EFFECT OF UBIDECARENONE ON WARFARIN ANTICOAGULATION AND PHARMACOKINETICS OF WARFARIN ENANTIOMERS IN RATS

Stephanie Zhou and Eli Chan*

Department of Pharmacy, National University of Singapore
Singapore, Republic of Singapore

SUMMARY

Interaction between the antioxidant ubidecarenone and the oral anticoagulant warfarin enantiomers was investigated in rats. The decreased hypoprothrombinemic response, assessed by means of percent changes of prothrombin complex activity and clotting factor VII activity, to warfarin, was observed following oral administration of 1.5 mg/kg racemic warfarin to rats during an 8-day oral regimen (10 mg/kg daily) of ubidecarenone. The antioxidant had no apparent effect on the *in vitro* rat serum protein binding of warfarin enantiomers. Treatment with ubidecarenone did not affect the absorption and distribution of the *S*- and *R*-enantiomers of warfarin, but produced a significant increase in the total serum clearance values of both *R*- and *S*-warfarin in rats. This effect was more pronounced with *R*-warfarin than with *S*-warfarin. The increased clearance values are attributable to acceleration of certain metabolic pathways and renal excretion of the warfarin enantiomers.

KEY WORDS

warfarin, ubidecarenone, pharmacokinetics, pharmacodynamics, drug interactions, rat

*Author for correspondence:
Associate Professor Eli Chan
Department of Pharmacy
National University of Singapore
9, Science Drive 2
Singapore 117546, Republic of Singapore
e-mail: phaelic@nus.edu.sg

INTRODUCTION

Ubidecarenone, 2,3-dimethoxy-5-methyl-6-decaprenyl benzoquinone or coenzyme Q_{10}, is a fat-soluble vitamin-like substance. It has an intrinsic role in the respiratory electron transport system in mitochondria /1/, and a direct regulatory role on succinyl and NADH dehydrogenases /2/. Ubidecarenone, which also functions as an unspecific antioxidant possessing membrane stabilizing properties, is important for its clinical effects in preventing cellular damage during myocardial ischemia and reperfusion /3,4/. Since the efficacy of ubidecarenone in the treatment of heart diseases has been well established /5-7/, people may take it as a natural health product, which is freely available to the public without restriction, to improve heart function. A patient report has pointed out that patients on warfarin anticoagulation therapy concurrently administered with ubidecarenone experience a decrease in the anticoagulant response to warfarin /8/.

Warfarin is a widely prescribed oral anticoagulant, which is available in the form of a racemic mixture consisting of equal amounts of *R*- and *S*-warfarin /9/. The *S*-enantiomer of warfarin is much more potent than the *R*-enantiomer /10,11/, and the elimination of warfarin, via oxidative and reductive pathways, is stereoselective /12,13/. Some properties of warfarin, such as narrow therapeutic index, high protein binding, and cytochrome P450-dependent metabolism, render it prone to many drug interactions /14/. Interacting drugs can affect the metabolism of warfarin in a stereochemical manner, thereby resulting in marked differences in modifying the anticoagulant response to warfarin /15,16/.

The present study was undertaken to investigate the potential interaction between warfarin and ubidecarenone. The interaction was assessed by quantification of the pharmacological response produced by warfarin, measurement of pharmacokinetic parameters associated with the *R*- and *S*-enantiomers, and determination of the respective metabolic fates of the two enantiomers with or without the concurrent administration of ubidecarenone in rats.

MATERIALS AND METHODS

Chemicals and reagents

Ubidecarenone was purchased from Asha Pharmaceuticals (Saskatoon, Canada). Racemic warfarin sodium salt, chlorowarfarin, barbital, carbobenzyloxy-L-proline, and dicyclohexylcarbodiimide were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Warfarin alcohols, 4′-, 6-, and 7-hydroxywarfarin were synthesized according to previously reported methods with some modification /17,18/. AnalaR grade dimethyl sulfoxide (DMSO), trisodium citrate dihydrate and sodium chloride were purchased from Merck Co. (Schuchardt, Germany). The solvents used included acetonitrile, *n*-butylamine, chloroform, diethyl ether, ethyl acetate, *n*-hexane and methanol, which were either of analytical grade for extraction or high-performance liquid chromatography (HPLC) grade for HPLC analysis. They were obtained from Labscan Limited Co. (Dublin, Ireland).

Factor VII deficient plasma (Lot no.: 0077H6185) was obtained from Sigma Diagnostics (St. Louis, MO, USA). Neoplastine CI Plus 5 (Lot no.: 992531) was supplied by Diagnostica Stago (France). Protein assay kits were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Tween 80 was provided by the Pharmaceutical Department, Health Ministry of Singapore. Milli-Q water was obtained using the Millipore® Continental Water System (Burlington, MA, USA).

Animals

Male Sprague-Dawley rats (220-250 g) were purchased from the Laboratory Animal Center, Singapore. Animal experiments were approved by the Institutional Animal Care and Use Committee of this Center. Animals were housed separately in a metabolic cage and kept on a 12-h light/dark cycle for a minimum of 3 days before being used in experiments. Food and water were available *ad libitum*.

In vitro effect of ubidecarenone on serum protein binding of warfarin enantiomers

Serum pooled from eight randomly selected male Sprague-Dawley rats was spiked with racemic warfarin, 0.05 and 0.15 μmol/ml (i.e., mM). To 1-ml aliquots of this serum were added 10-μl portions of

ubidecarenone (dissolved in DMSO) to yield final concentrations of 0, 0.005 and 0.025 μmol/ml. Serum protein binding of warfarin enantiomers was determined by equilibrium dialysis, using a Spectrum equilibrium dialyser (Spectrum Laboratories Inc., USA) with Teflon dialysis cells (type Semi-Micro) and dialysis membranes (Spectra/Por 3, 3500 MWCO) placed between two half-cells. One ml of serum containing racemic warfarin with or without ubidecarenone was equilibrated against 1 ml of isotonic 0.067 M sodium phosphate buffer (pH 7.4). Dialysis units were immersed in a 37°C temperature-controlled water bath and rotated at 10 rpm for 4 h. Samples were then obtained from both the buffer side (800 μl) and serum side (50 μl) and submitted to HPLC analysis. Total protein concentrations in serum were determined using Bradford's method /19/ with bovine serum albumin as a standard calibrator before and after dialysis. The observed unbound fraction ($f_{u,obs}$) was corrected for volume shift to calculate the true unbound fraction ($f_{u,true}$) using the previously established equation /20/:

$$f_{u,true} = \frac{f_{u,obs}}{f_{u,obs} + (1+\delta)(1-f_{u,obs})} \quad [1]$$

where δ is the fractional increase in the volume of the protein compartment, which was determined using the following equation /20/:

$$\delta = \frac{P_{predialysis}}{P_{postdialysis}} - 1 \quad [2]$$

where P is the protein concentration.

***In vivo* study protocol**

Two groups of male Sprague-Dawley rats (n=10 per group), in a parallel design, received ubidecarenone (10 mg/kg body weight per day) in 10% Tween 80 or an equal volume of 10% Tween 80 (as the control) by oral intubation for eight consecutive days. During this time animals had access to food and water *ad libitum* except when fasted overnight before administration of warfarin and for four hours afterwards. On day 4, each rat received a single oral dose of racemic warfarin (1.5 mg/kg, dose volume = 5 ml/kg) by oral intubation either

alone or with ubidecarenone. Blood samples were taken prior to, and at 1, 3, 6, 12, 24, 48, 72, and 96 h, and the urine samples were collected prior to, and over the 0-24, 24-48, 48-72, 72-96 and 96-120 h intervals after warfarin administration. About 400 µl of blood obtained without anticoagulation was centrifuged (10 min at 3000 *g*) to separate serum. Serum samples obtained were then stored at -20°C before HPLC analysis. ~50 µl of blood was used for diluted plasma preparation according to the established procedure /21/. Briefly, the blood sampling method consists of orbital bleeding, blood sample weighing, gravity calculation and sodium citrate buffer volume adjustment. The subsequent anticoagulant effect measurement was performed within one hour after blood sampling.

Assessment of anticoagulation

The degree of anticoagulation was assessed by means of the prothrombin time and the factor VII clotting time, measured using a coagulation testing system (BBL FibroSystem®, USA). The former was determined by the one-stage prothrombin time test, while the latter was determined by the prothrombin time test with the addition of a reagent factor VII-deficient plasma. Furthermore, the prothrombin time and factor VII clotting time were transformed to the prothrombin complex activity (PCA) and clotting factor VII activity (CFA), respectively, by use of the log-log functional relationship between the measured clotting time (in seconds) and the respective activities (in percent of normal activity).

The parameters used to assess the degree of blood coagulability were the minimum prothrombin complex activity (PCA_{min}) and clotting factor VII activity (CFA_{min}), and the area under the effect-time curve, calculated by the linear trapezoidal approximation.

Chemical analysis

By use of the previously established HPLC assay method /22/, warfarin enantiomers were determined simultaneously in serum samples, as were the enantiomers of the various warfarin metabolites, including *S,S*-, *S,R*-, *R,S*- and *R,R*-warfarin alcohols, *S*- and *R*-4′-hydroxywarfarin, *S*- and *R*-6-hydroxywarfarin, and *S*- and *R*-7-hydroxywarfarin, in urine samples. Briefly, the assay method involved solvent extraction of serum and urine samples followed by formation

of diastereoisomers of *S*- and *R*-warfarin and their metabolites, by reaction of the enantiomers with carbobenzyloxy-L-proline in the present of dicyclohexylcarbodiimide. The resultant diastereoisomers were separated on a silica HPLC analytical column and then, after post-column aminolysis with *n*-butylamine, quantified by fluorescence detection at an excitation wavelength of 310 nm and an emission wavelength of 370 nm. The HPLC system for the assay consisted of two sets of solvent delivery system (LC-10AT, Shimadzu, Japan), a fluorescence detector (RF-10AXL, Shimadzu, Japan), an autosampler (Bio-Rad Inc., CA, USA), an integrator (Hewlett Packard Inc., CA, USA), a Phenomenex® silica analytical column (250 mm × 5 mm i.d.), and a stainless steel post-column (250 mm × 5 mm i.d.) packed with glass beads (~40 μm).

Pharmacokinetic analysis

The serum drug concentration-time data were analyzed using WinNonlin Version 1.1 computer program (Scientific Consulting Inc., Lexington, KY, USA), an iterative curve-fitting program based on nonlinear regression analysis. The serum concentration-time data for *R*- and *S*-warfarin were described adequately in all animals by use of a one-compartment open model with first order absorption and elimination. A lag time (t_{lag}) was also included in the model to account for the delay in absorption. The administered dose (D) was divided by 2 to obtain the equivalent enantiomer dose /23/. It was assumed that the extent of warfarin absorption is complete (i.e., oral availability, F=1) and that the rate of absorption is not stereoselective /24/. Therefore, the following kinetic equations were used to describe the time profiles of serum concentrations of the *S*- and *R*-enantiomers of warfarin after oral administration of the racemic drug:

$$C_S = \frac{F * D * K_a}{2 * V_{d,S}(K_a - K_S)}\left(e^{-K_S(t-t_{lag})} - e^{-K_a(t-t_{lag})}\right) \qquad [3]$$

and

$$C_R = \frac{F * D * K_a}{2 * V_{d,R}(K_a - K_R)}\left(e^{-K_R(t-t_{lag})} - e^{-K_a(t-t_{lag})}\right) \qquad [4]$$

where C is the serum concentration of warfarin at time t, R,S are the enantiomeric species, F is the oral availability, D is the racemic dose, V_d is the apparent volume of distribution, K_a is the first-order absorption rate constant, and K is the first-order elimination rate constant. The pharmacokinetic parameters of the *R*- and *S*-enantiomers of warfarin were estimated by nonlinear regression analysis using Equations 3 and 4. Exact sampling times were used in the data fitting.

The peak serum concentration (C_{max}) and the peak time (t_{max}) were obtained by visual inspection of the serum concentration-time curve. The total area under the serum concentration-time curve ($AUC_{0\to t}$) from time zero to the last quantifiable time point was estimated by the linear trapezoidal approximation. The total area under the serum concentration-time curve from time zero to infinity ($AUC_{0\to\infty}$) was calculated as the sum of $AUC_{0\to t}$ and the extrapolated area, which was calculated from the final measurable concentration divided by the elimination rate constant. The elimination half-life ($t_{½}$) for individual warfarin enantiomers was calculated as $0.693/K_S$ and $0.693/K_R$ for *S*- and *R*-warfarin, respectively. The total serum clearance (CL) for individual warfarin enantiomers was calculated according to $CL = F*D/(2*AUC_{0\to\infty})$. The fractions of the administered dose eliminated from the body as the parent enantiomer (f_e) and its metabolite (f_m) were calculated according to the ratio of the respective cumulative amounts, expressed in warfarin equivalents, of the unchanged parent enantiomer and metabolite excreted in the 5-day urine collection and the administered amount of its parent enantiomer. Thus, the renal clearance (CL_R) of warfarin enantiomer and the formation clearance (CL_f) of metabolite were calculated using $CL_R = f_e*CL$ and $CL_f = f_m*CL$, respectively.

Statistical analysis

Data analyses were performed using SPSS 10.0 (SPSS Inc., USA). Sample data were expressed as means ± standard deviation (SD). Comparisons of means of related samples and from two independent groups were made using Student's paired-samples and independent-samples *t*-tests respectively. A value of $p < 0.05$ was taken to indicate statistical significance.

RESULTS

The results of the chemical assay validation are shown in Table 1. All values appeared to be within the acceptable limits.

The effect of ubidecarenone on the serum protein binding of warfarin

To investigate the effect of ubidecarenone on the protein binding of warfarin enantiomers in pooled rat serum, the binding study was carried out with relatively high concentrations of unlabeled racemic warfarin (50-150 μM), which permitted the direct determination of the respective unbound fraction values by use of the HPLC chiral separation technique. The unbound fraction of individual warfarin enantiomers in serum appeared to be concentration-independent as similar fraction values were found at the 25 and75 μM concentrations studied. The average enantiomeric ratio (R/S) of the unbound fraction for warfarin alone was 1.52, whereas in the presence of ubidecarenone the R/S ratio was slightly increased to 1.59. As shown in Table 2, no statistically significant differences in the unbound fraction of warfarin enantiomers were found between the groups in the absence and presence of ubidecarenone at either high or low concentrations ($p > 0.05$).

The effect of ubidecarenone on the anticoagulation of warfarin

Comparisons of PCA-time and CFA-time courses between the control and ubidecarenone treatment groups are shown in Figure 1. As expected, the PCA and CFA values were reduced following oral administration of racemic warfarin. The hypoprothrombinemic response to warfarin was maximal at 24 h in all rats. The mean PCA and CFA values for the ubidecarenone treatment group were significantly increased at several time points (over the first 48 h) when compared with the control group ($p < 0.01$ at 6, 12 and 24 h for PCA; $p < 0.05$ at 12 h, and $p < 0.01$ at 3, 24 and 48 h for CFA), especially the trough PCA and CFA values. As shown in Table 3, ubidecarenone tended to prolong the baseline prothrombin time (by 11%) after 3 consecutive days of treatment prior to warfarin administration, although no statistically significant difference in the baseline prothrombin time was found between the control and ubidecarenone treatment groups ($p > 0.05$). However, the baseline factor VII clotting

TABLE 1

Parameters associated with the chemical analysis of the warfarin enantiomers in serum and urine and their associated metabolites in urine

Species	Limit of detection (μmol/l)	Variability of lowest conc. on standard curve (n=6) (μmol/l; CV %)	Variability of highest conc. on standard curve (n=6) (μmol/l; CV %)	r^2
***S*-Warfarin** +	0.02	1; 6.0 [a]; 1.4 [b]	16; 11; 4.9	0.9961
***R*-Warfarin** +	0.02	1; 6.6; 3.1	16; 7.8; 7.1	0.9954
***S*-Warfarin** #	0.04	1; 4.2; 7.4	16; 5.9; 2.0	0.9838
***R*-Warfarin** #	0.04	1; 3.2; 7.8	16; 5.6; 1.8	0.9809
***S,R*-Warfarin alcohol** #	0.02	0.25; 10, 6.8	4; 3.7; 6.7	0.9971
***S,S*-Warfarin alcohol** #	0.02	0.25; 6.5; 1.0	4; 7.1; 5.0	0.9950
***R,S*-Warfarin alcohol** #	0.01	0.25; 6.8; 3.5	4; 3.8; 3.8	0.9967
***R,R*-Warfarin alcohol** #	0.02	0.25; 9.1; 6.0	4; 1.5; 2.7	0.9921
***S*-6-Hydroxywarfarin** #	0.04	0.25; 15; 12	4; 3.0; 12	0.9934
***S*-7-Hydroxywarfarin** #	0.11	0.25; 15; 6.3	4; 4.4; 10	0.9980
***R*-6-Hydroxywarfarin** #	0.07	0.25; 14; 9.4	4; 3.1; 7.9	0.9921
***R*-7-Hydroxywarfarin** #	0.14	0.25; 16; 12	4; 9.6; 7.9	0.9916
***S*-4′-Hydroxywarfarin** #	0.09	0.62; 11; 8.0	10, 6.3; 16	0.9945
***S*-4′-Hydroxywarfarin** #	0.11	0.62; 13; 5.1	10, 5.2; 4.4	0.9975

+: in serum; #: in urine; a: intraday coefficient of variation; b: interday coefficient of variation.

TABLE 2

In vitro effect of ubidecarenone on serum protein binding of individual warfarin enantiomers

Group	Percent unbound *S*-warfarin [a]			Percent unbound *R*-warfarin [a]		
	Mean	SD	n [b]	Mean	SD	n [b]
Control	0.59	0.15	19	0.90	0.31	19
Ubidecarenone (5 μM)	0.63	0.21	18	0.97	0.30	18
Ubidecarenone (25 μM)	0.54	0.16	18	0.88	0.33	18
Mean ± SD (n [b])	0.58 ± 0.19 (36)			0.92 ± 0.32 (36)		

a: ligand concentrations at 25 and 75 μM; b: number of samples.

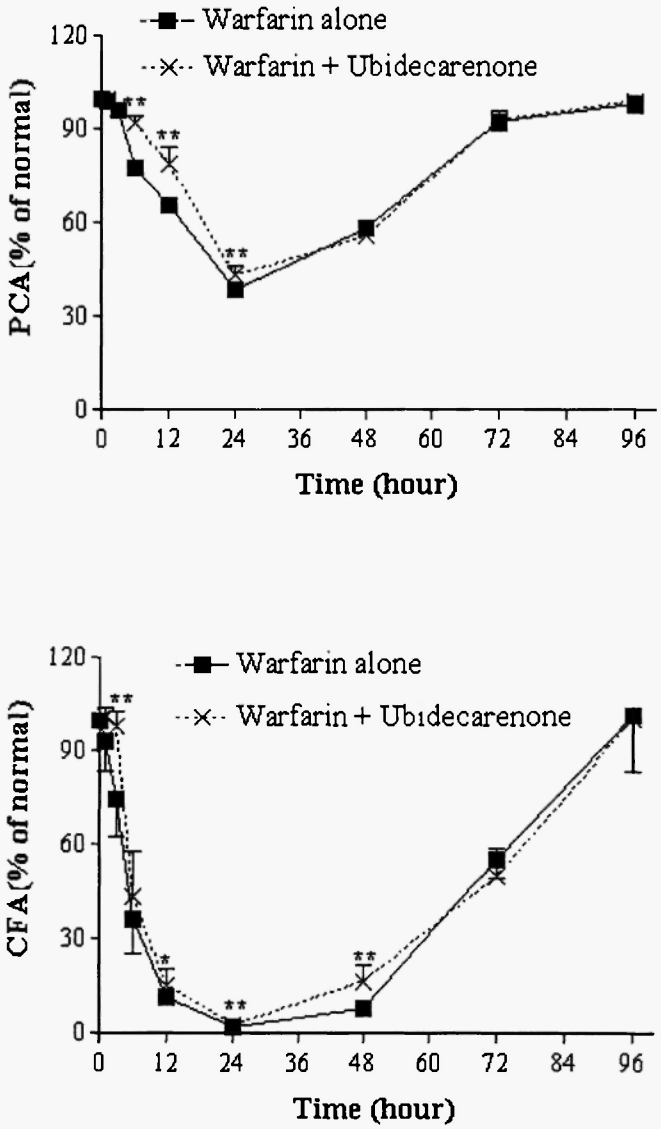

Fig. 1: Time course of percent changes (mean ± SD) of prothrombin complex activity (PCA) and clotting factor VII activity (CFA) following oral administration of racemic warfarin (1.5 mg/kg) to rats alone (control) and during ubidecarenone treatment. * $p < 0.05$, ** $p < 0.01$, comparing the control group (n=10) with the ubidecarenone treatment group (n=10).

TABLE 3

Estimated pharmacodynamic parameters (mean ± SD) following oral administration of racemic warfarin (1.5 mg/kg) to rats, alone (control) and 8 days into a regimen of ubidecarenone (10 mg/kg daily)

Parameter	Control	Ubidecarenone
PT_0 (sec)	38.0 ± 1.4	42.1 ± 0.7
PCA_{min} (% normal)	38.4 + 1.1	43.1 ± 3.1 **
$t_{PCA,min}$ (h)	24.0 ± 0.0	24.0 ± 0.0
AUC_{PCA} (% normal·h)	6866 + 141	7103 ± 138
Factor VII_0 (sec)	16.1 + 0.3	16.2 ± 0.1
CFA_{min} (% normal)	1.79 ± 0.38	2.46 ± 0.38 **
$t_{CFA,min}$ (h)	24.0 + 0.0	24.0 ± 0.0
AUC_{CFA} (% normal·h)	3399 + 361	3622 ± 361

PT_0 = baseline prothrombin time; PCA_{min} = trough prothrombin complex activity; $t_{PCA,min}$ = the time to achieve PCA_{min}; AUC_{PCA} = area under PCA-time curve from time 0 to last sampling time point; Factor VII_0 = baseline factor VII clotting time; CFA_{min} = trough clotting factor VII activity; $t_{CFA,min}$ = the time to achieve CFA_{min}; AUC_{CFA} = area under CFA-time curve from time 0 to last sampling time point. ** $p < 0.01$ comparing the control group (n=10) with the ubidecarenone treatment group (n=10).

time was not affected by the 3-day ubidecarenone pre-treatment. On the other hand, co-administration of ubidecarenone with warfarin significantly increased the mean PCA_{min} and CFA_{min} by 12% and 37%, respectively ($p < 0.01$). However, no statistically significant difference in AUC_{PCA} and AUC_{CFA} was found between the control and ubidecarenone treatment groups ($p > 0.05$) although ubidecarenone slightly increased the mean AUC_{PCA} and AUC_{CFA} by 3% and 6%, respectively.

Pharmacokinetic parameters

Warfarin was undetectable in the predose samples in all rats, confirming that ubidecarenone does not interfere with the warfarin

assay. The time profiles of serum concentrations of *S*- and *R*-warfarin in the presence and absence of ubidecarenone are shown in Figure 2. The serum concentrations of warfarin enantiomers were found to decline monoexponentially, with *R*-warfarin being eliminated more rapidly than its antipode. The peak concentrations of both the *S*- and

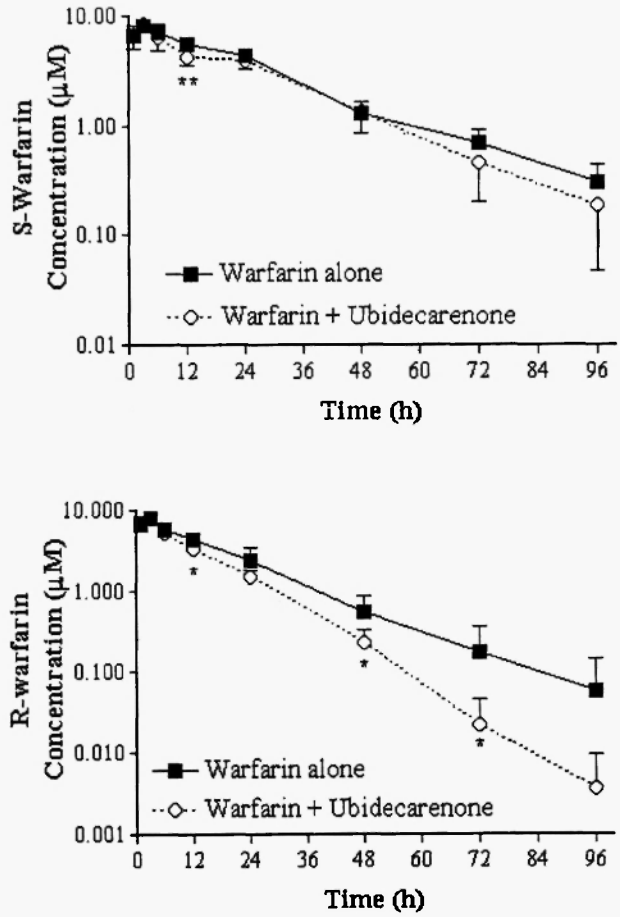

Fig. 2: Time course of total serum concentrations (mean ± SD) of *S*- and *R*-enantiomers of warfarin following oral administration of racemic dose (1.5 mg/kg) to rats alone (control) and during 8 days treatment with ubidecarenone. * $p < 0.05$, ** $p < 0.01$, comparing the control group (n=10) with the ubidecarenone treatment group (n=10).

R-enantiomers occurred 3 h after administration of a 1.5 mg/kg oral dose of racemic warfarin. Serum concentrations of both warfarin enantiomers tended to be somewhat lower from 3 h to 96 h in the presence of ubidecarenone. A statistically significant decrease in the serum concentrations of *S*- and *R*-warfarin was observed at several time-points between the control and ubidecarenone treatment groups.

The estimated pharmacokinetic parameters of warfarin enantiomers in the absence and presence of ubidecarenone are summarized in Table 4. With regard to those describing absorption kinetics, no statistically significant differences in C_{max} and t_{max} were found between *R*-warfarin and *S*-warfarin ($p > 0.05$). In addition, no statistically significant differences in the respective C_{max} and t_{max} values of *R*-warfarin and *S*-warfarin, or K_a and t_{lag} values, were found between the control and ubidecarenone treatment groups ($p > 0.05$). With respect to the parameters describing disposition kinetics, ubidecarenone decreased $AUC_{0\rightarrow 96h}$, $AUC_{0\rightarrow\infty}$ and $t_{½}$ of *R*-warfarin by 26%, 26%, and 34%, respectively, and those of *S*-warfarin by 13%, 14%, and 14%, respectively. On the other hand, ubidecarenone increased the CL of *S*- and *R*-warfarin by 19% and 34%, respectively. Statistically significant differences between the control and ubidecarenone treatment groups were found in the $t_{½}$, $AUC_{0\rightarrow 96h}$, $AUC_{0\rightarrow\infty}$ and CL of *R*-warfarin ($p < 0.01$) as well as in the CL of *S*-warfarin ($p < 0.05$). No differences in V_d were observed between the *R*- and *S*-enantiomers of warfarin or between the control and ubidecarenone treatment groups. It was noted that the elimination half-life ($t_{½}$) of *S*-warfarin was longer than that of *R*-warfarin (19.5 ± 3.4 h vs 11.9 ± 3.9 h, respectively, $p < 0.05$).

Urinary excretion data

The urinary excretion data for warfarin and metabolite (both oxidative and reductive) enantiomers obtained from the rats studied over the 0-120 h collection period are summarized in Table 5. For the control group, the total urinary recovery of the enantiomers of warfarin and metabolites (expressed as warfarin equivalents) was found to be 69% of the racemic dose administered (71% for *S*-warfarin and 66% for *R*-warfarin). With respect to the parent enantiomer excreted unchanged, *R*-warfarin was found recovered better than *S*-warfarin. The amount of 6- and 7-hydroxylated and reductive metabolites of *R*-warfarin recovered was greater than those of *S*-warfarin, whereas the

TABLE 4

Estimated pharmacokinetic parameters (mean ± SD) of *R*- and *S*-enantiomers of warfarin after oral administration of the racemic drug (1.5 mg/kg) to rats in the absence and presence of ubidecarenone

Parameter	Control		Ubidecarenone	
	S-Warfarin	*R*-Warfarin	*S*-Warfarin	*R*-Warfarin
C_{max} (μM)	8.21 ± 1.18	8.02 ± 1.17	8.36 ± 0.91	8.08 ± 1.17
t_{max} (h)	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
$AUC_{0\rightarrow 96}$ (μM·h)	245.2 ± 20.6	158.9 ± 31.0	213.9 ± 34.3	117.6 ± 17.8 **
$AUC_{0\rightarrow \infty}$ (μM·h)	254.6 ± 24.1	160.3 ± 32.6	219.1 ± 37.8	117.7 ± 17.8 **
K_a (h^{-1})	1.59 ± 0.58		1.93 ± 0.98	
t_{lag} (h)	$<10^{-4}$		0.081 ± 0.257	
$t_{½}$ (h)	19.5 ± 3.4	11.9 ± 3.9	16.8 ± 3.8	7.9 ± 1.3 **
V_d (l/kg)	0.256 ± 0.034	0.255 ± 0.036	0.270 ± 0.037	0.250 ± 0.041
CL ($ml \cdot h^{-1} \cdot kg^{-1}$)	9.0 ± 0.9	14.7 ± 3.1	10.7 ± 2.1 *	19.7 ± 2.8 **

C_{max} = peak serum concentration; t_{max} = peak time; AUC = total area under serum concentration-time curve; $AUC_{0\rightarrow 96}$ = AUC from time 0 to 96 h; $AUC_{0\rightarrow \infty}$ = AUC from time 0 to infinity; K_a = first-order absorption rate constant; t_{ag} = absorption lag time; $t_{½}$ = elimination half-life; V_d = apparent volume of distribution; CL = total serum clearance. * $p < 0.05$, ** $p < 0.01$, comparing the control group (n=10) with the ubidecarenone treatment group (n=10).

TABLE 5

Percent (mean ± SD) of the administered dose recovered from urine as the unchanged *S*- and *R*-enantiomers of warfarin and its respective metabolites in the absence and presence of ubidecarenone

Species [a]	*S*-Warfarin		*R*-Warfarin	
	Control	Ubidecarenone	Control	Ubidecarenone
Warfarin	1.02 ± 0.86	1.08 ± 0.36	5.95 ± 3.21	8.99 ± 3.43
***S*-Warfarin alcohol** [b]	2.23 ± 1.39	3.71 ± 1.96	3.39 ± 1.21	4.23 ± 1.47
***R*-Warfarin alcohol** [c]	0.66 ± 0.45	1.15 ± 1.09	1.40 ± 0.53	2.99 ± 1.30 *
6-Hydroxywarfarin	11.0 ± 2.8	11.4 ± 2.2	13.3 ± 3.4	12.6 ± 2.2
7-Hydroxywarfarin	28.3 ± 8.6	25.3 ± 6.4	29.2 ± 5.9	30.3 ± 4.9
4′-Hydroxywarfarin	28.2 ± 5.9	28.8 ± 3.6	12.9 ± 3.1	15.4 ± 2.7
Total hydroxyl [d]	67.5 ± 7.4	65.5 ± 5.8	55.4 ± 10.7	58.3 ± 9.1
Total dose recovered [e]	71.4 ± 7.5	71.4 ± 7.3	66.2 ± 13.8	74.5 ± 11.2

a: expressed in warfarin equivalent; b: metabolite formed by S-reduction; c: metabolite formed by R-reduction; d: total of all hydroxylated (oxidative) metabolites of warfarin; e: total of warfarin and all the reductive and oxidative metabolites. * $p < 0.05$, ** $p < 0.01$, comparing the control group (n=10) with the ubidecarenone treatment group (n=10).

recovery of the 4′-hydroxylated metabolite of *R*-warfarin was only half of that of *S*-warfarin. With respect to the total oxidative products, the recovery of the *R*-form products was less than that of *S*-form products. It was noted that metabolism of warfarin via reduction was of minor importance when compared with oxidative pathways, as the former only accounted for less than 6% of the recovered dose.

Ubidecarenone appeared to increase the urinary recovery of *R*-warfarin (by 51%) and its reductive (50%) and 4′-hydroxylated (20%) metabolites as well as of the *S*-form reductive metabolites (68%), but had no apparent effect on that of *S*-warfarin and its oxidative metabolites. Overall, there was an increase in the total recovery of the *R*-enantiomeric dose in the presence of ubidecarenone.

Combined serum and urinary excretion data

With the combined information of the cumulative amount of drug and metabolites excreted and the total area under the drug concentration-time curve, the effect of ubidecarenone on the clearances associated with the renal excretion of unchanged warfarin enantiomers and the formation of the respective metabolites could be examined in detail. Table 6 summarizes the estimates of the CL_R values of *S*- and *R*-warfarin as well as the CL_f of their respective metabolites. Ubidecarenone appeared to increase the CL_R of the *S*- and *R*-enantiomers by 23% and 101%, respectively. Also, there were increases in the CL_f of all reductive and oxidative metabolites of warfarin enantiomers, but to varying degrees: *S,R*-warfarin alcohol, 112%; *S,S*-warfarin alcohol, 96%; *R,R*-warfarin alcohol, 181%; *R,S*-warfarin alcohol, 69%; *S*-4′-hydroxywarfarin, 21%; *S*-6-hydroxywarfarin, 21%; *S*-7-hydroxywarfarin, 7%; *R*-4′-hydroxywarfarin, 60%; *R*-6-hydroxywarfarin, 28%; *R*-7-hydroxywarfarin, 42%. Statistically significant differences in the CL_f between the control and ubidecarenone treatment groups were found in *R,S*- and *R,R*-warfarin alcohol, *R*-4′- and *R*-7-hydroxywarfarin (see Table 6). With respect to the overall oxidative products, the CL_f values of both *S*- and *R*-form metabolites were increased in the presence of ubidecarenone (15% and 43%, respectively). Nonetheless, a statistically significant difference in the CL_f between the control and ubidecarenone treatment groups was found in the overall oxidative product of *R*-warfarin only ($p < 0.01$).

TABLE 6

Estimated values (mean ± SD) for the renal clearance of *S*- and *R*-warfarin and the respective formation clearance of metabolites in the absence and presence of ubidecarenone

Species	*S*-Warfarin		*R*-Warfarin	
	Control	Ubidecarenone	Control	Ubidecarenone
Renal clearance (ml/h/kg)	0.092 ± 0.076	0.113 ± 0 030	0.833 ± 0.343	1.817 ± 0.842 *
Formation clearance (ml/h/kg)				
***S*-Warfarin alcohol**	0.198 ± 0.114	0.389 ± 0.201	0.501 ± 0.191	0.846 ± 0 339 *
***R*-Warfarin alcohol**	0.057 ± 0.036	0.121 ± 0.111	0.208 ± 0.088	0 585 ± 0.226 **
6-Hydroxywarfarin	1.00 ± 0.32	1.21 ± 0 28	1.93 ± 0.46	2 48 ± 0 60
7-Hydroxywarfarin	2.56 ± 0.93	2.73 ± 0.92	4.21 ± 0.80	5.96 ± 1.29 **
4′-Hydroxywarfarin	2.53 ± 0.52	3.07 ± 0.70	1.89 ± 0 59	3.03 ± 0.62 **
Total hydroxy [a]	6.09 ± 1.09	7.01 ± 1.53	8.02 ± 1.63	11.5 ± 2.39 **

[a] presented as the formation clearance of total observable hydroxylated metabolites of warfarin enantiomers.
* $p < 0.05$, ** $p < 0.01$, comparing the control group (n=10) with the ubidecarenone treatment group (n=10).

DISCUSSION

Ubidecarenone is chemically related to vitamin K_2 /25/. A previous study /26/ showed that a synthetic analog of both vitamin K_1 and ubidecarenone caused an increase in prothrombin time when administered to rats. Vitamin K_1 could prevent this increase, but ubidecarenone had no effect to reverse the increase. The result implies that the analog of vitamin K_1 is most likely to become an antagonist of vitamin K_1, thereby decreasing synthesis of vitamin K_1-dependent clotting factors to a certain degree. Moreover, although so far no information is available concerning the *in vivo* and *in vitro* interaction of ubidecarenone with the vitamin K-dependent carboxylase, a synthetic derivative of ubidecarenone, decyl-ubiquinone, has been found to be a potent inhibitor of the vitamin K cycle, and its effect on carboxylase is stronger than that on vitamin K epoxide reductase /27/. As such, ubidecarenone may act as an antagonist of vitamin K_1 due to its structural similarity to vitamin K, thereby producing an anticoagulant effect like warfarin, rather than act as vitamin K to exhibit the "procoagulational" effect /8/. In this regard, the increase in the baseline prothrombin time observed in the ubidecarenone-pretreated group may be due to the warfarin-like anticoagulant effect of ubidecarenone. However, the difference in the baseline prothrombin time between the control and ubidecarenone treatment groups was of no statistical significance, suggesting that the synergistic effect of ubidecarenone with warfarin on the coagulation system is marginal.

Contrary to the expectation that ubidecarenone might augment the anticoagulant effect of warfarin, the present study showed that during the ubidecarenone regimen the anticoagulant effect of warfarin was diminished. This observation is consistent with the previous patient report that indicated that the anticoagulant effect of warfarin is decreased with the concurrent administration of ubidecarenone /8/. This finding suggests that the observed warfarin-ubidecarenone interaction may involve other mechanisms, e.g. altering the absorption and disposition of warfarin.

No change in either the f_u or V_d values in the presence of ubidecarenone (see Tables 2 and 4) suggests that the antioxidant might have a negligible displacement effect on the serum protein binding of the warfarin enantiomers as well as no apparent effect on their distribution in the rats studied, possibly due to lower ubidecarenone

concentrations in rat serum. The binding affinity of ubidecarenone to serum albumin has not yet been documented. A previous study in rats receiving an 8-day regimen of ubidecarenone (12 μmoles/100 g daily) found that the average serum concentration of the antioxidant was 0.0016 mM /28/. Thus, a lower average serum concentration of ubidecarenone was expected in the present *in vivo* study as the rats were given a lower dosage regimen of the antioxidant (12 μmoles/kg daily). It is possible that such a low serum molar concentration of the antioxidant renders it ineffective to compete with warfarin for the same protein binding sites. Due to its poor aqueous solubility, the concentrations of the antioxidant used in the present protein binding study (0.005 and 0.025 mM) were relatively low when compared to those of warfarin (0.05 and 0.15 mM).

Similar values of the K_a, t_{lag}, C_{max} and t_{max} found between the control and ubidecarenone treatment groups indicate no apparent effect of the antioxidant on the absorption of the warfarin enantiomers. However, ubidecarenone appeared to affect the elimination kinetics of *S*- and *R*-warfarin. The CL values of both *S*- and *R*-warfarin were increased by 14% and 34%, respectively. The AUC of *R*-warfarin was thus decreased by ubidecarenone to a greater extent than that of *S*-warfarin. As expected, a reduced elimination $t_{½}$ was found for both *S*- and *R*-warfarin (by 14% and 34%, respectively), but only the decrease of elimination $t_{½}$ for the latter was of statistical significance. Clearly, the effect of ubidecarenone is more pronounced with *R*-warfarin than with *S*-warfarin. Overall, the serum data indicate that the pharmacokinetic aspect of the warfarin-ubidecarenone interaction involves mainly the alteration of the elimination of both *S*- and *R*-warfarin, and the effect of ubidecarenone is selective towards the less-potent *R*-enantiomer.

Since the predominant excretion route of the enantiomers of warfarin and its metabolites is through the kidney, scrutiny of the urinary excretion data can reveal the extent to which the excretion and metabolism of the drug are affected. The findings of this urinary excretion study (see Table 6) demonstrate that the diminished anticoagulant effect of warfarin observed in the presence of ubidecarenone is accompanied partly by an increase in the renal clearance of *R*-warfarin and mostly by an increase in the formation clearance of the individual metabolites of the *R*-enantiomer. The consistency of these urinary excretion findings with the elimination kinetics seen in serum

led to the conclusion that ubidecarenone, when co-administered with warfarin, reduced the anticoagulation of warfarin by accelerating the excretion and metabolism of the warfarin enantiomers and of *R*-warfarin in particular.

Overall, the present findings are at variance with the assumption that ubidecareno has a pro-coagulational effect /8/. With concurrent ubidecarenone exposure, the anticoagulant effect of warfarin appeared to be reduced. This effect is attributed to the alteration of the excretion and metabolism of warfarin enantiomers, since neither literature reports nor the present study have provided evidence that ubidecarenone could displace warfarin from its protein binding sites. Endogenous ubidecarenone is generally recognized to be an important component in mitochondrial electron transport processes /25/, and it has a direct regulatory role on succinyl and NADH dehydrogenases /26/. Previous studies have indicated that daily oral or intraperitoneal administration of ubidecarenone to rats over a period of 2-10 weeks led to its accumulation in liver, concentrating in the nuclear and cytosolic fractions of the liver cells /29/. Therefore, ubidecarenone is likely to affect the hepatic metabolism of warfarin by accelerating the NAD(P)H-dependent cytochrome P450 catalyzed reaction. In addition, an increase of the content of ubidecarenone in renal tubular cells may accelerate the active renal secretion process by improving the respiratory electron transport system in mitochondria. It is possible that this accelerated secretion process may account for the increased renal excretion of warfarin.

CONCLUSION

The slight increase in the baseline prothrombin time observed in the ubidecarenone-pretreated group might be due to the possible vitamin-K-antagonistic properties of ubidecarenone. Nevertheless, concomitant ubidecarenone administration with warfarin decreased the degree of hypoprothrombinemia in response to warfarin in rats. Determinations of the effect of ubidecarenone on the pharmacokinetic parameters and *in vitro* serum protein binding of warfarin indicate that the absorption and distribution of warfarin are not affected by the ubidecarenone regimen, whereas the elimination of warfarin is altered in the presence of ubidecarenone. Ubidecarenone treatment decreased the elimination of *R*-warfarin to a greater extent than that of *S*-

warfarin, suggesting that the effect of ubidecarenone is selective towards the *R*-enantiomer. Quantitative analyses of the combined serum and urinary data indicate that the increased clearance of *R*-warfarin produced by concomitant ubidecarenone administration is a result of a significant increase in the formation clearance of the *R*-form reductive and oxidative metabolites as well as the renal clearance of *R*-warfarin. The overall findings suggest that the changes of the anticoagulant effect of warfarin in the presence of ubidecarenone represent the net effect of alterations in both pharmacokinetic and pharmacodynamic processes with opposite influences. The apparent effect of ubidecarenone to reduce the anticoagulant effect of warfarin is mainly attributable to the altered metabolism of warfarin, in particular, the metabolism of *R*-warfarin.

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