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Possible Influences of Ginseng on the Pharmacokinetics and Pharmacodynamics of Warfarin in Rats

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Abstract

We evaluated the significance of a reported clinical case of drug–drug interaction between ginseng and warfarin using a robust pharmacokinetic/pharmacodynamic approach in a rat model.

The influence of ginseng on the pharmacokinetics and pharmacodynamics of oral warfarin after a single dose ($2\,mg\,kg^{-1}$) and at steady state ($0{\cdot}2\,mg\,kg^{-1}$ daily $\times$ 6 days) was studied in male Sprague–Dawley rats. Prothrombin time was employed as a pharmacodynamic index. Warfarin plasma concentration and vitamin K content in the ginseng extract were assessed by validated HPLC assays.

The pharmacokinetics of warfarin after a single dose were not altered in the presence of ginseng; peak plasma concentration (control $7{\cdot}8 \pm 0{\cdot}5$; ginseng $7{\cdot}3 \pm 2{\cdot}5\,\mu g\,mL^{-1}$), time to peak (control $2{\cdot}6 \pm 1{\cdot}0$; ginseng $3{\cdot}1 \pm 1{\cdot}1$ h), elimination half-life (control $14{\cdot}3 \pm 5{\cdot}8$; ginseng $10{\cdot}6 \pm 3{\cdot}1$ h), and oral clearance (control $17{\cdot}5 \pm 3{\cdot}3$; ginseng $20{\cdot}2 \pm 5{\cdot}5\,mL\,h^{-1}$) were not significantly different ($P > 0{\cdot}05$). Similarly, alterations in the pharmacokinetics of warfarin were not detected under the multiple dosing paradigm. Under both dosing conditions, ginseng also showed no significant impact on the pharmacodynamics of warfarin as assessed by the area under the prothrombin time vs time curve (multiple dosing; control 3776 ± 619, ginseng 3830 ± 362 s h) and maximum prothrombin time (control $57{\cdot}2 \pm 11{\cdot}8$, ginseng $63{\cdot}3 \pm 9{\cdot}1$ s). Furthermore, the content of vitamin K was undetectable in the ginseng decoction.

In conclusion, current data obtained in the rat showed no significant impact of ginseng on the pharmacokinetics/pharmacodynamics of warfarin when they are concomitantly administered.

Ginseng, a commonly used natural product, is reputed as a "herb of eternal life". A survey of herbal-based over-the-counter products indicates that 28% of them contain ginseng; this frequency is much higher than any other single herbs (Tyler 1995). It has been estimated that ginseng comprises 15–20% of the total annual sales of botanical products in the United States (Murray 1995). As a result, it is may be expected that concomitant use of ginseng with other medications in clinical practice is common. However, information regarding drug interactions between ginseng and therapeutic agents available on the market remains very scanty.

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Warfarin is a widely used oral anticoagulant requiring a tight control in dosage; drug interaction is of primary concern when perturbations to its absorption, metabolism, or protein binding are suspected. As the use of herbal products is increasing, interactions between these natural remedies and warfarin have become a valid concern. A previous study has shown a significant pharmacokinetic/pharmacodynamic interaction between *Salvia miltiorrhiza* and warfarin (Chan et al 1995). In addition, a recent clinical case report has documented a probable interaction between ginseng and warfarin (Janetzky & Morreale 1997). In this regard, a transient decrease in the anticoagulation effect of warfarin has been observed in a patient maintained on stable warfarin therapy (>5 years) during a two-week period of self-medication

with a commercially available ginseng capsule (Ginsana). Such a decrease in the activity of warfarin was shown to be reversible upon cessation of ginseng use (Janetzky & Morreale 1997).

This case report highlights the potential danger of ginseng intake to patients receiving warfarin. As ginseng is easily accessible to the general public throughout the world and sold in various forms, e.g. dry roots, processed roots, herbal teas, chewing gums, candies and cosmetics, the potential risk of ginseng interacting with drugs having a narrow therapeutic window should not be underestimated. In this study, pharmacokinetic and pharmacodynamic evaluations on the significance of drug interaction between ginseng and warfarin were examined in detail using a rat model. Since vitamin K is a natural antagonist of warfarin (Fasco & Principe 1982), vitamin K content in ginseng roots was also analysed.

Materials and Methods

Preparation of ginseng extract

Ginseng roots, purchased from a local herbal shop in Hong Kong, were authenticated by macroscopic examination and microscopic identification in our pharmacognosy laboratory. For the preparation of ginseng extract used in animal studies, the powdered root was boiled with water for 3 h and the extract was evaporated to a concentration equivalent to 0·5 g crude drug mL^{-1}.

Chemical reagents and apparatus

Warfarin, phytomenadione (vitamin K1), menadione (vitamin K3) and thromboplastin reagent were purchased from Sigma Chemical Co., USA. Acenocoumarol, an internal standard for the warfarin assay, was supplied as a gift by Novartis Pharmaceuticals, NJ, USA. All other chemical reagents were obtained from commercial sources and organic solvents were either HPLC or analytical grade.

A Waters HPLC system consisting of a model 600 controller, a photodiode array detector, an autosampler and an in-line degasser, was employed for the quantitation of warfarin. A guard column packed with Novapak C_{18} (Waters) and a reversed-phase column (150 × 4·60 mm; particle size: 5 μm, Phenomenex) were employed.

Animal studies

Male Sprague–Dawley rats (220–240 g) were housed under controlled conditions (23 ± 2°C, 55% r.h. and 12-h light–dark cycle) and were allowed free access to food and water before the experiments. For the pharmacokinetic/pharmacodynamic study, rats were fasted overnight before oral warfarin administration and food supply was resumed 24 h after dosing. Rats were cannulated through the right jugular vein one day before blood sampling. Blood (0·5 mL) was withdrawn via the cannula at specific times after warfarin dosing. The blood sample collected was mixed with sodium citrate (3·8%, 0·05 mL) immediately before centrifugation (3000 *g* for 10 min), plasma was then harvested for the HPLC assay. For selected blood samples, prothrombin time (PT) measurements were performed along with the warfarin assay.

Single dose study. The rats were treated with the ginseng decoction (2 g crude drug kg^{-1}) orally twice daily for five days and were given a single oral dose of warfarin (2 mg kg^{-1}) on day 6. This design would allow enough time for the effects of ginseng to be fully expressed. Blood samples were collected at 0, 2, 4, 8, 12, 24, 36, 48 and 72 h after warfarin dosing for HPLC assay. Prothrombin time was also determined for blood samples collected at 0, 24, 48, 72 h post-dosing. In the control group, ginseng decoction was replaced by physiological saline while all other procedures remained identical.

Steady-state study. To mimic the condition of chronic dosing of these two agents, a multiple dosing paradigm was introduced. To achieve steady-state warfarin plasma concentrations, rats were orally dosed with warfarin (0·2 mg kg^{-1} once daily) and ginseng decoction (2 g crude drug kg^{-1} twice daily) for five days. The use of a lower dose for the steady-state study was to avoid over-exposure of the study animals to the anti-coagulation effect of warfarin after prolonged dosing. A blood sample (0·5 mL) was withdrawn once daily before each warfarin dose and also at time 0, 2, 4, 8, 12, 24, 48, 72, 96, 120 and 144 h after the last dose on day 6. These plasma samples were subjected to the HPLC assay for warfarin. Prothrombin time was assessed once daily for seven days commencing with the initial dose. In the control group, physiological saline was administered instead of ginseng and the same experimental procedures were followed.

HPLC assay for warfarin

For the quantitation of warfarin concentrations in plasma, methanol (400 μL) was added to the plasma sample (0·2 mL) for protein precipitation and was followed by the addition of distilled water (1·5 mL), hydrochloric acid (0·5 mL, 2 M) and

acenocoumarol (30 μL; 10 ng μL^{-1}). The mixture was extracted with chloroform (7 mL) and centrifuged at 3000 *g* for 15 min. The chloroform layer was collected and evaporated to dryness at 45°C under a stream of nitrogen. The residue was reconstituted with 200 μL phosphate buffer (pH 5·7) before injection (100 μL) onto the HPLC system.

The mobile phase used for the HPLC assay consisted of 45% methanol buffered with 10 mM phosphate (pH 5·7). The flow rate was set at 1 mL min^{-1} and detection was preformed at 300 nm. Complete separation of warfarin and acenocoumarol was achieved with retention times at 10 and 13 min, respectively. A linear calibration curve was obtained for warfarin in plasma with a range from 0·125 to 10 $\mu g\,mL^{-1}$. Linearity was reflected by the excellent correlation coefficient (0·9998) of linearity (r^2) obtained. The detection limit of the plasma assay for warfarin was 12·5 ng mL^{-1}. Adequacy of the drug assay was supported by the 11·8% coefficient of variation for the interday assay variability.

Measurement of prothrombin time

For the measurement of prothrombin time, a reconstituted thromboplastin reagent (PT reagent) and plasma samples (0·1 mL) were pre-warmed for 1–2 min at 37°C before mixing of the two components. Time (s) was recorded as soon as the PT reagent was introduced into the plasma sample till a clot was formed. Duplicated measurements were performed for quality control samples to ensure accuracy.

Evaluation of the vitamin K content in ginseng roots

Ginseng powder (2 g) was sonicated with ethanol (50 mL) at 60°C for 1·5 h. The solution was then filtered and concentrated to dryness at 65°C under vacuum. The residue was dissolved in chloroform (2 mL). HPLC analysis for vitamin K was afforded using a mobile phase consisting of 100% methanol (flow rate: 1·5 mL min^{-1}) for K1 and 40% methanol (flow rate: 1·0 mL min^{-1}) for K3. The ginseng extract (20 μL, 200 mg mL^{-1}) and standard solutions of vitamin K1 and K3 (20 μL, 0·2 mg mL^{-1},) were subjected to the HPLC system for analysis. The retention times and the corresponding UV spectra of the ginseng extract, vitamins K1 and K3 were recorded for comparison.

Analysis of pharmacokinetic/pharmacodynamic parameters of warfarin

The plasma concentration–time data of warfarin were assessed by non-compartmental analysis. The maximum plasma concentration (C_{max}) and the time achieving C_{max} (T_{max}) were directly observed from the data obtained for individual animals. Least-square regression analysis was employed on the terminal elimination phase for the estimation of elimination rate constant (k_{el}). Elimination half-life ($t_{\frac{1}{2}}$) of warfarin was computed as $0{\cdot}693/k_{el}$ and the area under the curve from time zero to infinity ($AUC_{0\rightarrow\infty}$) was estimated by trapezoidal integration as:

$$AUC_{0\rightarrow\infty} = AUC_{0-t} + C_t/k_{el} \qquad (1)$$

where AUC_{0-t} is the AUC from time zero to time t and C_t is the warfarin concentration for the last plasma sample collected at time t. Other parameter estimates including oral clearance (CL/F) and apparent volume of distribution (Vd/F) were estimated by standard procedures. The degrees of drug accumulation describing the absorption and elimination phases were estimated as C_{max-MD}/C_{max-SD} and C_{24-MD}/C_{24-SD}, respectively. The former is the ratio of the mean C_{max} observed in the multiple to that of the single dose study and the latter is the ratio of the mean plasma warfarin concentration measured at 24 h after warfarin dosing in the multiple to that of the single dose study. Pharmacokinetic estimates were corrected for the lower dose used in the steady-state study.

The degree of anticoagulation was assessed by the prothrombin time (PT). The maximum PT (PT_{max}) and the time achieving the PT_{max} ($T_{max,PT}$) were directly observed from the PT vs time data. Because of the indirect nature of the anti-coagulation effect and the different dosages employed for the single and multiple dosing, a direct comparison of the PT data between the two dosing conditions was not feasible. However, to allow better assessment of the effects of ginseng on the anticoagulation profiles, the respective area under the PT vs time curve from time zero to the time of the last measurement, i.e. AUC_{0-72} for the single dose study and AUC_{0-144} for the multiple dose study, was determined using linear trapezoidal summation. Statistical significant differences in the experimental data and derived pharmacokinetic/pharmaco-dynamic parameter estimates between the study and control groups were assessed by Student's *t*-test with the level of statistical significance set at 0·05.

Results

Identification of ginseng roots

Macroscopic examination of the purchased ginseng revealed that the surface of the roots was greyish-

yellow in colour with sparse and shallow transverse-striations and distinct longitudinal wrinkles. Microscopic identification of the powdered ginseng showed the presence of large quantities of parenchyma cells and fibres in various sizes, and the resin canals with yellow secretion. Simple or compound starch granules, cluster crystals of calcium oxalate and vessels with bordered pits were also observed. These microscopic features conformed to that of the authentic sample of *Panax ginseng* and were consistent with those described in the Chinese Pharmacopoeia (Anonymous 1992).

Single dose and steady-state pharmacokinetics of warfarin

Data obtained in the single dose ($2\,mg\,kg^{-1}$) study revealed that the rate of warfarin absorption was only moderate; the maximum plasma warfarin concentration ($7{\cdot}8 \pm 0{\cdot}5\,\mu g\,mL^{-1}$) was achieved at $2{\cdot}6 \pm 1{\cdot}0$ h (Table 1). The apparent volume of distribution (Vd/F) was $1{\cdot}48 \pm 0{\cdot}74\,L\,kg^{-1}$, i.e. approximately 2·5-fold of total body water, suggesting a significant amount of the drug was taken up by body tissues. In fact, such a large volume distribution might have a positive contribution to the long half-life ($14{\cdot}3 \pm 5{\cdot}8$ h) observed. Under steady-state conditions ($0{\cdot}2\,mg\,kg^{-1}$ for 6 days), dose-normalized pharmacokinetic parameter estimates (equivalent to $2\,mg\,kg^{-1}$) for warfarin were comparable to those obtained in the single dose study (Table 1), suggesting that the pharmacokinetic system was linear for the two doses employed and was stationary over time during multiple dosing. In addition, the similar trough warfarin levels obtained pre-dose and at 24 h post-dose confirmed that the steady-state condition had been achieved after six days of dosing.

Impact of ginseng on the pharmacokinetics of warfarin

Pharmacokinetic profile of warfarin after a single oral dose ($2\,mg\,kg^{-1}$) with or without ginseng co-administration is shown in Figure 1a. All pharmacokinetic parameter estimates including C_{max}, T_{max}, AUC, CL/F, $t_{\frac{1}{2}}$, and Vd/F for the different treatment groups are summarized in Table 1. These results indicate that the pharmacokinetics of warfarin were not altered and no statistically significant differences ($P > 0{\cdot}05$) were observed for the pharmacokinetic parameter estimates between the ginseng and control groups.

The mean plasma concentrations vs time profiles for warfarin obtained in both the control and ginseng-treated rats after six days of oral warfarin dosing ($0{\cdot}2\,mg\,kg^{-1}$ once daily) are presented in Figure 1b. These profiles do not suggest any substantial effects of ginseng on the pharmacokinetics of warfarin. Indeed, the pharmacokinetic profiles were essentially superimposable with each other under both dosing conditions suggesting a lack of acute or delayed effects of ginseng on the pharmacokinetics of warfarin. This observation is of particular relevance to the situation when both agents are taken on a long-term basis. Without any significant alterations ($P > 0{\cdot}05$) in C_{max}, T_{max}, $t_{\frac{1}{2}}$, Vd/F, and CL/F, all essential processes controlling the pharmacokinetic behaviour of warfarin were not subjected to changes by the co-administration of ginseng.

Table 1. Pharmacokinetic and pharmacodynamic parameter estimates (mean ± s.d.) of warfarin after a single oral dose ($2\,mg\,kg^{-1}$) or multiple dosing ($0{\cdot}2\,mg\,kg^{-1}$ once daily for 6 days) with or without ginseng coadministration.

Pharmacokinetics	Single dose		Steady-state (dose normalized data)	
	Warfarin	Warfarin + ginseng	Warfarin	Warfarin + ginseng
C_{max} ($\mu g\,mL^{-1}$)	7·8 ± 0·5	7·3 ± 2·5	9·4 ± 1·4	9·1 ± 1·2
T_{max} (h)	2·6 ± 1·0	3·1 ± 1·1	2·0 ± 0·0	2·0 ± 0·0
Vd/F ($mL\,kg^{-1}$)	1483 ± 746	1300 ± 743	1419 ± 294	1567 ± 305
$t_{\frac{1}{2}}$ (h)	14·3 ± 5·8	10·6 ± 3·1	16·3 ± 3·0	17·1 ± 3·6
k_{el} (h^{-1})	0·06 ± 0·03	0·07 ± 0·02	0·04 ± 0·01	0·04 ± 0·01
CL/F ($mL\,h^{-1}$)	17·5 ± 3·3	20·2 ± 5·5	15·0 ± 1·0	15·8 ± 1·9
AUC[a] ($mg\,h\,mL^{-1}$)	118 ± 22	104 ± 21	133 ± 10	127 ± 2
Pharmacodynamics				
PT_0 (s)	14·4 ± 2·4	15·1 ± 2·0	15·5 ± 5·1	17·5 ± 4·5
PT_{max} (s)	75·0 ± 10·9	75·7 ± 7·6	57·2 ± 11·8	63·3 ± 9·1
$T_{max,PT}$ (s)	24·0 ± 0·0	24·0 ± 0·0	144·0 ± 0·0	144·0 ± 0·0
AUC_{0-72} (s h)	2518 ± 324	2563 ± 199		
AUC_{0-144} (s h)			3776 ± 619	3830 ± 362

[a]$AUC_{0-\infty}$, and $AUC_{0-\tau}$ for single dose and multiple dose study, respectively. No statistically significant differences ($P > 0{\cdot}05$) were detected for any of the pharmacokinetic/pharmacodynamic parameters for warfarin.

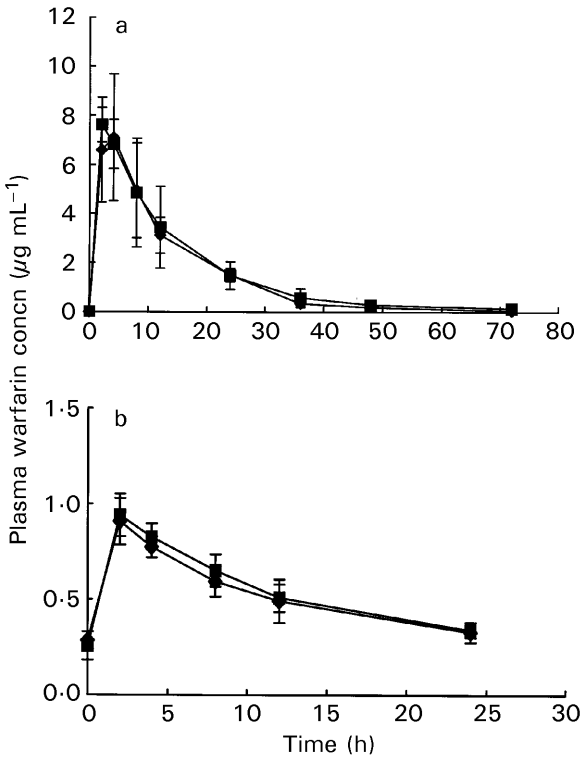

Figure 1. The plasma concentration vs time profiles for warfarin after a single oral dosing (2 mg kg^{-1}) (a) and multiple dosing (0·2 mg kg^{-1} once daily for 6 days) (b). Control rats ■, n = 6; ginseng-treated rats ◆, n = 6. Values are mean ± s.d.

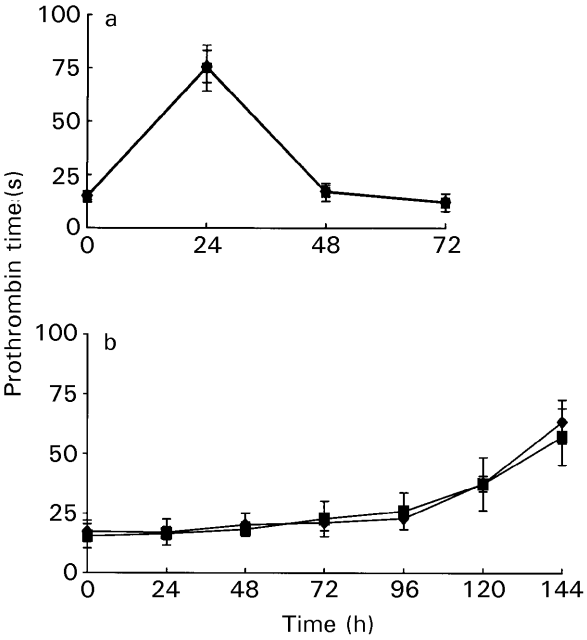

Figure 2. The prothrombin time vs time profiles for warfarin after a single oral dose (2 mg kg^{-1}) (a) and multiple dosing (0·2 mg kg^{-1} once daily for 6 days) (b). Control rats ■, n = 6; ginseng-treated rats ◆, n = 6. Values are mean ± s.d.

Impact of ginseng on the pharmacodynamics of warfarin

The time course of changes in prothrombin time following a single oral dose of warfarin (2 mg kg^{-1}) and five days of ginseng pretreatment was similar to that of the saline control (Figure 2a). Derived pharmacodynamic parameter estimates did not show significant differences between the two groups for either dosing conditions (Table 1). Under the single dose regimen, PT peaked at 21 h post warfarin dosing and returned to baseline value at 48 h. Baseline PT values in the ginseng (15·14 ± 2·03 s) and control groups (14·43 ± 2·37 s) were similar before warfarin dosing suggesting that the five-day ginseng pretreatment did not alter the coagulation function of the study rats.

The time course of changes in prothrombin time observed after multiple oral warfarin dosing (0·2 mg kg^{-1} daily for 6 days) concomitantly with ginseng or saline are shown in Figure 2b. The associated pharmacodynamic parameters are summarized in Table 1. Compared to the control, there was no significant change in the PT profile and the derived pharmacodynamic data in the ginseng group.

Evaluation of vitamin K content in ginseng root

Comparing the HPLC analysis of vitamin K1 with the analysis of the ginseng preparation, there were a few peaks in the ginseng preparation showing a similar retention time to K1 (data not shown). Further examination of the UV spectra of these peaks revealed that they possessed different absorption features and were not consistent to that of vitamin K1. No peak at the retention time corresponding to vitamin K3 could be found in the ginseng extract (data not shown). Thus, no detectable amounts of vitamin K1 and K3 were present in the ginseng extract.

Discussion

The case report of Janetzky & Morreale (1997) suggested that ginseng consumption may negatively affect the therapeutic effect of warfarin. However, data obtained in this study suggest that ginseng did not alter the pharmacokinetic and pharmacodynamic characteristics of warfarin, thereby implying that inhibition of the vitamin K-dependent carboxy-lation of coagulation Factors II, VII, IX and X by warfarin (Nicholas & Andrew 1992) was not affected. Furthermore, as warfarin exists naturally as a mixture of *R*- and *S*-enantiomers with different potencies and in-vivo characteristics, the lack of an interaction suggests

ginseng did not induce any changes in the pharmacokinetics/pharmaco-dynamics of these individual enantiomers.

In both single and multiple dose studies, maximum plasma concentration (C_{max}) and T_{max} were similar in the ginseng and control groups suggesting that the rate of warfarin absorption was not altered. In addition, ginseng did not cause a change in the extent of warfarin absorption as the AUC values were similar under the two dosing schemes. As warfarin is a highly protein-bound drug, i.e. $99 \pm 1\%$ (Benet 1996), changes in its degree of protein binding should be reflected in modifications of tissue distribution. The stable Vd/F estimates obtained under both dosing conditions suggest protein drug binding and thus tissue distribution of warfarin were not affected by ginseng. After a five-day treatment of ginseng, perturbation of hepatic P450 activity by ginseng, if any, should be fully expressed. However, the lack of effect of ginseng co-administration on the $t_{\frac{1}{2}}$ value of warfarin suggests that ginseng did not alter hepatic metabolism. Similarly, CL/F, i.e. the parameter estimate describing warfarin's disposition, was not modified by the presence of ginseng (Table 1). Because pharmacokinetic perturbations were not detected under either of the dosing conditions, the degrees of drug accumulation describing both the absorption and elimination phases remained unchanged, i.e. C_{max-MD}/C_{max-SD} for absorption: 1·20 for control group vs 1·25 for ginseng group; C_{24-MD}/C_{24-SD} for elimination: 2·30 vs 2·20.

The maximum hypothrombinaemic effect (PT_{max}) of a single oral dose of warfarin ($2\,mg\,kg^{-1}$) occurred approximately 21 h after the peak plasma concentration of warfarin. This confirms that the anticoagulant effect of warfarin is delayed until the content of vitamin K-dependent clotting factors is depleted. This delay in response further justifies the need for the steady-state study design. Interestingly, there was no detectable amount of vitamin K in the ginseng extract and this is in agreement with the lack of change in the prothrombin time profiles even when ginseng was added.

Data obtained in the present animal study suggest the processes governing absorption, distribution, metabolism, and elimination of warfarin as well as its pharmacological effect are not altered by the presence of ginseng. However, it is advisable not to over-interpret these data because of the possible limitations of extrapolating animal data to man. Further clinical investigations are necessary to rule out the existence of such an interaction in man.

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