Steady-State Clearance Rates of Warfarin and Its Enantiomers in Therapeutically Dosed Patients

SARAH D. McALEER,[1] ABOO S. FOONDUN,[2] MORGAN FEELY,[2] AND HENRY CHRYSTYN[1]*
[1]*Pharmacy Practice, Postgraduate Studies in Pharmaceutical Technology, School of Pharmacy, University of Bradford, Bradford, United Kingdom*
[2]*Clinical Pharmacology Unit, Department of Medicine, Leeds General Infirmary, Leeds, United Kingdom*

ABSTRACT Previous studies to identify the pharmacokinetics of R- and S-warfarin have not used steady-state area under the curve (AUC) data during therapeutic doses of racemic warfarin. Instead they have used high single doses of either racemic warfarin or a single enantiomer in volunteers or have taken a single blood sample from anticoagulated patients and assumed full compliance and a steady-state status. In this study, a series of steady-state racemic warfarin, R-warfarin, and S-warfarin serum concentrations, during a 24 h dosage interval, was measured in 10 compliant patients (5 females and 5 males) taking racemic warfarin. The anticoagulation status of all 10 patients according to the International Normalised Ratio (INR) was stable. Their mean (SD) age and weight were 67.0 (9.9) yr and 63.9 (15.4) kg. The mean (SD) clearances derived from steady-state AUC values, following therapeutic dosing, for racemic warfarin, R-warfarin, and S-warfarin were 2.40 (0.82), 2.30 (0.65), and 2.80 (1.17) ml/h/kg, respectively. The mean (SD) ratio of S-:R-warfarin clearance was 1.24 (0.40). Comparison of the clearance measured from the AUC, of these patients, to one point determinations assuming steady state for the samples drawn at either 6, 15, or 20 h after dosage (during the dosing interval) showed some statistical differences. Most single point determinations of warfarin clearance assume that a sample 12 h postdose is equivalent to that of the steady-state concentration, but in this study the steady-state concentration of only 6 patients occurred between 6 and 15 h postdose. This could explain why these studies demonstrate differences in the clearance of R- and S-warfarin compared to the values we have derived from steady-state AUC data using patients with proven compliance and therapeutic doses. *Chirality 9:13–16, 1997.*

KEY WORDS: R-warfarin; S-warfarin; steady-state clearance; patients; AUC data

The pharmacodynamics and the pharmacokinetics of the warfarin enantiomers have been shown to be stereoselective. When patients received separate large single doses of R- and S-warfarin the duration of the anticoagulant response was greater following administration of the latter.[1] These findings were confirmed in 4 anticoagulated patients at steady state by Breckenridge et al.,[2] suggesting that S-warfarin was 4 times more potent than R-warfarin. Metabolism of the enantiomers is stereospecific.[3–5]

All previous studies reporting the pharmacokinetics of warfarin and its enantiomers have limitations because area under the curve (AUC) data at steady state in warfarin-treated patients have not been used. These studies have used either very high single doses ranging from 0.5 to 1.5 mg/kg (compared to usual total therapeutic daily doses of less than 10 mg)[6–10] or have used a one point determination following repeated administration.[11–13] One point determinations assume that the patient is always taking the medication and that the sample was drawn at a time corresponding to the average concentration at steady state and are thus prone to error.

We have validated an achiral/chiral coupled high performance liquid chromatographic method[14] for racemic, R-, and S-warfarin, and have used this to measure steady-state serum concentrations during a dosage interval of racemic warfarin in compliant anticoagulant patients to calculate their steady-state AUC. No other report has used steady-state AUC data to derive clearance values of R- and S-warfarin in compliant anticoagulated patients receiving therapeutically controlled racemic warfarin therapy. In addition, because one point determinations of clearance are prone to error, we have compared the clearance derived from the steady-state AUC to those which can be calculated (during the study dosage interval) at 6, 15, and 20 h postdose by assuming steady-state conditions.

S.D.M. was supported by a Ph.D. studentship from the Science and Engineering Research Council (SERC).
*Correspondence to: Professor Henry Chrystyn, Pharmacy Practice, Postgraduate Studies in Pharmaceutical Technology, School of Pharmacy, University of Bradford, Bradford BD7 1DP, United Kingdom.
Received 1 February 1996; accepted 20 May 1996

TABLE 1. Demographic and serum concentration time data at steady state for rac-W

Patient	Age (yr)	Weight (kg)	Warfarin daily dose (mg)	Mean INR[a]	C_{ss} (rac-W)[b] (mg/1)
A	69	57.2	6.0	4.0	1.08
B	70	70.0	3.0	1.8	0.93
C	54	79.4	5.0	3.1	1.85
D	69	70.8	3.0	2.5	0.79
E	61	92.5	6.0	2.6	1.49
F	65	47.6	5.0	1.8	1.72
G	75	52.2	4.0	3.8	1.17
H	85	42.6	4.5	2.6	1.83
I	71	69.9	2.0	2.7	0.38
J	51	57.2	8.0	3.5	2.30
Mean	67.0	63.9	4.7	2.8	1.35
(SD)	(9.9)	(15.4)	(1.8)	(0.81)	(0.06)

[a]Mean of 3 measurements drawn at 0, 3, and 15 h postdose, in steady state
[b]C_{SS} = AUC/24.

MATERIALS AND METHODS

Ten patients prescribed long-term (racemic) warfarin therapy as anticoagulation, whose dose and International Normalised Ratio (INR) had been stable over a period of 6 wk, were recruited. All patients gave written informed consent, were clinically stable, and serum biochemistry revealed no hepatic or renal dysfunction. Serum albumin levels were normal. Concurrent medications did not interact with warfarin. Local Research Ethics Committee approval was obtained. The patients were admitted to the study when their INR remained constant (less than 0.5 variation) on three occasions over the previous 14 days. Allard et al.[15] have identified that this criterion indicates consistent compliance with anticoagulant therapy. Their (racemic) warfarin therapy was given at a time equivalent to that at which the patient usually self-administered the medication at home. Blood samples were drawn through an indwelling cannulae kept open with normal saline, at 0, 1, 2, 3, 6, 15, 20, and 24 h after dosage. Each 10 ml blood sample was allowed to clot, and the serum was removed and kept at −20°C prior to analysis. At t = 0, 3, and 15 h an extra 5 ml sample of blood was drawn for INR determination by the hematology department.

For each of the blood samples the serum concentrations of racemic warfarin (rac-W), R-warfarin (R-W), and S-warfarin (S-W) were measured using an achiral/chiral high performance liquid chromatographic assay.[14] Using this assay, rac-W concentrations were measured following achiral resolution using a C_8 spherisorb (FSA, UK) stationary phase. The enantiomeric ratio of each sample was then determined using a chiral AGP (Chromtech AB, Sweden) stationary phase. The lower limit of quantification of this assay for either R-W or S-W concentrations in serum was 0.05 mg/l. The intra- and inter-day coefficients of variation of the chiral assay for serum R-W or S-W concentrations of 0.5 mg/ml were 1.77 and 3.42%, respectively. For a 1.0 mg/l serum rac-W concentration using the achiral separation the coefficients of variations were 0.69 and 5.43%, respectively. Analysis of the tablets used in the study revealed 50% of each enantiomer.

Oral clearances (Cl) of Rac-W, R-W, and S-W were calculated by dividing the dose (half the racemic dose for R-W and S-W) by the steady-state AUC value. Bioavailability was assumed to be 100%. The AUC (during each steady-state dosage interval) was obtained using the linear trapezoid method. The average concentration at steady state (Css) was calculated from the AUC divided by the duration of the dosage interval (24 h).

The serum concentrations of rac-W, R-W, and S-W at 6, 15, and 20 h after dosage were used to calculate one point steady-state oral clearance values (Cl_6, Cl_{15}, and Cl_{20}). These values were obtained by dividing the dose by the respective concentration and the dosage interval (24 h) with an assumption that the bioavailability was 100%. Statistically significant differences between Cl measured by the AUC and those of Cl_6, Cl_{15}, and Cl_{20} were determined using the paired Student's t-test and the mean difference (95% confidence interval) of the comparisons was calculated.

RESULTS

Table 1 shows that the mean (±SD) age and weight of the 10 patients (5 females and 5 males) were 67.0 (9.9) yr and 63.9 (15.4) kg, respectively. The calculated serum drug concentrations at steady state (C_{ss}), from the AUC data, together with individual dosing and INR data are also shown in Table 1. Comparison of the calculated C_{ss} for each patient, to their measured individual serum rac-W concentration time profile, revealed that (during the elimination phase) for 6 (A, B, D, F, H, and J) this value occurred between 6 and 15 h postdose, for 3 (E, G, and I) between 15 and 20 h, and for 1 (C) between 3 and 6 h.

Figure 1 shows the mean (±SD) steady-state serum concentrations of rac-W, R-W, and S-W during the steady-state

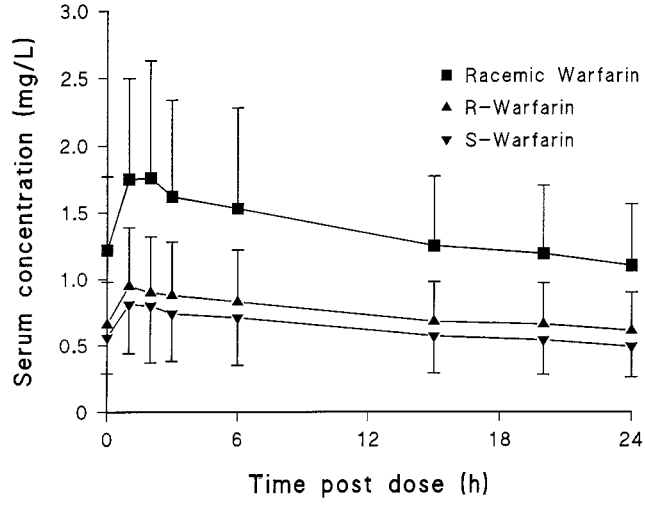

Fig. 1. Mean (±SD) steady-state serum concentrations of rac-W, R-W, and S-W in 10 anticoagulated patients [mean (±SD) racemic dose of 4.7 (±1.8) mg/day].

TABLE 2. AUC clearance data following therapeutic rac-W dosing

Patient	Clearance (ml/h/kg)		
	rac-W	R-W	S-W
A	4.06	3.21	5.62
B	1.91	1.68	2.20
C	1.42	1.21	1.73
D	2.23	2.31	2.16
E	1.18	1.88	1.74
F	2.55	2.87	2.28
G	2.73	2.55	2.95
H	2.40	1.78	3.68
I	3.09	3.05	3.24
J	2.44	2.47	2.41
Mean (SD)	2.40 (0.82)	2.30 (0.65)	2.80 (1.17)

therapeutic once daily dosing of the rac-W tablets. The mean (±SD) daily rac-W dose was 4.7 (±1.8) mg. There was no difference between rac-W concentration at t = 0 and 24 h with a mean difference (95% confidence interval) of –0.03 (–0.14, 0.01) mg/l. The median tmax (time to maximum serum concentration) and Cmax (serum drug concentration at tmax) of rac-W were 1.5 h and 1.84 mg/l, respectively, with ranges of 1–6 h and 0.59–3.54 mg/l. Similar Cmax values for R-W and S-W were 0.90 and 0.87 mg/l with ranges of 0.3–1.75 and 0.29–1.79 mg/l, respectively.

The mean (SD) INRs measured at 0, 3, and 15 h postdose during the steady-state concentration profiles were 2.12 (0.79), 2.78 (0.63), and 2.93 (0.90), respectively. The median INR was 2.65 with a range of 1.8–4.0. Oral clearance values, derived from the steady-state AUC, of rac-W, R-W, and S-W are shown in Table 2. This table shows that the mean (SD) clearance values were 2.40 (0.82), 2.30 (0.65), and 2.80 (1.17) ml/h/kg, respectively. There was no significant difference between the clearance values of the two enantiomers with a mean difference (95% confidence interval) of –0.50 (–1.18, 0.17) ml/h/kg. The mean (SD) S-W:R-W clearance ratio was 1.24 (0.40).

Table 3 shows that there were some statistical differences between the clearance of rac-W, R-W, and S-W calculated by the AUC and by the single point determination methods using the serum concentrations measured at 6, 15, and 20 h postdose (Cl_6, Cl_{15}, and Cl_{20}, respectively).

DISCUSSION

Stable INR values in the 2 wk prior to the study day indicate consistent compliance with therapy as identified by Allard et al.[15] This is further substantiated by similar serum warfarin concentrations measured at 0 and 24 h. No direct correlation existed between serum concentrations of rac-W, R-W, and S-W and the clotting response (INR). This may be due to the inter- and intra-individual variability in the biological degradation of the clotting factors, to the effect of warfarin on decreasing the synthesis of the clotting factors, and to uncertainty on the anticoagulant potency of each enantiomer.

Single point determinations for the calculation of clearance may be inaccurate because they rely on compliance and the assumption that the sample was taken at a time corresponding to the steady-state concentration. The single point methods reporting the clearance of warfarin and its enantiomers in patients taking rac-W have drawn blood samples approximately 12 h postdose.[11–13] It has been suggested that a sample taken 12 ± 2 h after drug administration could provide concentrations within 1–6% of the average drug concentration observed over that dosage interval.[13] The statistical analysis in Table 3 together with the C_{ss}, calculated from the AUC data (in Table 1), occurring between 6 and 15 h postdose in only 6 of the 10 subjects highlights the limitation of the single point determinations of clearance. The previously reported values using a single point determination[11–13] from patients taking rac-W do not, therefore, reflect an accurate value for the clearance of warfarin and its enantiomers and should not be used.

The mean (SD) clearance values from steady-state AUC data of patients receiving a therapeutic dose of rac-W were 2.40 (0.82), 2.30 90.65), and 2.80 (1.17) ml/h/kg for rac-W, R-W, and S-W, respectively. There was a much larger variability of the R:S clearances of the enantiomers in these 10 anticoagulated patients than previously reported in volunteers[6–10] receiving high single doses of rac-W. In 6 patients S-W clearance was higher than that of R-W, 1 patient had similar values, and in the other 3 patients R-W was cleared faster. The greater variability of the steady-state clearance may be due to the clinical status of the patients. Furthermore, unlike previous studies, these patients were studied in steady state, their compliance was proven, and they received therapeutic doses according to their INR response and thus in clinical practice the pharmacokinetic variation of R-W and S-W may be greater than in volunteers.

TABLE 3. Mean difference [95% confidence interval (CI)] between clearance values calculated by AUC (Cl) and one point serum concentration methods (Cl_6, Cl_{15}, and Cl_{20})[a]

	Mean difference (95% CI)		
	C1 vs. $C1_6$	C1 vs. $C1_{15}$	C1 vs. Cl_{20}
rac-W	-0.20 (–0.42, 0.02)	0.29 (0.03, 0.55)*	0.52 (0.24, 0.80)*
R-W	-0.26 (–0.41, –0.11)*	0.19 (-0.01, 0.39)	0.39 (0.14, 0.63)*
S-W	-0.30 (–0.42, –0.18)*	0.33 (-0.09, 0.74)	0.60 (0.10, 1.00)*

[a]All units are in ml/h/kg.
*$P < 0.05$.

LITERATURE CITED

1. Hewick, D.S., McEwen, J. Plasma half-lives, plasma metabolites and anticoagulant efficacies of the enantiomers of warfarin in man. J. Pharm. Pharmacol. 25(6):458–465, 1973.
2. Breckenridge, A., Orme, M., Wesseling, H., Lewis, R.J., Gibbons, R. Pharmacokinetics and pharmacodynamics of the enantiomers of warfarin in man. Clin. Pharmacol. Ther. 15(4):424–430, 1974.
3. Lewis, R.J., Trager, W.F. Warfarin metabolism in man: Identification of metabolites in urine. J. Clin. Invest. 49:907–913, 1970.
4. Lewis, R.J., Trager, W.F., Chan, K.K., Breckenridge, A., Orme, M., Rowland, M., Schary, W. Warfarin: Stereochemical aspects of its metabolism and the interaction with phenylbutazone. J. Clin. Invest. 53:1607–1617, 1970.
5. Chan, K.K., Lewis, R.J., Trager, W.F. Absolute configurations of the four warfarin alcohols. J. Med. Chem. 15(12):1265–1270, 1972.
6. O'Reilly, R.A. Studies on the optical enantiomorphs of warfarin in man. Clin. Pharmacol. Ther. 16(2):348–354, 1974.
7. Wingard, L.B., O'Reilly, R.A., Levy, G. Pharmacokinetics of warfarin enantiomers: A search for intrasubject correlations. Clin. Pharmacol. Ther. 23(2):212–217, 1978.
8. Hignite, C., Uetrecht, J., Tschanz, C., Azarnoff, D. Kinetics of R- and S-warfarin isomers. Clin. Pharmacol. Ther. 8(1):99–105, 1980.
9. Banfield, C., Rowland, M. Stereospecific high-performance liquid chromatographic analysis of warfarin and its metabolites in plasma and urine. J. Pharm. Sci. 73:1392–1396, 1985.
10. Toon, S., Holt, B.L., Mullins, G.F.P., Bullinham, R., Aarons, L., Rowland, M. Investigations into the potential effects of multiple dose keterolac on the pharmacokinetics and pharmacodynamics of racemic warfarin. Br. J. Clin. Pharmacol. 30:743–750, 1990.
11. Hallack, H.U., Wedlund, P.J., Modi, M.W., Patel, H., Lewis, G.L., Woodruff, B., Trowbridge, A.A. High clearance of S-warfarin in a warfarin-resistant subject. Br. J. Clin. Pharmacol. 35:327–330, 1993.
12. Chu, Y.U., Wainer, I.W. The measurement of warfarin enantiomers in serum using coupled achiral/chiral high performance liquid chromatography (HPLC). Pharm. Res. 5:680–683, 1988.
13. Chan, E., McLachlan, A.J., Pegg, M., Mackay, A.D., Cole, R.B., Rowland, M. Disposition of warfarin enantiomers and metabolites in patients during multiple dosing with rac-warfarin. Br. J. Clin. Pharmacol. 37:536–569, 1994.
14. McAleer, S.D., Chrystyn, H., Foondun, A.S. Measurement of the (R) and (S) isomers of warfarin in patients undergoing anticoagulant therapy. Chirality 4:488–493, 1992.
15. Allard, S., Peaker, S., Roberts, B.E., Davies, J.A., Feely, M.P. Patient compliance and control of anticoagulation with warfarin. Br. J. Haematol. 77(Suppl):P148, 1991.