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Brief report

Differential effects of 2C9*3 and 2C9*2 variants of cytochrome P-450 CYP2C9 on sensitivity to acenocoumarol

José Hermida, José Zarza, Ignacio Alberca, Ramón Montes, María Luz López, Eva Molina, and Eduardo Rocha

The 2C9*3 and 2C9*2 polymorphisms of cytochrome P-450 CYP2C9 are associated with hypersensitivity to warfarin and bleeding. The effect of these polymorphisms on sensitivity to acenocoumarol is unknown. Three groups of patients, with low, medium, or high acenocoumarol-dose requirements, were studied. Age influenced the acenocoumarol sensitivity. Bearing the 2C9*3 allele was associated with the need for a lower acenocou-

marol dose (odds ratio [OR], 6.02; 95% confidence interval [CI], 1.50-24.18); 80% of carriers of the 2C9*3 allele required a low dose. The 2C9*2 allele was associated with a lower acenocoumarol-dose requirement (OR, 2.70; 95% CI, 1.11-6.58) because of a reduced risk of the need for a high acenocoumarol dose (4.8% of the patients in the high-dose group carried the 2C9*2 allele versus 34.1% and 30.2%, respectively, in the medium-dose and low-

dose groups). Therefore, carriers of 2C9*3 may need a low initial loading dose of acenocoumarol. Because acenocoumarol sensitivity with the 2C9*2 variant does not seem to be clinically relevant, the drug could be an alternative to warfarin in 2C9*2 carriers. (Blood. 2002;99:4237-4239)

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Introduction

Oral anticoagulant therapy with coumarin derivatives is used broadly to prevent recurrent arterial and venous thrombosis. The response to coumarin derivatives depends on several factors, such as sex, age, diet, interacting drugs, and probably other unknown individual and ethnic factors.^{1,2} Warfarin is the main coumarin derivative used in the United Kingdom and United States. The biotransformation and subsequent elimination of warfarin are due mainly to oxidation by cytochrome P-450 CYP2C9. Recently, 2 variant alleles of this cytochrome that reduce in vitro enzymatic activity have been identified: 2C9*2 (Arg144Cys) and 2C9*3 (Ile359Leu).^{3,4} These variant alleles have been strongly associated with increased sensitivity to warfarin and, less clearly, with the risk of bleeding during anticoagulant treatment.⁵⁻⁷

Acenocoumarol is a coumarin derivative used widely for oral anticoagulant therapy in many European and Latin American countries. Mannucci⁸ has suggested that acenocoumarol be used instead of warfarin in patients carrying the hyperresponsive alleles 2C9*2 and 2C9*3. Acenocoumarol, like warfarin, is administered as a racemate mixture of (R)- and (S)-acenocoumarol. Cytochrome P-450 CYP2C9 is involved in the oxidation of both enantiomers, whereas cytochrome P-450 CYP2C19 acts on (R)-acenocoumarol only.⁹ Because the (S)-enantiomer is more active than the (R)-enantiomer, allelic variations of cytochrome P-450 CYP2C9 could play an important role in sensitivity to acenocoumarol. In this context, the 2C9*3 allele has been linked to raised plasma levels of (S)-acenocoumarol in a small group of patients with low acenocoumarol requirements.¹⁰

Because of the broad clinical use of acenocoumarol in many countries and the possible use of acenocoumarol as an alterna-

tive to warfarin in patients bearing the 2C9*2 and 2C9*3 variants, we studied the role of these polymorphisms in modulating the acenocoumarol requirement in patients receiving anticoagulation treatment.

Study design

Patients

The study included 113 outpatients attending the Hospital Clínico Universitario de Salamanca, Spain, who had been receiving a stable acenocoumarol dose for at least 3 months to achieve an International Normalized Ratio between 2 and 3.2. The patients were classified into the following 3 groups according to the acenocoumarol dose prescribed in the previous month: a group of 43 consecutive patients with low dose requirements (≤ 7 mg/week), a group of 45 consecutive patients with medium dose requirements (> 7 mg and < 28 mg/week), and a group of 21 consecutive patients with high dose requirements (≥ 28 mg/week). Age, sex, weekly dose required, and intake of drugs affecting acenocoumarol metabolism were recorded. Two patients with liver disease and 2 with thyroid disease were excluded from the study. The percentages of patients in the low-, medium-, and high-dose acenocoumarol groups in the whole population of patients receiving anticoagulation treatment were 11%, 83.5%, and 5.5%, respectively. All subjects gave informed consent to participation in the study.

Genotyping

Genotyping for the 2C9*3 and 2C9*2 alleles was done by using polymerase chain reaction followed by digestion with restriction enzymes according to methods previously described,⁶ with minor changes. The primers used for 2C9*3 analysis were 5'-TGCACGAGGTCC-AGAGATGC-3' and 5'-AATGATACTATGAATTTGGGACTT CGA-3',

From the Hemostasis and Thrombosis Research Unit, School of Medicine, University of Navarra, Pamplona, Spain; the Haematology Service, University Clinic of Navarra, Pamplona, Spain; and the Department of Haematology, University Hospital of Salamanca, Spain.

Reprints: Eduardo Rocha, Haematology Service, University Clinic of Navarra, Avenida Pío XII 36, 31008 Pamplona, Spain; e-mail: erocha@unav.es.

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and the restriction enzyme was *NsiI*. The primers used for 2C9*2 analysis were 5'-CAATGGAAAGAAATGGAAGGAGGT-3' and 5'-GACTCATA-ATGAAAGATATG-3', and the restriction enzyme was *AvaII*.

Statistical analysis

Dose was treated as an ordinal variable (low, medium, and high). Values for age and dose, when analyzed as a continuous variable, were expressed as means \pm SD. A multivariate ordered logistic regression model was used to assess the effect of allelic variants and age (independent variables) on the acenocoumarol requirement. Thus, the relation between the ordinal variable dose (low, medium, or high) and allelic variants and age could be established, with controlling for sex and use of other drugs.¹¹ The adequacy of the model was assessed by using the Pearson goodness-of-fit test. Subjects heterozygous and homozygous for the allelic variants were included in the same category because of the low prevalence of homozygous subjects. One-way analysis of variance was used to compare age between groups. Differences in the acenocoumarol requirement between patients who were carriers and noncarriers of the 2C9*3 allele in the low-dose group were assessed with the Student *t* test. Statistical analyses were done with SPSS software (version 10.0; SPSS, Chicago, IL).

Results and discussion

Fifteen of 108 patients carried the 2C9*3 allele, with 14 being heterozygous and one homozygous; one patient could not be analyzed. Twenty-nine of 108 patients carried the CYP2C9*2 allele, with 26 being heterozygous and 3 homozygous; one patient could not be analyzed (Table 1). Three patients in the low-dose group and one in the medium-dose group were double heterozygous. No double-heterozygous patients were found in the high-dose group. The mean age in the low-, medium-, and high-dose groups was significantly different (74.3 ± 7.7 years, 65.7 ± 10.5 years, and 58.3 ± 11.0 years, respectively; $P < .001$).

We analyzed the effect of 2C9*3 and 2C9*2 variants, age, sex, and interacting drugs by using a multivariate ordered logistic regression model. Neither sex nor drugs affected the acenocoumarol requirement. This finding could have been due to the low number of patients taking other drugs: only 14 patients were taking metabolism-decreasing drugs (8 in the low-dose, 3 in the medium-dose, and 3 in the high-dose group) and only 2 were taking metabolism-increasing drugs (one in the low-dose and one in the high-dose group). Because there were no significant interactions among the analyzed variables (data not shown), the final model was formed by using 2C9*3 and 2C9*2 variants and age (Table 2).

Age was an important factor modulating the sensitivity to acenocoumarol. For every year of age, there was a 12% increase in

Table 2. Adjusted odds ratios for 2C9*3 and 2C9*2 variants and patient age

Variable	Adjusted OR	95% CI	P*
CYP2C9*3	6.02	1.50-24.18	.0114
CYP2C9*2	2.70	1.11-6.58	.0284
Year of age	1.12	1.07-1.17	< .0001

Adjusted ORs are measurements of being at risk of needing a lower dose of acenocoumarol when carrying an allelic variant or when being 1 year older. Patient sex and use of other drugs were finally excluded from the multivariate model because they did not show significant effects ($P = .41$ and $P = .56$, respectively) or play a role in any interactions.

CI indicates confidence interval. *On Wald testing.

the risk of needing a lower acenocoumarol dose (odds ratio [OR], 1.12; 95% confidence interval [CI], 1.07-1.17).

Carrying the 2C9*3 allele was an important independent factor modulating the sensitivity of patients to the anticoagulant effect of acenocoumarol. Patients with this allele were at a higher risk of needing a lower dose of acenocoumarol than patients without this variant (adjusted OR, 6.02; 95% CI, 1.50-24.18). This effect was due mainly to the remarkable number of carriers of the 2C9*3 allele among the patients in the low-dose group. Eighty percent of patients carrying at least one 2C9*3 allele had a very low acenocoumarol requirement (< 7 mg/week); moreover, the mean weekly dose of acenocoumarol required by patients in the low-dose group with at least one 2C9*3 allele was lower than that of patients in the same group without this allelic variant (5.04 ± 1.26 mg versus 6.15 ± 0.94 mg; $P = .04$). Together, these results suggest a remarkable effect of the 2C9*3 allele on sensitivity to acenocoumarol, possibly one even greater than the previously observed effect on warfarin metabolism.^{5,6} This finding could be relevant applied to clinical practice: because it has been suggested that hyperresponsiveness to warfarin is linked to a high risk of bleeding at the beginning of treatment, patients bearing the 2C9*3 allele that are to be treated with acenocoumarol should start treatment with a reduced initial loading dose to avoid overanticoagulation and subsequent bleeding risk.

The 2C9*2 variant also played an independent and significant role on patients' sensitivity to acenocoumarol. Carrying at least one 2C9*2 allele also increased the risk of requiring a lower dose of acenocoumarol (OR, 2.70; 95% CI, 1.11-6.58). Interestingly, this effect was due mainly to the remarkably reduced risk of needing an acenocoumarol dose higher than 28 mg/week when this variant was present: only 4.8% of patients in the high-dose group carried the 2C9*2 allele, whereas it was present in 34.1% of patients in the medium-dose group and 30.2% in the low-dose group. Nevertheless, the effect of the 2C9*2 allele on the acenocoumarol requirement was much smaller than the effect exerted by the 2C9*3 allele. In the clinical setting, because the 2C9*2 allele has been described as a major factor conditioning warfarin sensitivity, it may be advisable for patients bearing the 2C9*2 allele to start anticoagulant treatment with acenocoumarol instead of warfarin to avoid overanticoagulation risk and subsequent bleeding.⁵⁻⁷

In conclusion, we demonstrated that the 2C9*3 allele importantly and independently modulates sensitivity to acenocoumarol, a finding indicating that a reduced initial loading dose of acenocoumarol should be used in carriers of this allele. The weak effect on acenocoumarol sensitivity exerted by the 2C9*2 allele makes this drug a potential alternative to warfarin in patients carrying this variant.

Table 1. Frequencies of cytochrome P-450 2C9 allelic variants in patients with different acenocoumarol-dose requirements

Variant	Low dose (n = 43)	Medium dose (n = 45)	High dose (n = 21)
CYP2C9*1/*1	30 (71.4)	43 (95.6)	20 (95.2)
CYP2C9*1/*3	11 (26.2)	2 (4.4)	1 (4.8)
CYP2C9*3/*3	1 (2.4)	—	—
CYP2C9*1/*1	30 (69.8)	29 (65.9)	20 (95.2)
CYP2C9*1/*2	11 (25.6)	14 (31.8)	1 (4.8)
CYP2C9*2/*2	2 (4.6)	1 (2.3)	—

Values are numbers (%) of patients.

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