Dose-Dependent Pharmacokinetics of Warfarin in Healthy Volunteers

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Purpose. To examine the pharmacokinetics of warfarin after administration of single oral doses (2, 5, and 10 mg) to healthy male volunteers.

Methods. A sensitive reverse-phase HPLC method was used to quantify warfarin plasma concentrations as low as 6 ng/ml. Blood samples were collected for up to 120 hours following administration of these doses.

Results. As the dose decreased from 5 to 2 mg, the apparent volume of distribution (V/F) increased from 12 to 21 liters and the terminal half-life ($t_{1/2}$) increased from 47 to 71 hours. Oral clearance remained unchanged over the examined dose range. These apparent dose-dependent changes in warfarin's $t_{1/2}$ and V/F may be due to saturable tissue binding of this drug. It appears that a previously undetected and prolonged terminal phase may exist but can not be adequately characterized with the 120-hour sampling interval. To evaluate this long $t_{1/2}$, a follow-up study was conducted to examine warfarin's pharmacokinetics for up to 21 days following a 10-mg dose. The prolonged terminal phase started to become apparent when plasma levels declined to less than 100 ng/ml. The $t_{1/2}$ of this terminal phase was determined to be approximately one week.

Conclusions. This is the first report that documents the dose-dependent pharmacokinetics of warfarin and the previously unreported long $t_{1/2}$ of one week for warfarin in humans.

KEY WORDS: warfarin; human; dose-dependent pharmacokinetics; saturable tissue binding; HPLC.

INTRODUCTION

Warfarin is a widely used oral anticoagulant in the treatment of thromboembolic disorders (1). The clinical pharmacokinetics of warfarin and its R- and S-enantiomers have been extensively studied following the administration of oral doses usually ranging from 10 to 25 mg (2). HPLC (high-performance liquid chromatographic) methods using UV detection with assay limits ranging from 60 to 80 ng/ml were employed to characterize warfarin's pharmacokinetics in humans. Warfarin is completely absorbed after oral dosing; is a low clearance drug (total plasma clearance of ca. 3 ml/min); is highly plasma protein bound (>99% bound); and is distributed into a small apparent volume of ca. 10 liters. The apparent terminal half-life has been reported to be between 25 to 50 hours. Warfarin's pharmacokinetics appear to be dose-independent over the dose range of 10 to 25 mg.

In the past decade, new approaches to anticoagulation using low doses of oral warfarin (usually 1 to 3 mg once daily) have been shown to prevent venous thrombosis after surgeries, recurrent cardiac complications, and pulmonary embolism in patients with acute myocardial infarction (1). However, clinical pharmacokinetic information after oral administration of low doses of warfarin is scarce. Therefore, we used a sensitive HPLC assay to examine the plasma concentrations resulting from low dose warfarin tablets. Evidence of dose-dependent pharmacokinetics in humans was observed. This phenomenon of dose-dependent pharmacokinetics has been reported in rats (3).

MATERIALS AND METHODS

Materials

Warfarin sodium tablets (2-, 5-, and 10-mg Coumadin®) and analytical reference standard were obtained from The DuPont Merck Pharmaceutical Company (Wilmington, Delaware). The internal standard for the HPLC method, p-chlorowarfarin was purchased from Aldrich (Milwaukee, Wisconsin). Both warfarin and p-chlorowarfarin were used as received. All solvents and reagents were either HPLC or reagent grade.

Clinical Protocols

Pharmacokinetics after Oral Administration of 2-, 5-, and 10-mg Doses of Warfarin

This study employed an open-label, parallel-group, single-dose administration design. Healthy, non-smoking male volunteers (19–40 years old; 19 subjects for both 2- and 5-mg groups; 18 subjects for 10-mg group) were enrolled in this study. Blood samples were collected immediately prior to and at 1, 2, 4, 8, 10, 12, 24, 36, 48, 72, 96, and 120 hours post dose. The blood samples were collected in 10-ml heparinized collection tubes and immediately centrifuged at 3000 rpm for 15 min. The plasma was transferred to a polyethylene test tube, and samples were stored frozen (−20°C) pending assay.

Follow-up Single Dose Pharmacokinetics of Warfarin

This study was designed to determine the plasma concentration versus time profile of warfarin for up to 21 days after administration of a 10-mg oral dose. This study used an open label, single dose administration design. Six healthy, non-smoking male volunteers (21–37 years old) were enrolled. Blood samples were collected immediately prior to and at 0.25, 0.5, 0.75, 1, 2, 4, 8, 10, 12, 24, 36, 48, 72, 96, 120, 144, 168, 240, 336, 432, and 504 hours post dose. Prepared plasma samples were stored frozen (−20°C) pending assay.

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Determination of Racemic Warfarin in Human Plasma

A reverse-phase HPLC method utilizing a post-column reaction system before fluorescence detection was developed and validated for quantifying warfarin in plasma. After adding the internal standard (p-chlorowarfarin), reference standard, quality control and subject samples (1.0 ml) were acidified with 0.4 ml of 1.0 M sulfuric acid and extracted with 12 ml of ethyl acetate. Samples were shaken for 30 min and centrifuged for 10 min at 1500 rpm. Ten ml of the organic layer was placed into a clean test tube and evaporated to dryness under nitrogen. The samples were reconstituted with 0.2 ml of 57% acetonitrile in water (v/v) prior to HPLC analysis.

The HPLC system consisted of an automatic injector (WISP 710B, Waters Chromatography Division, Millipore, Milford, Massachusetts), a solvent delivery system (Beckman 110A pump, Beckman Instruments, Berkeley, California), a post-column reactor equipped with a 2 ml reaction coil (Applied Biosystems, Ramsey, New Jersey), a fluorescence detector (Applied Biosystems 980 detector), and a computer data system (Nelson Analytical 4400 Data System equipped with XTRACHROM software, Nelson Analytical, Cupertino, California). The chromatographic conditions of the HPLC system were as follows: an octyl column (MAC-MOD Zorbax C-8, 25 cm × 4.6 mm, i.d.); flow rate of 1.0 ml/min for the mobile phase of acetonitrile/water/glacial acetic acid (57/42.6/0.4, v/v/v); ambient (22°C) column temperature; a post-column reagent of 12% triethanolamine (4) with a flow rate of 0.3 ml/min; and fluorescence detection at excitation and emission wavelengths of 310 and 370 nm, respectively. The retention times of warfarin and p-chlorowarfarin were 9 and 12 minutes, respectively. The validated standard curve ranged from 6.3 to 450 ng per 1.0 ml plasma. The extraction efficiency of warfarin from plasma was 76 ± 4% (mean ± SD, n = 24) over the concentration range of extracted standards.

Pharmacokinetic and Statistical Analyses

Model-independent methods (5) were used to analyze warfarin plasma concentration versus time data. Maximum plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were observed values. The terminal rate constant (λ_n) was estimated by linear regression of the terminal log-linear phase of the plasma concentration versus time curve. The terminal half-life ($t_{1/2}$) was calculated as $0.693/\lambda_n$. The area under the plasma concentration versus time curve from zero to infinity ($AUC_{0-\infty}$) was the sum of the area from time zero to the last sampling time (AUC_{0-t}) and the extrapolated area. The AUC_{0-t} was calculated using the linear trapezoidal rule. The extrapolated area was estimated by dividing the last plasma concentration by λ_n. The apparent oral plasma clearance (CL/F) was calculated as dose/$AUC_{0-\infty}$. The apparent volume of distribution (V/F) was calculated as dose/($\lambda_n \cdot AUC_{0-\infty}$). A two sample, unpaired t-test and one-way analysis of variance were used to test for significant differences between groups.

RESULTS

Warfarin concentrations in plasma following oral administration of 2-, 5-, and 10-mg single doses decline polyexponentially with time for up to the last sampling time of 120 hours (Figure 1). Literature data following a 25-mg dose (6) are included here for comparison purpose (Figure 1 and Table I). The curvature of the plasma concentration versus time curves became more pronounced as the dose decreased. The pharmacokinetic parameters of warfarin obtained after dosing with 5 mg and higher doses (Table I) were similar to the parameter values reported in the literature (2). However, the values of $t_{1/2}$ and V/F following administration of the 2-mg warfarin dose were different from the parameter values obtained in the 5- and 10-mg dose groups. The $t_{1/2}$ and V/F values increased as the dose decreased from 5 to 2 mg. However, the CL/F remained unchanged over the examined dose range.

The apparent $t_{1/2}$ values after administration of 5- and 10-mg doses were estimated using the observed terminal plasma concentration versus time data for up to 120 hours post dose. This 120-hour sampling interval may not be long enough to allow for proper detection of the prolonged terminal phase observed following administration of the lower 2-mg dose. Therefore, it is possible that a previously undetected and prolonged terminal phase may exist with an apparent $t_{1/2}$ possibly in the range of 80 to 100 hours or longer. To confirm the presence of this prolonged terminal phase, a follow-up study was initiated to characterize the plasma concentration versus time profile of warfarin for up to 21 days following a 10-mg dose. When the sampling interval was extended to 504 hours, the plasma concentration versus time curve became polyphasic in shape (Figure 2). The prolonged terminal phase became apparent when the drug plasma levels declined to less than 100 ng/ml (Figures 1 and 2). The $t_{1/2}$ of this terminal phase was approximately one week (Table II).

DISCUSSION

This is the first report that documents the dose-depen-

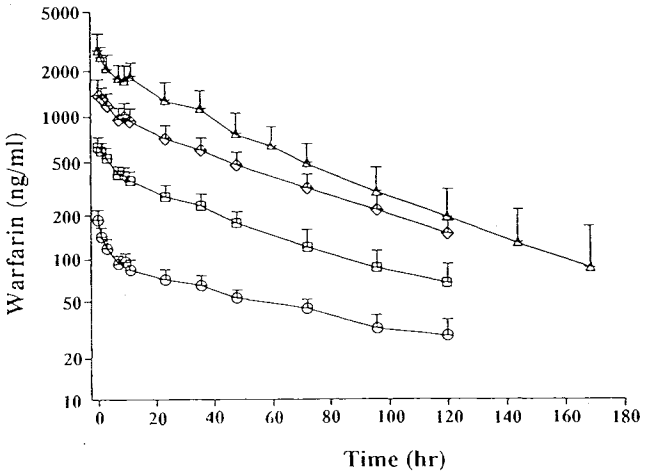

Fig. 1. Profiles of warfarin plasma concentration versus time after oral administration of single doses of 2- (○), 5- (□), 10- (◇), and 25-mg (△) Coumadin tablets (literature data following a 25-mg dose (6) are included for comparison purpose). All data are expressed as mean ± SD and the number of subjects in each dose group are specified in Table I.

Table I. Pharmacokinetic Parameters of Warfarin After Administration of Single Oral Doses of Coumadin Tablets in Healthy Volunteers[a]

Parameter	2-mg (n = 19)[c]	5-mg (n = 19)[c]	10-mg (n = 18)[c]	25-mg[b] (n = 12)[c]
C_{max} (μg/ml)	0.19 ± 0.03	0.65 ± 0.08	1.4 ± 0.3	3.0 ± 0.8
t_{max} (hr)[d]	1.0 (1.0, 1.0)	1.0 (1.0, 4.0)	1.0 (1.0, 4.0)	1.0 (1.0, 2.0)
$AUC_{0-\infty}$ (μg · hr/ml)	10 ± 2	29 ± 6	62 ± 16	120 ± 30
$\lambda_n \times 100$ (hr^{-1})	0.98 ± 0.19[e]	1.5 ± 0.2	1.7 ± 0.3	1.8 ± 0.4
$t_{1/2}$ (hr)[f]	71 ± 14[e]	47 ± 8	41 ± 7	38 ± 8
CL/F (ml/min)	3.3 ± 0.7	3.1 ± 0.6	2.8 ± 0.7	3.7 ± 1.3
V/F (l)	21 ± 4[e]	12 ± 2	10 ± 2	12 ± 4

[a] All data are expressed as mean ± SD.
[b] Literature data following a 25-mg dose (6) are included for comparison purpose.
[c] Number of subjects per dose group.
[d] Median (min, max).
[e] Significantly different from 5- and 10-mg dose groups at $p < 0.01$ by one-way analysis of variance.
[f] Harmonic mean ± pseudo SD (7).

dent pharmacokinetics of warfarin (i.e., a concentration-dependent change in warfarin's disappearance rate in plasma) and the previously unreported and prolonged $t_{1/2}$ of one week for warfarin in humans. The V/F increased about 3-fold when the rate constant of this prolonged terminal phase was used to calculate the distribution volume (Table II). However, the mean AUC value calculated using the plasma data for up to 504 hours was not different ($p>0.05$) from the mean AUC value calculated using the data for up to 168 hours (Table II) because the AUC under the prolonged terminal phase only represented less than 20% of the total AUC. This result indicates that this long $t_{1/2}$ of one week makes a negligible contribution to the total plasma clearance of warfarin. Also, the rate of warfarin accumulation on a multiple-dosing regimen and the steady-state plasma level of warfarin should be minimally affected by this long $t_{1/2}$. The steady-state plasma level of warfarin can be achieved after once daily dosing for a week (2, 8), and this one week of time required to reach steady-state depends on the reported $t_{1/2}$ of about 50 hours instead of the long $t_{1/2}$ of one week. Therefore, this prolonged terminal phase is likely to represent saturable tissue binding of warfarin to tissue enzymes. This result is consistent with the pharmacokinetic principle that changes in a drug's tissue binding will affect apparent volume of distribution and $t_{1/2}$, and will not affect the total clearance which is influenced by plasma protein binding only (9). Thereby, a likely reason to explain the apparent concentration-dependent change in warfarin's disappearance rate in plasma may be the presence of a saturable tissue binding site (a low capacity and high affinity site). This kind of dose-dependent pharmacokinetics has been observed in humans for imirestat (an aldose reductase inhibitor) (10), and angiotensin converting enzyme inhibitors such as enalapril (11).

Dose-dependent pharmacokinetics of warfarin have been shown to occur in rats (3). The nonlinear hepatic uptake of warfarin may explain the decrease in the apparent volume of distribution with increasing warfarin dose. In subsequent reports (12, 13), nonlinear tissue distribution of warfarin to liver and other tissues was demonstrated in a group of rats in which warfarin free fraction in serum was independent of drug concentration over a wide concentration range. These results (12,13) have been shown to be consistent with the presence of two classes of tissue binding sites, one with very high affinity and low capacity and the other one with

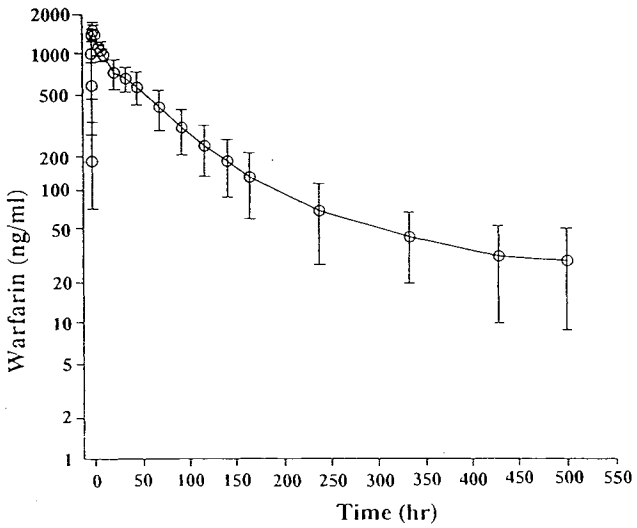

Fig. 2. Warfarin plasma concentration versus time profile after oral administration of a 10-mg dose. All data are expressed as mean ± SD, n = 6.

Table II. Comparative Pharmacokinetics of Warfarin Calculated by Using Plasma Level Data for up to 168 and 504 Hours After Oral Administration of a 10-mg Dose in Healthy Volunteers

Parameter[a]	t_{last} = 168 hours	t_{last} = 504 hours
$AUC_{0-\infty}$ (μg · hr/ml)	82 ± 24	97 ± 34
$\lambda_n \times 100$ (hr^{-1})	1.4 ± 0.2[b]	0.43 ± 0.13
$t_{1/2}$ (hr)[c]	51 ± 9[b]	163 ± 50
CL/F (ml/min)	2.2 ± 0.6	1.9 ± 0.6
V/F (l)	9.6 ± 1.5[b]	27 ± 7

[a] All data are expressed as mean ± SD, n = 6.
[b] Significantly different from the 504-hr group at $p < 0.01$ by unpaired t-test.
[c] Harmonic mean ± pseudo SD.

lower affinity and apparently unlimited capacity. However, the identity of the saturable tissue binding site(s) has not yet been identified. Pratt and co-workers (14) have demonstrated a possible presence of a saturable tissue binding site in hepatic microsomal fraction in rats. In another study conducted by Thijssen and Baars (15), vitamin K 2,3-epoxide reductase, a hepatic microsomal enzyme, has been shown to be a probable site of high affinity tissue binding for warfarin in rats.

The presence of a saturable tissue binding site or "deep compartment" for warfarin has been shown to be related to a phenomenon of "first-dose effect" in rats (3, 15). The "first-dose effect" means that the first administered dose saturates the high affinity and low capacity binding site and "normal" pharmacokinetics (i.e., the prolonged terminal phase is not detectable until drug concentration in plasma declines to a certain level) are observed after administration of subsequent doses. Since warfarin's pharmacokinetics are dose-dependent probably due to saturable tissue binding of the drug, this "first-dose effect" phenomenon may also occur in humans and underscore the need to carefully consider the effects of this nonlinear characteristic on the design of bioequivalence and drug interaction studies, e.g., use steady-state instead of single dose study design.

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REFERENCES

1. J. Hirsh. Oral anticoagulant drugs. *N. Engl. J. Med.* 324: 1865–1875 (1991).
2. R. S. Porter and W. T. Sawyer. Warfarin. In W. E. Evans, J. J. Schentag, W. J. Jusko (eds.), *Applied Pharmacokinetics, Principles of Therapeutic Drug Monitoring*, 3rd Ed, Applied Therapeutics, Inc., Vancouver, WA, 1992, Chap. 31.
3. K. Takada and G. Levy. Comparative pharmacokinetics of coumarin anticoagulants XLIV: Dose-dependent pharmacokinetics of warfarin in rats. *J. Pharm. Sci.* 69: 9–14 (1980).
4. J. M. Steyn and H. M. Van Der Merwe. Reversed-phase high-performance liquid chromatographic method for the determination of warfarin from biological fluids in the low nanogram range. *J. Chromatogr.* 378: 254–260 (1986).
5. M. Gibaldi and D. Perrier. *Pharmacokinetics*, 2nd Ed., Marcel Dekker, New York, 1982, Chap. 11.
6. I. H. Benedek, S.-Y. P. King, R. J. Powell, A. M. Agra, W. L. Schary, and H. J. Pieniaszek, Jr. Effect of moricizine on the pharmacokinetics and pharmacodynamics of warfarin in healthy volunteers. *J. Clin. Pharmacol.* 32: 558–563 (1992).
7. F. C. Lam, C. T. Hung, and D. G. Perrier. Estimation of variance for harmonic mean half-lives. *J. Pharm. Sci.* 74: 229–231 (1985).
8. P. Unge, L.-E., Svedberg, A. Nordgren, H. Blom, T. Andersson, P.-O. Lagerström, and J.-P. Idström. A study of the interaction of omeprazole and warfarin in anticoagulated patients. *Br. J. Clin. Pharmacol.* 34: 509–512 (1992).
9. M. Gibaldi, G. Levy, and P. J. McNamara. Effect of plasma protein and tissue binding on the biological half-life of drugs. *Clin. Pharmacol. Ther.* 24: 1–4 (1978).
10. R. K. Brazzell, P. R. Mayer, R. Dobbs, P. J. McNamara, R. Teng, and J. T. Slattery. Dose-dependent pharmacokinetics of the aldose reductase inhibitor imirestat in man. *Pharm. Res.* 8: 112–118 (1991).
11. R. J. MacFadyen, P. A. Meredith, and H. L. Elliott. Enalapril clinical pharmacokinetics and pharmacokinetic-pharmacodynamic relationships: An overview. *Clin Pharmacokinet.* 25: 274–282 (1993).
12. W. K. Cheung and G. Levy. Comparative pharmacokinetics of coumarin anticoagulants XLIX: Nonlinear tissue distribution of S-warfarin in rats. *J. Pharm. Sci.* 78: 541–546 (1989).
13. G. Levy. Pharmacologic target-mediated drug disposition. *Clin. Pharmacol Ther.* 56: 248–252 (1994).
14. S. K. Pratt, M. J. Winn, and B. K. Park. The disposition of the enantiomers of warfarin following chronic administration to rats: Relationship to anticoagulant response. *J. Pharm. Pharmacol.* 41: 743–746 (1989).
15. H. H. W. Thijssen and L. G. M. Baas. Hepatic uptake and storage of warfarin. The relation with the target enzyme vitamin K 2,3-epoxide reductase. *J. Pharmacol. Exp. Ther.* 243: 1082–1088 (1987).