the pharmacokinetics and pharmacodynamics of warfarin.

Methods
This was an open-label, three-way crossover randomized study in 12 healthy male subjects, who received a single 25-mg dose of warfarin alone or after 14 days' pretreatment with St John's wort, or 7 days' pretreatment with ginseng. Dosing with St John's wort or ginseng was continued for 7 days after administration of the warfarin dose. Platelet aggregation, international normalized ratio (INR) of prothrombin time, warfarin enantiomer protein binding, warfarin enantiomer concentrations in plasma and S-7-hydroxywarfarin concentration in urine were measured. Statistical comparisons were made using ANOVA and 90% confidence intervals are reported.

Results
INR and platelet aggregation were not affected by treatment with St John's wort or ginseng. The apparent clearances of S-warfarin after warfarin alone or with St John's wort or ginseng were, respectively, 198 ± 38 ml min^{-1}, 270 ± 44 ml min^{-1} and 220 ± 29 ml min^{-1}. The respective apparent clearances of R-warfarin were 110 ± 25 ml min^{-1}, 142 ± 29 ml min^{-1} and 119 ± 20 ml min^{-1}. The mean ratio and 90% confidence interval (CI) of apparent clearance for S-warfarin was 1.29 (1.16, 1.46) and for R-warfarin it was 1.23 (1.11, 1.37) when St John's wort was coadministered. The mean ratio and 90% CI of AUC_{0-168} of INR was 0.79 (0.70, 0.95) when St John's wort was coadministered. St John's wort and ginseng did not affect the apparent volumes of distribution or protein binding of warfarin enantiomers.

Conclusions
St John's wort significantly induced the apparent clearance of both S-warfarin and R-warfarin, which in turn resulted in a significant reduction in the pharmacological effect of *rac*-warfarin. Coadministration of warfarin with ginseng did not affect the pharmacokinetics or pharmacodynamics of either S-warfarin or R-warfarin.

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Introduction

The anticoagulant warfarin has an important place in the management of cardiovascular disease in the community where people have free access to herbal medicines. Warfarin's narrow therapeutic range and metabolism by cytochrome P450 (CYP) make it prone to potentially life-threatening interactions and result in warfarin being one of the most frequently investigated drugs with respect to drug interactions [1]. St John's wort (*hypericum*) is a herbal medicine widely used in the community for the management of a range of conditions, including depression. Numerous drug interactions with St John's wort have been reported based on case reports, and *in vitro* and *in vivo* study [2–4]. There are a number of case reports suggesting that coadministration of St John's wort decreases the effects of warfarin [3]. The Medical Products Agency (MPA, Uppsala, Sweden) has received seven case reports of a reduced anticoagulant effect and decreased international normalized ratio (INR) of warfarin associated with coadministration of St John's wort [2]. Similarly, over the period October 1992 to September 2000, the UK Committee on Safety of Medicines and the Medicines Control Agency received 35 reports of suspected interactions between St John's wort and conventional medicines of which four were related to potential interactions with warfarin [4]; two reported an increase in INR and two cases reported a decrease in INR. Despite these observations, the possible interaction between warfarin and St John's wort has not been systemically investigated. Similarly, ginseng is also widely used in the community for a variety of indications, but few drug interaction studies have been undertaken. There is a case report of decreased effect of warfarin in a patient receiving ginseng [5], but a study in rats found no effect of ginseng on the pharmacokinetics or pharmacodynamics of warfarin [6]. The conclusion from a recent systematic review was that patients who take warfarin with ginseng should regularly monitor their INR [7]. The aim of the present study was to investigate the possible drug interactions between warfarin and these two widely used herbal medicines.

Materials and methods

Herbal medicines

The commercial products of St John's wort and ginseng used in this study were chosen according to an assessment of the quality of various brands and also conformity of the dose with that recommended in the Herbal Medicine-Expanded Commission E Monographs. High-performance thin-layer chromatography (HPTLC) was used to characterize the constituents of proprietary preparations of St John's wort and ginseng according to methods described in the Hypericum and Ginseng monographs of the British Pharmacopeia (2001). Proprietary products Bioglan (St John's wort, each tablet containing standardized dry extract equivalent to 1 g *Hypericum perforatum* flowering herb top, 0.825 mg hypericin and 12.5 mg hyperforin; Batch 1331–2) and Golden Glow (Korean ginseng, each capsule containing extract equivalent to 0.5 g *Panax ginseng* root and 8.93 mg ginsenosides as ginsenoside Rg1; Batch K01251) were used in this clinical trial.

Subjects

Twelve healthy male subjects were recruited to three study groups. Subjects were aged 20–40 years, and were within 15% of ideal body weight for height and build. Subjects came from a diverse ethnic mix (eight Caucasians, four Asians). All subjects were nonsmokers and were selected on the basis of medical history, physical examination, and clinical laboratory test results (including INR, platelet aggregation, creatinine, bilirubin, albumin and total protein). Subjects with current or past medical conditions that might affect the pharmacokinetic or pharmacodynamic response to warfarin were excluded from the study. Furthermore, subjects had not taken any medication for at least 2 weeks before commencing the study. Power calculations indicated that 12 subjects in a crossover study would provide an 80% chance of detecting a 20% difference in the pharmacokinetic parameters of S-warfarin. All participants gave written informed consent before entering the study. The study was approved by the St Vincent's Hospital Human Research Ethics Committee and the Human Ethics Committee of the University of Sydney.

Study design

An open-label, three-treatment, three-period, three-sequence, randomized, crossover study was conducted with at least a 14-day washout between treatment periods. A single 25-mg dose of *rac*-warfarin (Coumadin™, 5×5-mg tablets; Boots HealthCare Australia Pty Ltd, North Ryde, NSW, Australia) was administered to each subject with and without pretreatment with multiple doses of either St John's wort (one tablet, three times daily for 2 weeks) or ginseng (two capsules, three times daily for 1 week). Dosing of St John's wort or ginseng was continued for a further 1 week after warfarin administration. Blood samples were collected via an indwelling cannula or by venepuncture into both sodium citrate and EDTA tubes. Sampling times in relation to warfarin dosing were: −48, −24, 0, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 h. Whole blood was used

to measure platelet aggregation, and plasma was harvested by centrifugation (at 1500 g for 10 min) to determine the INR. A portion was stored frozen until the time of drug concentration analysis. Urine was collected before and after administration of warfarin dose for 3 days. The volume of urine was recorded and a portion was stored frozen for subsequent analysis.

Analytical techniques

The concentrations of S-warfarin and R-warfarin in plasma and S-7-hydroxywarfarin in urine were determined using a modified version of the HPLC assay by Naidong *et al.* [8] which employs a chiral HPLC column (Silica-bonded β-cyclodextrin, Cyclobond™, Astec, Alltech Associates Australia Pty Ltd, Baulkham Hills, NSW, Australia) with fluorescence detection. In brief, aliquots (0.5 ml) of plasma were spiked with the internal standard (naproxen) and H_2SO_4 solution (0.35 ml, 0.5 M) and then extracted with dichloromethane : hexane (4 ml, 1 : 5). Urine samples (0.1 ml) were also spiked with internal standard (naproxen) and H_2SO_4 solution (0.1 ml of 0.5 M) and then extracted with dichloromethane : hexane (4 ml, 1 : 2). The organic phase was decanted after separation by centrifugation (300 g, 10 min) and freezing the aqueous phase in dry ice. The organic layer was evaporated to dryness under nitrogen and reconstituted in acetonitrile (200 µl) and an aliquot (20 µl) injected onto the column. The mobile phase comprised acetonitrile : methanol : triethylamine : glacial acetic acid (95 : 5 : 0.2 : 0.3, v/v/v/v) with a flow rate of 1 ml min^{-1}. Measurements were made using a Shimadzu RF 535 fluorescence detector (excitation wavelength 310 nm, emission wavelength 400 nm) (Shimadzu Scientific Instruments (Oceana) Pty Ltd, Rydalmere, NSW, Australia). The assay was linear for S-warfarin and R-warfarin over the range 20–2500 ng ml^{-1} in plasma and was linear for S-7-hydroxywarfarin over the range of 33–1650 ng ml^{-1} in urine. The precision of the assay, as indicated by the percent coefficient of variation, was <12% for S-warfarin, R-warfarin and S-7-hydroxywarfarin. The interday and intraday accuracies of the assay were within 15% of the actual value. The recovery of warfarin following extraction and reconstitution ranged from 86% to 93% for the S-enantiomer, and 88% to 93% for the R-enantiomer from plasma, and ranged from 85% to 90% for S-7-hydroxywarfarin from urine.

Plasma protein binding

The unbound fractions of S-warfarin and R-warfarin in plasma were assessed by ultrafiltration (Centrifree® YM-30; Millipore Australia Pty Ltd, North Ryde, NSW, Australia) after 10 µg *rac*-warfarin were added to 1 ml of pooled plasma obtained from each subject at 4, 18, 60, 108 and 156 h after warfarin dose. This approach relies on the assumption of concentration-independent protein binding as established by Banfield *et al.* [9]. The fraction unbound (fu) was calculated as the ratio of the concentration of each warfarin enantiomer in the ultrafiltrate to that in plasma.

INR measurement

INR was measured using a BFT™ II analyser (Dade Behring Diagnostics Pty Ltd, Lane Cove, NSW, Australia) with Thromborel® S reagent (human thromboplastin/calcium reagent for one-stage prothrombin time; Dade Behring).

Platelet aggregation measurement

Platelet aggregation was measured using a whole-blood aggregometer (Chrono-par®; Chrono-log Corp., USA, Edward Keller Australia Pty Ltd, Hallam, VIC, Australia) according to the manufacturer's instructions. Briefly, prewarmed whole blood (1 ml) diluted 1 in 2 (one part of blood to one part of saline) with normal saline was incubated at 37 °C for 2 min. Platelet aggregation was induced by adding arachidonic acid (10 µl, 50 mM concentration, Chrono-par®, Chrono-log Corp., USA, purchased from EKA). A change in impedance was recorded for 6 min after stimulation with arachidonic acid and reported as impedance aggregation (Ω).

Data treatment

The pharmacokinetic parameters for warfarin enantiomers were estimated using noncompartmental methods. The elimination rate constant (k) was obtained by linear regression analysis of warfarin enantiomer log concentration–time data over the terminal linear portion of the concentration–time curve. Elimination half-life ($t_{1/2}$) was calculated as ln2/k. The area under the plasma S-warfarin and R-warfarin concentration–time curves until the last concentration observation (AUC_{0-t}) were calculated using the trapezoidal rule. The AUC was extrapolated to infinity ($AUC_{0-\infty}$) using C_t/k where C_t is the last measured S-warfarin or R-warfarin concentration. The highest S-warfarin and R-warfarin concentrations (C_{max}) and the time that these occurred (t_{max}) were obtained by observation without interpolation. Apparent total clearance (CL/F) and apparent volume of distribution (V/F) for the warfarin enantiomers were calculated as dose/2/$AUC_{0-\infty}$ and CL/F/k, respectively. INR was reported as the area under the curve of the INR (AUC_{INR}) and calculated by the trapezoidal method. Urine excre-

tion rate (UER) was calculated as the amount (Ae,m) of metabolite eliminated over the sample collection time interval (*T*).

Statistical analysis

The pharmacokinetic and pharmacodynamic parameters were analysed by analysis of variance (ANOVA) followed by the *post hoc* multiple comparisons with Tukey using Stata® 5.0 (Stata Corp., TX, USA) and SPSS® 10.0 (SPSS Inc., Chicago, IL, USA) for subjects nested in sequence, sequence, period and treatment effects. The 95% confidence interval (CI) was used for description presentation of study parameters and the 90% CI of the ratio of logarithmically transformed parameters was used to compare control (warfarin alone) and treatment (warfarin with herbal medicine) phases and it was deemed that an interaction lacked clinical significance if the 90% CI values fell within the range 80–125%. A *P*-value of <0.05 was considered significant.

Results

Pharmacokinetics of S-warfarin and R-warfarin

There was a significant difference in AUC, $t_{1/2}$ and CL/F for both S-warfarin (Figure 1, Table 1 and 2) and R-warfarin (Figure 2, Table 1 and 2) following treatment with St John's wort. By contrast, there were no significant changes in these parameters following treatment with ginseng (Figures 1 and 2; Tables 1 and 2). Neither St John's wort nor ginseng affected the apparent volume of distribution, C_{max} or t_{max} of the warfarin enantiomers. The pharmacokinetic parameters for warfarin enantiomers were in close agreement with previous literature [10].

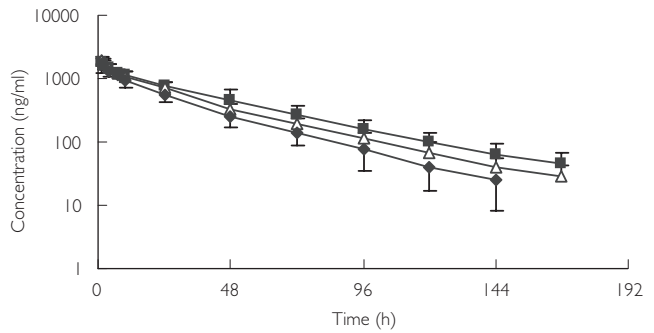

Figure 1

Mean S-warfarin log concentration-time profiles following single oral 25 mg *rac*-warfarin dose with warfarin only, Warfarin + ginseng (GS) and Warfarin + St John's wort (SJW) (error bars show SD, n = 12). S-warfarin (■), S-warfarin + GS (△), S-warfarin + SJW (◆)

Urine excretion rate of S-7-hydroxywarfarin

The UER of S-7-hydroxywarfarin after administration of warfarin alone (40 ± 22 μg h^{-1}), was not significantly reduced following treatment with St John's wort (31 ± 19 μg h^{-1}), but was reduced by treatment with ginseng (29 ± 17 μg h^{-1}). The ratio of geometric mean and 90% CIs for the UER was 0.82 (0.61, 1.12) for St John's wort and 0.68 (0.50, 0.91) for ginseng.

Table 1

Warfarin pharmacokinetic parameters following a single oral 25-mg *rac*-warfarin (WF) alone, in combination with either St John's wort (WF + SJW) or ginseng (WF + GS) (mean ± SD, 95% CI, $n = 12$)

Treatment	WF alone	WF + SJW	WF + GS
fu (%)			
S-warfarin	0.34 ± 0.11 (0.27, 0.41)	0.36 ± 0.10 (0.30, 0.43)	0.39 ± 0.16 (0.29, 0.49)
R-warfarin	0.48 ± 0.06 (0.44, 0.51)	0.47 ± 0.09 (0.41, 0.52)	0.46 ± 0.08 (0.41, 0.51)
$AUC_{0-\infty}$ *(μg ml^{-1} h^{-1})*			
S-warfarin	65.4 ± 13.8 (56.6, 74.1)	47.7 ± 8.3 (42.4, 53.0)	57.8 ± 7.4 (53.1, 62.5)
R-warfarin	120.9 ± 32.9 (99.0, 142.7)	91.1 ± 15.4 (80.8, 101.3)	108.1 ± 18.3 (95.9, 120.2)
t_{max} *(h)*			
S-warfarin	1.29 ± 0.51 (0.97, 1.62)	1.26 ± 0.46 (0.97, 1.55)	1.30 ± 0.55 (0.95, 1.65)
R-warfarin	1.34 ± 0.48 (1.02, 1.66)	1.34 ± 0.48 (1.10, 1.73)	1.30 ± 0.52 (0.95, 1.65)
C_{max} *(μg ml^{-1})*			
S-warfarin	1.89 ± 0.26 (1.7, 2.0)	1.82 ± 0.34 (1.6, 2.0)	1.93 ± 0.31 (1.7, 2.1)
R-warfarin	1.92 ± 0.32 (1.7, 2.1)	1.84 ± 0.36 (1.6, 2.1)	1.89 ± 0.29 (1.7, 2.0)
$t_{1/2}$ *(h)*			
S-warfarin	31.7 ± 4.5 (28.8, 34.5)	25.1 ± 4.3 (22.4, 27.9)	29.2 ± 5.2 (25.9, 32.4)
R-warfarin	51.7 ± 9.6 (45.6, 57.8)	40.3 ± 3.9 (38.0, 42.7)	47.9 ± 7.8 (42.9, 52.9)
CL/F (ml min^{-1})			
S-warfarin	198 ± 38 (174, 223)	270 ± 44 (241, 297)	220 ± 29 (201, 238)
R-warfarin	110 ± 25 (94, 126)	142 ± 29 (123, 161)	119 ± 20 (106, 131)
V/F (l kg^{-1})			
S-warfarin	0.12 ± 0.03 (0.11, 0.14)	0.13 ± 0.03 (0.11, 0.15)	0.13 ± 0.03 (0.11, 0.15)
R-warfarin	0.10 ± 0.02 (0.09, 0.13)	0.10 ± 0.02 (0.10–0, 13)	0.10 ± 0.02 (0.10, 0.12)

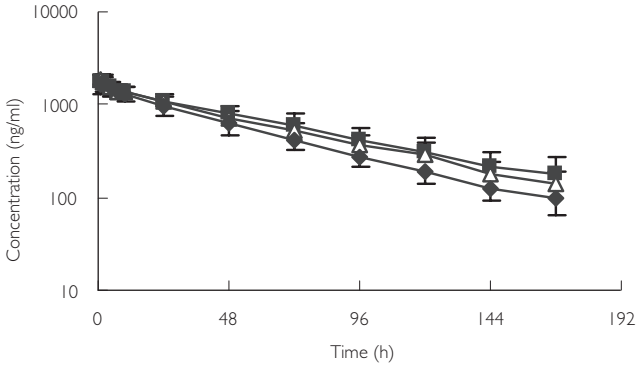

Figure 2
Mean R-warfarin log concentration-time profiles following single oral 25 mg *rac*-warfarin dose with warfarin only, warfarin + ginseng (GS) and warfarin + St John's wort (SJW) (error bars show SD, n = 12). R-warfarin (■), R-warfarin + GS (△), R-warfarin + SJW (◆)

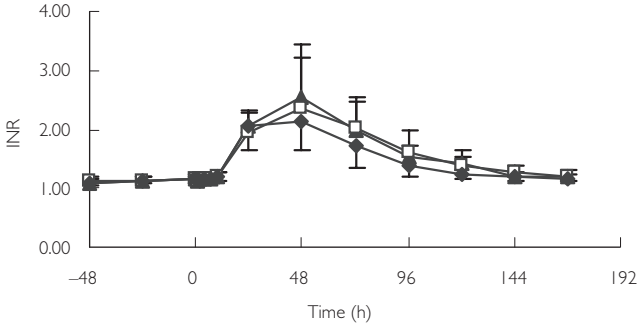

Figure 3
Mean INR-time profiles following single oral 25 mg *rac*-warfarin dose with warfarin only, Warfarin + ginseng (GS) and Warfarin + St John's wort (SJW) (error bars show SD, n = 12, warfarin dose administered on time 0). Warfarin + GS (▲), Warfarin only (□), Warfarin + SJW (◆)

Protein binding

The fraction unbound of S-warfarin and R-warfarin was 0.34 ± 0.11% and 0.48 ± 0.06%, respectively, for warfarin alone, and 0.36 ± 0.10% and 0.47 ± 0.09% for warfarin following administration with St John's wort, was 0.39 ± 0.16% and 0.46 ± 0.08% following treatment with ginseng, respectively, and protein binding of warfarin enantiomers did not change during the sample schedule. None of these differences was significant.

Pharmacodynamics

The ratios of the $AUC_{0–168}$ of INR and the 90% CIs were 0.79 (0.70, 0.95) for treatment with St John's wort, and 1.01 (0.88, 1.16) for ginseng (Table 3). There was a significant difference in warfarin pharmacodynamics between warfarin alone and coadministration with St John's wort (Table 4). However, there was no significant difference in warfarin pharmacodynamics when warfarin was ingested following treatment with ginseng. Neither St John's wort nor ginseng alone affected baseline INR or platelet aggregation.

Table 2
Mean ratios and 90% confidence intervals (CI) for log-transformed S-warfarin and R-warfarin pharmacokinetic parameters comparing herb treatment with warfarin-only controls ($n = 12$, $^*P < 0.05$)

Treatment	St John's wort (90% CI)	Ginseng (90% CI)
fu (%)		
S-warfarin	1.12 (0.96, 1.31)	1.10 (0.85, 1.42)
R-warfarin	1.04 (0.95, 1.14)	1.03 (0.94, 1.13)
$AUC_{0-\infty}$ (μg ml^{-1} h^{-1})		
S-warfarin	0.73 (0.65, 0.83)*	0.89 (0.82, 0.98)
R-warfarin	0.77 (0.67, 0.87)*	0.91 (0.84, 0.99)
t_{max} (h)		
S-warfarin	1.13 (0.76, 1.49)	1.20 (0.77, 1.62)
R-warfarin	1.17 (0.80, 1.54)	1.11 (0.78, 1.44)
C_{max} (μg ml^{-1})		
S-warfarin	0.95 (0.86, 1.05)	1.01 (0.90, 1.12)
R-warfarin	0.96 (0.84, 1.09)	0.98 (0.88, 1.09)
$t^l/_2$ (h)		
S-warfarin	0.79 (0.72, 0.87)*	0.92 (0.85, 0.99)
R-warfarin	0.79 (0.72, 0.86)*	0.93 (0.88, 0.99)
CL/F (ml min^{-1})		
S-warfarin	1.29 (1.16, 1.46)*	1.12 (1.03, 1.22)
R-warfarin	1.23 (1.11, 1.37)*	1.10 (1.01, 1.20)
V/F (l kg^{-1})		
S-warfarin	1.10 (0.97, 1.24)	1.04 (0.94, 1.14)
R-warfarin	1.06 (0.88, 1.24)	1.03 (0.95, 1.10)

Adverse events

Twelve subjects completed the study. No significant adverse events were observed. Three subjects reported changes in sleeping habits (waking up early in the morning) during St John's wort treatment.

Discussion

This study investigated the effect of two commonly ingested herbal medicines on the pharmacokinetics and pharmacodynamics of warfarin and their independent effect on INR and platelet aggregation using a standard study design widely used in investigating warfarin–drug interactions. The major finding was that the coadministration of St John's wort at recommended doses increased the apparent clearance of the warfarin enantiomers leading to a reduction in the pharmacodynamic effect of *rac*-warfarin.

In assessing potential herb–drug interactions, it is essential to use herbal medicines of known quality to

provide the best chance of being able to rigorously detect significant effects. In this study, HPTLC was used to characterize the constituents of proprietary preparations of St John's wort and ginseng prior to the study. As some variability in the composition of different brands of commercial herbal medicines products of St John's wort and ginseng has been described, it is important to establish the quality of the herbal medicines under investigation. There remains debate about which constituents of St John's wort and ginseng [3, 4, 7] might be involved in potential drug interactions, so the products employed in this study were not assessed for the specific content of individual constituents but for their overall content and quality.

In this study, warfarin, a racemic mixture, was administered and the pharmacokinetics of the individual enantiomers was studied. It is known that S-warfarin exhibits two to five times the anticoagulant activity of the R-enantiomer, and there are stereoselective differences in the pharmacokinetics of the enantiomers [11]. Elucidation of the pharmacokinetics of the warfarin enantiomers thus allows greater insight into the mechanism of the possible pharmacokinetic and pharmacodynamic interactions with this drug. S-warfarin is metabolized to S-7-hydroxywarfarin by CYP2C9 [12] while R-warfarin is metabolized by CYP1A2 and CYP3A4 [13]. The simultaneous investigation of warfarin enantiomer pharmacokinetics thus provided insight into the effect of St John's wort and ginseng on these drug metabolism pathways.

In vitro studies have demonstrated that St John's wort inhibits CYP2C9, CYP2D6 and CYP3A4 activity [14, 15]. Paradoxically St John's wort has also been reported to induce CYP1A2, CYP2E1 and CYP3A4 based on *in vivo* studies [16–23]. This effect has been attributed to the activation of the human pregnane X receptor (PXR) by the St John's wort constituent hyperforin which has been demonstrated both *in vitro* [24] and *in vivo* [25]. The present study employed a regimen of 14-day pretreatment with St John's wort which was based on the study by Wang *et al.* [23], who reported that following administration of St John's wort to healthy volunteers for 14 days, there was induction of CYP3A4 activity in the intestinal wall and liver, but no alteration in the activities of CYP2C9, CYP1A2, or CYP2D6. This conclusion was based on administration of a cocktail of probes substrate for the specific cytochromes (tolbutamide, CYP2C9; caffeine, CYP1A2; dextromethorphan, CYP2D6; oral midazolam, intestinal wall and hepatic CYP3A; and intravenous midazolam, hepatic CYP3A). This study also employed (at least) a 14-day washout period between treatment periods. As little is known about the offset of the induction of drug-metabolizing enzymes, this period was based on the half-lives of warfarin enantiomers [11] and the St John's wort constituents hypericin and hyperforin [4]. The analysis of variance in this randomized three-period study demonstrated no period effect, suggesting the inductive effect of St John's wort on drug-metabolizing enzymes had dissipated in the 14 days between treatments.

In the present study, treatment with St John's wort significantly induced not only CYP1A2 and/or CYP3A4 as evidenced by the effects on R-warfarin, but also CYP2C9 as determined by the effects on the pharmacokinetics of the S-enantiomer. The induction of CYP2C9 was in contrast to the observations of Wang *et al.* [23] and confirms the suggestions raised by Henderson *et al.*

Table 3
Mean ratios and 90% confidence intervals (CI) for warfarin pharmacodynamic parameters ($n = 12$, $^*P < 0.05$)

Treatment	St John's wort (90% CI)	Ginseng (90% CI)
INR baseline	0.99 (0.96, 1.01)	0.99 (0.97, 1.02)
AUC_{0-168} of INR	0.79 (0.70, 0.95)*	1.01 (0.88, 1.16)
Platelet aggregation	1.0 (0.88, 1.14)	1.0 (0.85, 1.06)

Table 4
Warfarin pharmacodynamic parameters following a single oral 25-mg *rac*-warfarin (WF) alone, in combination with either St John's wort (WF + SJW) or ginseng (WF + GS) (mean ± SD, 95% CI, $n = 12$)

Treatment	WF alone	WF + SJW	WF + GS
INR baseline	1.14 ± 0.07 (1.08, 1.20)	1.12 ± 0.06 (1.08, 1.20)	1.13 ± 0.05 (1.10, 1.12)
AUC_{0-168} of INR	111.0 ± 49.3 (79.6, 142.3)	88.3 ± 30.7 (68.8, 107.8)	111.1 ± 43.1 (83.9, 138.7)
Platelet aggregation (Ω)	7.7 ± 2.2 (5.6, 9.1)	7.5 ± 1.1 (6.5, 8.2)	7.1 ± 1.4 (6.0, 7.8)

[3] in their review of St John's wort drug interactions. Surprisingly, St John's wort did not significantly affect the UER of S-7-hydroxywarfarin while it induced the clearance of S- and R-warfarin; the mechanism of this effect is not clear and needs further study. Treatment with St John's wort did not have an independent effect on INR or platelet aggregation, nor did it influence warfarin enantiomer protein binding or distribution.

A few ginseng–drug interactions have been reported in the literature. One case report found that coadministration of ginseng led to a decline in INR during warfarin therapy [5]. However, no significant effect was found in a trial in humans, where the activities of various cytochrome P450 isoenzymes were assessed using the phenotypic ratios of probe drugs that included midazolam (CYP3A4), caffeine (CYP1A2), chlorzoxazone (CYP2E1), and debrisoquin (CYP2D6) [17]. Furthermore, Donovan *et al.* [26] demonstrated that Siberian ginseng did not affect CYP3A4 and CYP2D6 activity using alprazolam and dextromethorphan, respectively, as model substrates in healthy subjects. A warfarin–ginseng interaction study in rats using both single and multiple doses of warfarin found no effect of ginseng on warfarin pharmacokinetics [6]. Consistent with these latter reports, the present study confirmed that ginseng did not affect the pharmacokinetics and pharmacodynamics of warfarin in human subjects. Furthermore, ginseng had no effect on the activity of CYP1A2, CYP3A4 or CYP2C9 in the healthy volunteers pretreated with this herb. Ginseng did affect the UER for S-7-hydroxywarfain, but did not induce the clearance of warfarin; the explanation for this finding is not clear and needs further study.

Several articles have suggested that constituents of ginseng may inhibit thrombin, collagen or arachidonic acid-induced platelet aggregation *in vitro* using human platelet-rich plasma [27–29]. However, contrary to these reports, ginseng did not significantly affect the INR and platelet aggregation in the present study. This discrepancy between *in vitro* and *in vivo* effects could be related to metabolic biotransformation of ginseng constituents after oral dosing as suggested by a bioavailability study of ginsenoside Rb1 and Rg1 in rats [30].

In summary, this study found that St John's wort, when administered to healthy male subjects in a single-ingredient herbal product at recommended doses, induced the metabolism of both S-warfarin and R-warfarin in humans with a subsequent effect on INR. By contrast, Korean ginseng at recommended doses had little effect on warfarin metabolism or effect in healthy subjects. These findings suggest that there is a potential for interactions with St John's wort for drugs that are the substrates for CYP2C9 and/or CYP3A4. This finding thus provides rigorous evidence to support the recommendation that close monitoring of INR should be undertaken in patients receiving this herb–drug combination. Further research is needed to clarify the implications of these finding for elderly patients (who are likely to receive warfarin) and for people using a range of herbal and complementary medicines (often in a variety of doses).

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