Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications

Guruprasad P Aithal, Christopher P Day, Patrick J L Kesteven, Ann K Daly

Summary

Background The cytochrome P450 CYP2C9 is responsible for the metabolism of S-warfarin. Two known allelic variants CYP2C9*2 and CYP2C9*3 differ from the wild type CYP2C9*1 by a single amino acid substitution in each case. The allelic variants are associated with impaired hydroxylation of S-warfarin in in-vitro expression systems. We have studied the effect of CYP2C9 polymorphism on the in-vivo warfarin dose requirement.

Methods Patients with a daily warfarin dose requirement of 1.5 mg or less (low-dose group, n=36), randomly selected patients with a wide range of dose requirements from an anticoagulant clinic in north-east England (clinic control group, n=52), and 100 healthy controls from the community in the same region were studied. Genotyping for the CYP2C9*2 and CYP2C9*3 alleles was done by PCR analysis. Case notes were reviewed to assess the difficulties encountered during the induction of warfarin therapy and bleeding complications in the low-dose and clinic control groups.

Findings The odds ratio for individuals with a low warfarin dose requirement having one or more CYP2C9 variant alleles compared with the normal population was 6.21 (95% CI 2.48–15.6). Patients in the low-dose group were more likely to have difficulties at the time of induction of warfarin therapy (5.97 [2.26–15.82]) and have increased risk of major bleeding complications (rate ratio 3.68 [1.43–9.50]) when compared with randomly selected clinic controls.

Interpretation We have shown that there is a strong association between CYP2C9 variant alleles and low warfarin dose requirement. CYP2C9 genotyping may identify a subgroup of patients who have difficulty at induction of warfarin therapy and are potentially at a higher risk of bleeding complications.

Lancet 1998; 353: 717–19
See Commentary page xxxx

Introduction

Warfarin is the oral anticoagulant of choice in the UK and many other countries and is being prescribed to an increasing number of patients. The risk of serious haemorrhage during warfarin therapy ranges from 1.3 to 4.2 per 100 patient years of exposure.1,2 Haemorrhage incidence is associated with the intensity of anticoagulation, with the deviation in prothrombin time ratio shown to be the strongest risk factor for bleeding complications.3

Widespread interindividual variation in the response to a given dose of warfarin makes the prediction of an accurate maintenance dose difficult, with an effective daily dose ranging from 0.5 mg to 60 mg.4,5 Standardised induction regimens, with monitoring of International Normalised Ratio (INR) over the first 4 days, have only a 69% success rate in predicting the correct maintenance dose,6 and, although complex normograms and computer programs requiring protein C and S estimations have been shown to improve the prediction they are not suitable for routine use.7 An understanding of the genetics of warfarin metabolism and the identification of genetic factors that contribute to the individuality of the warfarin dose response may help to ameliorate clinical difficulties associated with warfarin therapy.

The asymmetric carbon of warfarin (C9) gives rise to two enantiomeric forms, R-warfarin and S-warfarin, which are differentially metabolised. When administered as a racemate, S-warfarin is about three times as potent as R-warfarin.8 The cytochrome P450 CYP2C9 is the principal enzyme that catalyses the conversion of S-warfarin to inactive 6-hydroxy and 7-hydroxy metabolites whereas the oxidative metabolism of R-warfarin is mainly catalysed by CYP1A2 and CYP3A4.9 The existence of genetic polymorphisms in CYP2C9 giving rise to functionally significant effects on enzyme activity is now well established. In addition to the wild-type (CYP2C9*1) allele, point mutations in the CYP2C9 gene result in two allelic variants—CYP2C9*2, where cysteine substitutes for arginine at amino acid 144, and CYP2C9*3, where leucine substitutes for isoleucine at residue 359.10 Both allelic variants have impaired hydroxylation of S-warfarin when expressed in vitro.11,12 The CYP2C9*3 variant is less than 5% as efficient as the wild-type enzyme, while CYP2C9*7 shows about 12% of wild-type activity, apparently as a result of the amino acid substitution altering the interaction of the enzyme with cytochrome P450 oxidoreductase.13,14 Because these in-vitro data and some preliminary in-vivo data15 have suggested that the known polymorphisms in CYP2C9 significantly impair S-warfarin metabolism, we have now investigated whether either CYP2C9 allelic variant was more common in patients with a lower than average warfarin dose requirement and have also examined whether the incidence of bleeding complications is associated with CYP2C9 genotype.

Methods

The investigation was approved by the Newcastle upon Tyne Joint Ethics Committee and all the participants gave informed consent.

Participants

Patients whose warfarin dose requirement was 1.5 mg per day or less, had a stable warfarin dose requirement for at least three

---

The Lancet • Vol 353 • February 27, 1999

717
consecutive clinic visits with a target INR of 2·0 to 3·0, and no apparent cause for low-dose requirement (eg, drug interactions or liver disease) were recruited from an anticoagulation clinic in north-east England. Of a total of 828 patients registered with the clinic at the time of recruitment, 76 fulfilled the requirements for the study. Of these, 57 patients had prescheduled clinic visits during the study period (July 1, 1997–July 31, 1997) and were invited to take part in the study. The other 19 patients either had clinic visits prescheduled for a later date (n=14) or failed to attend the scheduled visit during the study period (n=5) and were not invited to take part in the study. Of 57 patients, 36 (17 male, 19 female) aged 55–88 (median 73) years, who agreed to take part in the study, formed the low-dose group. Reasons for refusing consent in the other 21 patients were fear of needles, lack of time, and lack of interest in research.

From a group of 124 consecutive attendees to the anticoagulant clinic invited to take part in the study, 52 individuals (26 men, 26 women) aged 33–94 (median 70·5) years, with a wide range of daily warfarin dose requirement, were recruited to the clinic control group during the month starting June 5, 1998. One patient previously included in the low-dose group was excluded. The community control group consisted of 100 individuals (58 men, 42 women) aged 38–91 (median 70·5) years for patients in the low-dose group and 0·01 for patients in the clinic control group. In the low-dose group, six minor, five serious, and six life-threatening bleeding episodes occurred in 11 patients, two life-threatening bleeding episodes in 11 patients during 311 patient years of warfarin treatment in the low-dose group ranged from 2·0 to 10·0 (median 4·4). 20 of 36 patients (two patients with *1/*1 genotype and 18 with one or more of the mutant alleles) had a peak INR above the therapeutic range of greater than 4·0 during induction of warfarin therapy when compared with nine of 52 patients in the clinic control group (odds ratio 5·97 [2·26–15·82]). Nine of 20 patients in the low-dose group with supra-therapeutic INR during induction had their inpatient stay prolonged for 1–9 days (median 2 days) while optimum anticoagulation was achieved. In seven additional patients, raised INR resulted in frequent visits to the anticoagulant clinic or additional investigations, or both. No patient in the clinic control group had their inpatient stay prolonged because of poor control of anticoagulation.

Duration of warfarin treatment was 0·2–17 (median 2) years for patients in the low-dose group and 0·1–30 (3·1) years for patients in the clinic control group. In the low-dose group, during 132·8 patient years of warfarin treatment, seven minor, five serious, and six life-threatening bleeding episodes occurred in 11 patients, including one patient where bleeding contributed to death. This compares with six minor, five serious, and two life-threatening bleeding episodes in 11 patients during 311·1 patient years of warfarin therapy in the clinic control group. In the low-dose group, six minor and one major bleeding episode occurred in five patients within the first 4 weeks of induction of warfarin therapy. Each of these patients had one or more variant CYP2C9 alleles.

**Table 1:** CYP2C9 genotype distribution

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Low-dose group (n=36)</th>
<th>Random clinic control group (n=52)</th>
<th>Community control group (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*1/*1</td>
<td>7 (0·19)</td>
<td>32 (0·62)</td>
<td>60 (0·60)</td>
</tr>
<tr>
<td>CYP2C9*1/*2</td>
<td>12 (0·33)</td>
<td>9 (0·17)</td>
<td>20 (0·20)</td>
</tr>
<tr>
<td>CYP2C9*1/*3</td>
<td>10 (0·28)</td>
<td>10 (0·19)</td>
<td>17 (0·17)</td>
</tr>
<tr>
<td>CYP2C9*2/*2</td>
<td>5 (0·14)</td>
<td>0 (0·00)</td>
<td>2 (0·02)</td>
</tr>
<tr>
<td>CYP2C9*2/*3</td>
<td>2 (0·06)</td>
<td>1 (0·02)</td>
<td>0 (0·00)</td>
</tr>
<tr>
<td>CYP2C9*3/*3</td>
<td>0 (0·00)</td>
<td>1 (0·00)</td>
<td>1 (0·01)</td>
</tr>
</tbody>
</table>

**Results**

CYP2C9 genotype distributions in the low-dose warfarin group compared with the clinic controls and the community controls are summarised in table 1. In the low-dose warfarin group, 29 (81%) of the 36 patients had one or more of the variant alleles present compared with 40 (40%) of 100 in the control group. The odds ratio for individuals with a low warfarin dose requirement having one or more CYP2C9 variant alleles compared with the normal population was 6·21 (95% CI 2·48–15·6). When possession of either one or two variant alleles was considered separately, the odds ratio for a patient on low-dose warfarin with one variant allele only compared with the general population was 2·68 (95% CI 1·22–5·86) and with two variant alleles was 7·8 (95% CI 1·90–32·1). Genotype and allele frequencies were measured in the clinic controls to test whether particular CYP2C9 genotypes were associated with an increased risk of requiring anticoagulant treatment. There was no significant difference in CYP2C9 genotype frequencies between the clinic controls and the community control group. Age and sex were not confounders between the low-dose group and clinic controls because the groups were not significantly different with respect to these factors. A further control group of 37 patients randomly selected from patients attending another anticoagulant clinic in the same region had the same CYP2C9 genotype frequencies as the community control group. Allele frequencies of 0·785 for CYP2C9*1, 0·110 for CYP2C9*2, and 0·05 for CYP2C9*3 in our control groups are comparable to those of 0·79, 0·125, and 0·085 for CYP2C9*1, *2 and *3 alleles, respectively, reported in a study of a white British population.

All the patients in the study group were started on warfarin as inpatients. Each patient received an initial dose of 10 mg warfarin, with further dose requirements determined by a modified Fennerity formula1 with regular estimations of INR. Peak INR during the first week of warfarin treatment in the low-dose group ranged from 2·0 to 10·0 (median 4·4). 20 of 36 patients (two patients with *1/*1 genotype and 18 with one or more of the mutant alleles) had a peak INR above the therapeutic range of greater than 4·0 during induction of warfarin therapy when compared with nine of 52 patients in the clinic control group (odds ratio 5·97 [2·26–15·82]). Nine of 20 patients in the low-dose group with supra-therapeutic INR during induction had their inpatient stay prolonged for 1–9 days (median 2 days) while optimum anticoagulation was achieved. In seven additional patients, raised INR resulted in frequent visits to the anticoagulant clinic or additional investigations, or both. No patient in the clinic control group had their hospital stay prolonged because of poor control of anticoagulation.
The incidence of minor bleeding episodes was higher in the low-dose group (seven in 1328 patient years) when compared with that in random clinic controls (six in 3111 patient years) though this did not reach significance (rate ratio 2.73 [95% CI 0.92–8.1], p=0.07). Significantly higher numbers of major (serious and life-threatening) bleeding episodes occurred in the low-dose group (11 in 1328 patient years) when compared with the random clinic controls (seven in 3111 patient years; rate ratio 3.68 [1.43–9.50], p=0.007). Comparison between the low-dose group and the random clinic controls is shown in Table 2.

**Table 2: Comparison of low-dose group with random clinic controls**

<table>
<thead>
<tr>
<th></th>
<th>Low-dose group (n=36)</th>
<th>Random clinic control group (n=52)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male; female</td>
<td>17:29</td>
<td>26:26</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>55–88 (median 73)</td>
<td>39–95 (median 70.5)</td>
<td>0.11</td>
</tr>
<tr>
<td>Follow up (years)</td>
<td>0–2–17 (median 2)</td>
<td>0–1–30 (median 3.1)</td>
<td>0.35</td>
</tr>
<tr>
<td>Number with INR &gt;4.0</td>
<td>20</td>
<td>9</td>
<td>0.0002</td>
</tr>
<tr>
<td>at induction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minor bleeding episodes</td>
<td>5:27</td>
<td>1:93</td>
<td>0.07</td>
</tr>
<tr>
<td>(% per person years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major bleeding episodes</td>
<td>8:28</td>
<td>2:25</td>
<td>0.007</td>
</tr>
<tr>
<td>(% per person years)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

In this study we show that there is a strong association between CYP2C9 genotype and warfarin sensitivity. An individual requiring a low warfarin dose is six times more likely to be positive for one or more of the variant alleles associated with impaired S-warfarin metabolism (CYP2C9*2 and CYP2C9*3) compared with the general population. CYP2C9 genotyping appears to have the potential to identify a subgroup of individuals who are poor metabolisers of warfarin, hence they require a very low dose of drug. Our findings also indicate that a significant proportion of these individuals have difficulties at the time of induction of warfarin therapy and have an increased risk of bleeding complications when compared with clinic controls.

With the exception of homozygosity for CYP2C9*3, all the other combinations of variant alleles occurred more frequently in the low-dose group compared with the controls. One of 100 controls was homozygous for CYP2C9*3 while none in the low-dose group had this genotype. It is predicted from the control genotype frequencies that eight individuals attending the clinic were homozygotes, making the absence of CYP2C9*3 homozygotes in the low-warfarin-dose group unexpected. However, because in-vitro studies have shown extremely slow hydroxylation of S-warfarin when CYP2C9*3 is expressed, it is possible that individuals homozygous for this allele have such a low warfarin dose requirement that stabilisation is not successful and treatment with warfarin is abandoned. Interestingly, diminished clearance of S-warfarin with exacerbated response to normal doses of warfarin has been recently reported in a patient who was homozygous for CYP2C9*3.14

Impaired metabolism of a low therapeutic index drug such as warfarin has important clinical consequences. Difficulty in establishing optimum anticoagulation was experienced in 20 (56%) of 36 patients in the low-dose group with their peak INR rising above the target range after a fixed-dose regimen. 18 of these patients carried one or more variant CYP2C9 alleles. Difficult induction resulted in delayed discharges, multiple visits to the clinicians, and additional investigations in an attempt to seek an explanation for warfarin sensitivity. A smaller warfarin dose requirement may be associated with greater variability of INR and this, coupled with warfarin’s low therapeutic index, may lead to bleeding complications. These are likely to occur later during the course of warfarin therapy, presumably when monitoring is relaxed. Consistent with this, the incidence of major bleeding complications in the low-dose group was four times higher than that in the clinic controls.

We suggest that knowledge of CYP2C9 genotype when deciding on treatment with warfarin may help to reduce problems during induction. In addition, the likely increased risk of bleeding in patients with mutations may influence the decision to start warfarin therapy, particularly in patients in whom the benefit is perceived to be small.

**Contributors**

Guruprasad Aithal was involved in genotyping and wrote the paper. Christopher Day and Ann Daly jointly supervised the study and revised the paper. Patrick Kesteven contributed to the study design and data analysis.

**Acknowledgments**

This study was supported in part by Eurohepatox Biomed 2 programme of the European Union. We are grateful to Annette Steward, Selahaddin Tekes, and Catherine Lamb for their assistance in genotyping.

**References**