High clearance of (S)-warfarin in a warfarin-resistant subject

HUSSEIN O. HALLAK¹, PETER J. WEDLUND¹, MARLENE W. MODI², INDRA H. PATEL², G. LUKE LEWIS³, BERNADETTE WOODRUFF³ & ARTHUR A. TROWBRIDGE³

¹College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082, USA, ²Hoffmann La Roche Inc., Nutley, NJ 07110-1199, USA and ³Scott and White Clinic, Texas A&M University, College of Medicine, Temple, TX 76508, USA

A 30 year old black male required a 60 mg daily dose of warfarin to elicit a therapeutic anticoagulant response (normal warfarin dose 2.5–10 mg day⁻¹; maximum 15 mg day⁻¹). Hereditary warfarin resistance was suspected after compliance, diet, concurrent medication and any gastrointestinal disorder were eliminated as contributory causes. The disposition of vitamin K and vitamin K epoxide was examined in the propositus, his two sisters and 13 control black male subjects. Each subject was given an i.v. bolus dose (5 mg) of vitamin K prior to and after 2 weeks of warfarin therapy (5 mg day⁻¹). The oral clearances of (S)- and (R)-warfarin were also measured in each subject during the last day of warfarin therapy. The mean ($\pm$ s.d.) systemic clearance of vitamin K was similar in all subjects before (114 ± 35 ml min⁻¹) and after (112 ± 40 ml min⁻¹) warfarin therapy. The mean ($\pm$ s.d.) AUC value for vitamin K epoxide was increased by warfarin treatment (6.5 ± 5.4 μ g ml⁻¹ min before and 139 ± 78 μ g ml⁻¹ min after) in all subjects. In the propositus, the oral clearance of (S)-warfarin (14.5 ml min⁻¹) and the clearance ratio for (S)/(R)warfarin (2.6) differed by more than 7 standard deviations from the control group (4.3 ± 1.1 ml min⁻¹ and 1.2 ± 0.2 , respectively). In one sister of the propositus, the stereoselective disposition of warfarin was comparable with that of her brother ((S)-warfarin clearance = 16.2 ml min⁻¹; and (S)/(R)-warfarin clearance ratio = 2.7).

Keywords warfarin resistance stereoselective vitamin K

Introduction

Warfarin resistance can be either acquired or hereditary. Possible causes of acquired resistance include enzyme induction of warfarin metabolism by other drugs (Corn, 1966; O'Reilly *et al.*, 1980); enhanced dietary intake of vitamin K (Lee *et al.*, 1981; O'Reilly & Rytand, 1980; Parr *et al.*, 1982; Walker, 1984; Zallman *et al.*, 1981); noncompliance with therapy (Bently *et al.*, 1986; O'Reilly, 1966); and hypothyroidism (Hansten, 1980; Kellett *et al.*, 1986). Although malabsorption has been considered as a possible cause for acquired resistance, because the absorption of warfarin is a passive process malabsorption is unlikely in the absence of severe gastrointestinal disease. There is also one report of a patient developing resistance to warfarin gradually over months of therapy (Barnett & Hancock, 1975).

Genetic differences in coumarin elimination are considered to contribute to differences in the dosing requirements of anticoagulants (Weiner *et al.*, 1950;

Wingard *et al.*, 1978), although to a limited extent (Lewis *et al.*, 1967). Hereditary differences in the response to vitamin K are thought to be more important (Alving *et al.*, 1985; Arnold *et al.*, 1977; Holt & Freytes, 1983; Kueh *et al.*, 1982; O'Reilly, 1970; O'Reilly & Aggeler, 1970; O'Reilly *et al.*, 1964; Warriar *et al.*, 1986; Zager *et al.*, 1973).

The resistance to warfarin exhibited by a 30 year old black male appeared to represent a typical case of hereditary warfarin resistance. Due to the autosomal dominant inheritance of this trait, two sisters of the propositus were also investigated. To examine the mechanism of this resistance, the disposition of vitamin K in the absence and presence of warfarin was studied in these subjects and in a control group of 13 black male volunteers. The stereoselective disposition of warfarin was also examined in each subject under steady state conditions.

Methods

Patient history

The propositus (Subject 1) was a 30 year old black male who was admitted to the hospital on November 7, 1988 with progressive oedema and discomfort of the right upper extremity of a few days duration. A venogram showed thrombosis involving the cephalic and basilic vein about the elbow and mid-arm and shoulder thrombosis involvement of the proximal axillary vein. The patient was treated initially with heparin i.v followed by warfarin beginning on the second day of admission. The patient was noted to be 'resistant' to warfarin. The prothrombin time during warfarin therapy remained unchanged and further attempts at anticoagulation were stopped at 20 mg day⁻¹ of warfarin (last day of warfarin November 19, 1988). The patient was readmitted from November 30 to December 14, 1988 with complaints of swelling of the left lower extremity. The left lower extremity venogram on December 14, 1988 showed occlusion of the deep femoral vein in the left groin with multiple collaterals at the level of the occlusion. The patient was treated initially with heparin. Warfarin was started on the second day of admission and the dose was increased progressively. A total dose of 60 mg warfarin daily was needed to achieve a therapeutic prothrombin time (the normal warfarin dose required to achieve a therapeutic response ranges from 2.5 to 10 mg day⁻¹). The patient responded to therapy, and warfarin was discontinued on June 12, 1989 at which time he was asymptomatic and has remained so since. Evaluation of this subject and available family members showed no evidence of a hypercoagulable state, i.e. normal antithrombin III, protein C, protein S, and plasminogen levels. There was also no family history of thromboembolic disease.

Subject selection

The propositus (Subject 1) and two of his sisters (Subjects 2 and 3) were black and aged 30 to 35 years. They were not receiving any medications that might interfere with warfarin therapy for at least 2 weeks prior to the beginning of the study. Thirteen nonsmoking, black males, 25 to 35 years old, who were not receiving any medications were recruited as a control group. Physical examination and clinical laboratory tests revealed that all of the subjects had normal thyroid function, prothrombin time and were clinically healthy.

The investigation was approved by the Investigational Review Board, and written informed consent was obtained from the subjects prior to their enrollment in the study. All the subjects were phenotyped for mephenytoin and dextromethorphan oxidative metabolism and were found to be extensive metabolizers (EM) of both drugs (Schmid *et al.*, 1985; Wedlund *et al.*, 1984; Wilkinson *et al.*, 1989). Subjects were also screened for drugs of abuse with negative findings.

Study design

Study 1: vitamin K-vitamin K epoxide kinetics After an overnight fast, each subject received a 5 mg dose of

vitamin K diluted in 10 ml of 0.9% saline solution, given intravenously over a period of 10 min. Blood samples were drawn in 10 ml foil-covered heparinized vacuum tubes at predose and at 5, 10, 15, 30 and 60 min and 2, 3, 4, 5, 7, 9, 12, 16, 18 and 24 h after vitamin K administration. Plasma was harvested under yellow light, placed in amber-coloured, foil-wrapped tubes and frozen at -20° C until analyzed.

Study II: stereoselective warfarin disposition and vitamin K-vitamin K epoxide concentration-time profile in the presence of warfarin After study I the subjects were started on 2 weeks of warfarin therapy (one 5 mg tablet of warfarin at 08.00 h each day). After the first week of warfarin therapy, the subjects had a brief clinical examination and a 5 ml blood sample was collected for the measurement of prothrombin time. During the 2 weeks, the dietary habits of the subjects were monitored by a daily diary and individuals were reminded not to ingest green leafy vegetables, liver or broccoli.

Two weeks after the first warfarin dose the subjects were admitted to the hospital. After an overnight fast, a blood sample was collected to determine the levels of vitamin K-dependent clotting factors (II, VII, IX, X and plasma proteins C and S).

At 08.00 h the next morning, the 5 mg warfarin dose was given orally along with a 5 mg dose of vitamin K diluted in 10 ml of 0.9% saline solution infused intravenously over 10 min. Blood samples were drawn in 10 ml foil-covered heparinized vacuum tubes at predose and at 5, 10, 15, 30 and 60 min and at 2, 3, 4, 5, 7, 9, 12, 16, 18 and 24 h after dosing. Plasma was harvested under yellow light, placed in amber-coloured, foil-wrapped tubes and frozen at -20° C until analyzed.

H.p.l.c. assays

The samples were assayed for warfarin enantiomers by the method of Banfield & Rowland (1984) with the modification that the ether extract was filtered through a silica-packed solid-phase extraction column (Maxi-Clean Cartridges, Alltech Associates, Deerfield, IL). Vitamin K and vitamin K epoxide concentrations in plasma were measured by the method of Hirauchi *et al.* (1988) except for the use of vitamin K₂ as internal standard.

Data analysis

AUC(0-24 h) values for (R)- and (S)-warfarin were estimated using the linear trapezoidal method. The AUC(0-24 h) for vitamin K was estimated by the trapezoidal method with extrapolation to infinite time. The AUC of vitamin K epoxide was measured from time zero up to the last assayable concentration (phase I) or until blood sampling ceased (24 h; phase II).

Steady-state plasma concentrations of warfarin enantiomers were determined by dividing the AUC of each isomer by the dosing interval (i.e. 24 h). The oral clearances of (R)- and (S)-warfarin were calculated from (0.5 × Dose)/AUC. The systemic clearance of vitamin K was calculated by dividing the dose of vitamin K by its corresponding AUC.

Statistical significance was assessed by determining the mean value and associated standard deviation for the parameter of interest in the control group. The 95% confidence intervals were established for each parameter by multiplying the standard deviation for the control group by 2. A statistical difference in the parameter of interest was presumed if the parameter in the resistant subject was two or more standard deviations from the mean value in the control group. Regression analysis was performed assuming a linear relationship between (S)-warfarin and vitamin K epoxide AUC.

Results

The AUC for vitamin K epoxide was increased significantly by warfarin therapy (Table 1) and there was a weak but significant correlation between S-warfarin concentration and vitamin K epoxide AUC ($r^2 = 0.36$). However, the propositus and his two sisters were *not* significantly different from the control group with respect to vitamin K clearance or the vitamin K epoxide AUC in the presence or absence of warfarin (Table 1).

The oral clearances for (R)- and (S)-warfarin and the clearance ratio for (S)/(R)-warfarin for the propositus and one of his sisters differed from the control group by more than two standard deviations (Table 2).

Discussion

O'Reilly *et al.* (1964) and subsequent others (Alving, 1985; Holt & Freytes, 1983; Kueh *et al.*, 1982; Warrier *et al.*, 1986; Zager *et al.*, 1973) have provided strong evidence that warfarin resistance is a pharmacogenetic trait mediated by changes at the receptor level. O'Reilly (1970) has even proposed that a genetic alteration in

Table 2 Stereoselective disposition of warfarin in the propositus, his two sisters and a group of control subjects

<i>Control male subjects</i>				
Subject	S-warfarin clearance (ml min ⁻¹)	R-warfarin clearance (ml min ⁻¹)	Warfarin (S)/(R) clearance ratio	PT time (Warfarin-treated)
Controls*	4.3 ± 1.1	3.6 ± 1.0	1.2 ± 0.2	13.8 ± 2.8
1. Propositus	14.5	5.8	2.6	11.3
2. Sister	16.2	6.0	2.7	11.2
3. Sister	5.2	2.7	1.9	10.7

*Values are mean ± s.d.

vitamin K epoxide reductase may mediate the altered response to warfarin in the resistant subjects he has studied. A significant change in vitamin K epoxide reductase efficiency and/or binding to warfarin, however, ought to be reflected in a significant change in the disposition of vitamin K and/or its epoxide metabolite. The findings of this study do not support such a mechanism for warfarin resistance in the subject we examined. The systemic clearances of vitamin K and the AUC of vitamin K epoxide were comparable in the resistant subject, his two sisters and the control group and with previously reported estimates (Bjornsson, 1984).

Warfarin enantiomers are known to be highly metabolized and highly protein bound. The low warfarin concentrations observed in the propositus and one of his sisters may be the result of low protein binding and/or a more enhanced metabolism. However, warfarin exhibits minor stereoselectivity in its plasma protein binding (Toon & Trager, 1984; Yacobi & Levy, 1977). Hence, the clearances of (R)- and (S)-warfarin should be affected similarly by any abnormalities in plasma proteins (i.e. the (S)/(R)-clearance ratio should not change). Nevertheless, the data shown in Table 2 indicate that the (S)/(R)-clearance ratio for the propositus and one of his sisters was increased significantly relative to the control group. This suggests that an abnormally low plasma protein binding is unlikely to explain the low warfarin concentrations observed in the propositus and his sister. Rather the difference observed provides support for an alteration in warfarin metabolism. The absence of drugs or any pathological process which might affect warfarin absorption or disposition, indicates that the resistance reported in this subject is a manifestation of an intrinsically high oral clearance of the more active (S)-warfarin isomer.

No correlations were observed between the clearances of warfarin enantiomers and the mephenytoin or dextromethorphan metabolic ratios. This indicates that these enzymes are not the source of the marked variability in the metabolism of warfarin. This is not surprising, however, since Rettie *et al.* (1992) have shown recently that cytochromes P-450 2C9 and 1A2 are the principle isoenzymes modulating the metabolism of warfarin enantiomers.

Table 1 Disposition of vitamin K and vitamin K epoxide in the absence and presence of warfarin

Subject	Vitamin K AUC (µg ml ⁻¹ min)	Vitamin K epoxide AUC (µg ml ⁻¹ min)	Vitamin K clearance (ml min ⁻¹)
<i>Without warfarin</i>			
Controls*	49 ± 17	4.9 ± 2.3	113 ± 37
1. Propositus	35	2.6	143
2. Sister	38	11.5	131
3. Sister	58	22.8	86
<i>With warfarin</i>			
Controls*	55 ± 26	156 ± 76	109 ± 42
1. Propositus	59	50	85
2. Sister	35	36	144
3. Sister	33	114	154

*Values are mean ± s.d.

References

- Alving, B. M., Strickler, M. P. & Knight, R. D. (1985). Hereditary warfarin resistance. *Arch. Int. Med.*, **145**, 499–501.
- Arnold, R. S., Israilli, Z. H., Dayton, P. G. & Wenger, N. K. (1977). Coumarin resistance: A diagnostic and therapeutic approach. *Southern med. J.*, **70**, 1191–1192.
- Banfield, C. & Rowland, M. (1984). Stereospecific fluorescence HPLC analysis of warfarin and its metabolites in plasma and urine. *J. pharm. Sci.*, **73**, 1392–1396.
- Barnett, D. B. & Hancock, B. W. (1975). Anticoagulant resistance: An unusual case. *Br. med. J.*, **1**, 608–609.
- Bently, D. P., Backhouse, G., Hutching, A., Haddon, R. L., Spragg, B. & Routledge, P. A. (1986). Investigation of patients with abnormal response to warfarin. *Br. J. clin. Pharmacol.*, **22**, 37–41.
- Bjornsson, T. D. (1984). Vitamin K and vitamin K antagonists. In *Drugs and Nutrients*, eds Roe, D. A. & Campbell, T. C., pp. 429–473. New York: Marcel Dekker Inc.
- Corn, M. (1966). Effect of phenobarbital and glutethimide on biologic half-life of warfarin. *Thromb. Diath. Haemorrh.*, **16**, 606–612.
- Hansten, P. D. (1980). Oral anticoagulants and drugs which alter thyroid function. *Drug Intell. clin. Pharm.*, **14**, 331–333.
- Hirauchi, K., Sakano, T., Nagaoka, T. & Morimoti, A. (1988). Simultaneous determination of vitamin K₁, vitamin K₁ 2,3 epoxide and menaquinone in human plasma by high performance liquid chromatography with fluorimetric detection. *J. Chromatogr.*, **430**, 21–29.
- Holt, R. J. & Freytes, C. O. (1983). Familial warfarin resistance. *Drug Intell. clin. Pharm.*, **17**, 281–283.
- Kellett, H. A., Sawers, J. S. A., Boulton, F. E., Cholerton, S., Park, B. K. & Toft, A. D. (1986). Problems of anticoagulation with warfarin in hyperthyroidism. *Quart. J. Med.*, **58**, 43–51.
- Kueh, Y. K., Ee, B. K. H. & Teoh, P. C. (1982). Resistance to oral anticoagulant therapy—case report and review of the literature. *Singapore med. J.*, **23**, 114–115.
- Lee, M., Schwartz, R. N. & Sharifi, R. (1981). Warfarin resistance and vitamin K. *Ann. Intern. Med.*, **94**, 140–141.
- Lewis, R. J., Spivack, M. & Spaet, T. H. (1967). Warfarin resistance. *Am. J. Med.*, **42**, 620–623.
- O'Reilly, R. A. (1966). Discussion: Resistance to coumarin anticoagulant drugs in man. *Thromb. Diath. Haemorrh.*, **21** (Suppl.), 280–283.
- O'Reilly, R. A. (1970). The second reported kindred and hereditary warfarin resistance to oral anticoagulant drugs. *New Engl. J. Med.*, **282**, 1448–1451.
- O'Reilly, R. A. & Aggeler, P. M. (1970). Determinants of the response to oral anticoagulant drugs in man. *Pharmac. Rev.*, **22**, 35–96.
- O'Reilly, R. A. & Rytand, D. A. (1980). Resistance to warfarin due to unrecognized vitamin K supplementation. *New Engl. J. Med.*, **303**, 160–161.
- O'Reilly, R. A., Aggeler, P. M., Hoag, M. S., Leong, L. S. & Kropatkin, M. L. (1964). Hereditary transmission of exceptional resistance to coumarin anticoagulant drugs. *New Engl. J. Med.*, **271**, 809–815.
- O'Reilly, R. A., Trager, W. F., Motley, C. H. & Howald, W. (1980). Interaction of secobarbital with warfarin pseudo-racemates. *Clin. Pharmac. Ther.*, **28**, 187–195.
- Parr, M. D., Record, K. E., Griffith, G. L., Zeok, J. V. & Todd, E. P. (1982). Effect of parenteral nutrition on warfarin therapy. *Clin. Pharm.*, **1**, 274–276.
- Rettie, A. E., Korzekwa, K. R., Kunze, K. L., Lawrence, R. F., Eddy, A. C., Aoyama, T., Gelboin, H. V., Gonzalez, F. J. & Trager, W. F. (1992). Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: A role for P-4502C9 in the etiology of (S)-warfarin drug interactions. *Chem. Res. Toxicol.*, **5**, 54–59.
- Schmid, B., Bircher, J., Preisig, R. & Kupfer, A. (1985). Polymorphic dextromethorphan metabolism: co-segregation of oxidative O-demethylation with debrisoquin hydroxylation. *Clin. Pharmac. Ther.*, **489**, 618–624.
- Toon, S. & Trager, W. F. (1984). Pharmacokinetic implications of stereoselective changes in plasma-protein binding: warfarin/sulfinpyrazone. *J. pharm. Sci.*, **73**, 1671–1673.
- Walker, F. B. (1984). Myocardial infarction after diet induced warfarin resistance. *Ann. Intern. Med.*, **144**, 2089–2090.
- Warrier, I., Brennan, C. A. & Lusher, J. M. (1986). Familial warfarin resistance in a black child. *Am. J. Ped. Hemat. Oncol.*, **8**, 346–357.
- Wedlund, P. J., Aslanian, W. S., McAllister, C. B., Wilkinson, G. R. & Branch, R. A. (1984). Mephenytoin hydroxylation deficiencies in Caucasians: frequency of a new oxidative drug metabolism polymorphism. *Clin. Pharmac. Ther.*, **36**, 773–780.
- Weiner, M., Shapiro, S., Axelrod, J., Cooper, J. R. & Brodie, B. B. (1950). The physiological disposition of dicumarol in man. *J. Pharmac. exp. Ther.*, **99**, 409–420.
- Wilkinson, G. R., Guengerich, F. P. & Branch, R. A. (1989). Genetic polymorphism of (S)-mephenytoin hydroxylation. *Pharmac. Ther.*, **43**, 53–76.
- Wingard, L. B., O'Reilly, R. A. & Levy, G. (1978). Pharmacokinetics of warfarin enantiomers: A search for intrasubject correlations. *Clin. Pharmac. Ther.*, **23**, 212–217.
- Yacobi, A. & Levy, G. (1977). Protein binding of warfarin enantiomers in serum of humans and rats. *J. Pharmacokin. Biopharm.*, **5**, 123–131.
- Zager, J., Tjandramaga, T. B., Cucinell, S. A. & Dayton, P. G. (1973). Coumarin resistance in a negro woman. *Ann. Intern. Med.*, **78**, 775–776.
- Zallman, J. A., Lee, D. P. & Jeffrey, P. L. (1981). Liquid nutrition as a cause of warfarin resistance. *Am. J. Hosp. Pharm.*, **38**, 1174.

(Received 29 April 1992,
accepted 10 September 1992)