Warfarin Therapy – Why One Dose Does Not Fit All!

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Abstract

We report an Indian adult female patient with Deep Vein Thrombosis (DVT), in whom it was difficult to achieve and maintain target INR on warfarin (oral anticoagulant) by conventional doses. Pharmacogenomics study for warfarin revealed that she had Homozygous mutant for CYP2C9 *3(CYP2C9 *3/*3) and Heterozygous mutant for VKORC 1(1639G>A) [genetic polymorphism double defect]. This conferred a greater sensitivity to her warfarin therapy in an otherwise conventional dose regime used in most patients, making her management challenging. This sensitivity (or resistance in other cases) can be assessed by this evidence based test and warfarin dosing could be individualised to avoid toxicity.

Introduction

Warfarin is an oral anticoagulant used for prophylaxis as well as treatment of thromboembolism. Warfarin treatment is dependent upon interaction between physiological, environmental and genetic factors.1 Cytochrome P 450 2C9 (CYP2C9) is a highly polymorphic liver enzyme involved in the metabolism of Warfarin. Due to a narrow therapeutic index of Warfarin, the risk of bleeding is high if the dose of Warfarin exceeds the needed amount. Impairment in CYP2C9 metabolic activity might cause difficulty in dose adjustment as well as toxicity. Vitamin K epoxide reductase complex subunit 1 (VKORC 1) is the site of action of Warfarin.2 Inherited differences in VKORC 1, increase or decrease the amount of Warfarin required to inhibit the formation of functional clotting factors. Thus CYP2C9 and VKORC1 mutations affect Warfarin clearance and therapeutic response.3 Combination of mutations of one or more genes reduces the average daily warfarin requirements further. Physicians have to use individualised approach while using warfarin to achieve efficacy with safety.

Case Summary

A 45 year old married female presented to surgery department with painful swelling of the left lower limb. She was a known case of hypertension controlled on tablet Amlodipine. On examination, her vitals were stable and systemic examination was normal. Local examination of the left leg revealed swelling with tenderness of calf muscles. Homan’s sign was positive suggesting the possibility of deep vein thrombosis.

Investigations revealed a normal CBC, normal liver and renal parameters, mild hepatomegaly on ultrasound of abdomen. Chest x-ray revealed cardiomegaly. ECG was normal. A trivial mitral regurgitation was noted on 2-D echo cardiography. Venous Doppler of the left lower limb revealed presence of a thrombus in left external iliac, common femoral and deep femoral veins. Her serum homocysteine level was 32.68 µmol/L (normal =3.36 to 20.44 µmol/L).

She was started on low molecular weight heparin with overlap of tablet warfarin 5 mg per day for 5 days. Tablet Folic acid was given for hyperhomocysteinaemia. She was referred to Haematology department for management of anticoagulation as her INR was 5. In view of the risk of bleeding, she was advised to stop Warfarin and to repeat PT/INR. Despite stopping her oral anticoagulant, her INR remained 5. After 10 days her INR was 2.23. She was started on Warfarin 2 mg per day and was discharged.

She was on a regular follow up for PT/INR. In the next month she was readmitted in the same surgical unit for generalised weakness and bleeding per vaginum for 7 days. She was on the same dose of 2 mg per day of Warfarin and INR on admission was 1.69. In view of bleeding, Warfarin was stopped. Local cause for bleeding was ruled out when she was referred to Gynaecology department. Bleeding was controlled with tranexamic acid. She was transfused 2 units of packed cells as Haemoglobin had significantly dropped by 4 gm%. She was started on 2 mg of Warfarin when INR was 1.05. In the next follow-up as INR was 1.9 (<2) dose of Warfarin was increased to 3 mg per day. Then INR increased to 4.29 so again the dose was reduced to 2 mg per day. INR remained above 4, hence dose was decreased to 1 mg alternate day. This was not enough to maintain INR between 2 and 3.

It is evident from Table 1 that there were marked fluctuations in the INR as well as the dose of Warfarin. It was very difficult to achieve and maintain a therapeutic INR with a small dose of Warfarin. Increasing the dose of Warfarin by 1 mg also was associated with increase in INR above 4 and the risk of bleeding.

Patient’s compliance was confirmed and other non-genetic factors as also drug intake for interaction affecting the metabolism of Warfarin were ruled out. In view of initial episode of bleeding with target INR and subsequent inability to achieve and maintain the target INR, we thought of doing the gene profile for CYP2C9 and VKORC 1 (pharmacogenomics).

Gene Profile Report

Gene tested: CYP2C9 (*2 and *3) and VKORC 1(1639G>A)

Result

Wild type for CYP2C9 *2
Homozygous mutant for CYP2C9 *3(CYP2C9 *3/*3)
Heterozygous mutant for VKORC 1(1639G>A)

Methodology

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)
Table 1: Showing patient’s PT/INR and the corresponding doses of Warfarin

<table>
<thead>
<tr>
<th>Date</th>
<th>PT (Sec)</th>
<th>INR</th>
<th>Dose of Warfarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>05.03.09</td>
<td>&gt; 60</td>
<td>&gt; 5</td>
<td></td>
</tr>
<tr>
<td>16.03.09</td>
<td>29</td>
<td>2.23</td>
<td>2 mg od</td>
</tr>
<tr>
<td>08.04.09</td>
<td>20.7</td>
<td>1.69</td>
<td></td>
</tr>
<tr>
<td>25.05.09</td>
<td>13.7</td>
<td>1.05</td>
<td>2 mg od</td>
</tr>
<tr>
<td>17.06.09</td>
<td>24.7</td>
<td>1.90</td>
<td>3 mg od</td>
</tr>
<tr>
<td>30.06.09</td>
<td>57.4</td>
<td>4.29</td>
<td>2 mg od</td>
</tr>
<tr>
<td>06.07.09</td>
<td>54.2</td>
<td>4.17</td>
<td>1 mg alt day</td>
</tr>
<tr>
<td>20.07.09</td>
<td>15.6</td>
<td>1.20</td>
<td>1 mg od</td>
</tr>
<tr>
<td>27.07.09</td>
<td>17.5</td>
<td>1.35</td>
<td>1 mg and 2 mg on alt day</td>
</tr>
<tr>
<td>10.08.09</td>
<td>55.1</td>
<td>4.24</td>
<td>1 mg od</td>
</tr>
<tr>
<td>22.08.09</td>
<td>47.9</td>
<td>3.68</td>
<td>1 mg alt day</td>
</tr>
<tr>
<td>27.08.09</td>
<td>35.6</td>
<td>2.69</td>
<td>1 mg alt day</td>
</tr>
<tr>
<td>17.09.09</td>
<td>21.8</td>
<td>1.68</td>
<td>1 mg for 2 days, off next day</td>
</tr>
<tr>
<td>05.10.09</td>
<td>14.9</td>
<td>1.15</td>
<td>1 mg for 2 days, 2 mg next day</td>
</tr>
</tbody>
</table>

These results were further confirmed by DNA sequencing.

As our patient was homozygous for mutation of CYP2C9 *3(CYP2C9 *3/*3) and heterozygous for mutation of VKORC 1(1639G > A), it was a therapeutic challenge to achieve the maintenance dose of Warfarin.

**Discussion**

Clinical use of Warfarin is complicated by unpredictable dose response which depends on multiple factors. Non-genetic factors such as demographics, diet, interacting drugs account for 30%. Genetic factors such as polymorphism play a role in 30% cases and some unknown factors are responsible for the remaining 40% cases. Genes encode proteins or enzymes. Change of amino acid can cause difference in the sequence of the gene. These single nucleotide polymorphisms (SNPs) change the form of the enzyme along with its activity. So the same drug is processed in different way by each individual.

Pharmacogenetics is the study of inherited variations in genes for drug metabolism and response. Pharmacogenomics is the whole genome application of pharmacogenetics. Pharmacogenomics of Warfarin is of special importance. Warfarin has a narrow therapeutic index. Anticoagulant benefits are countered by this narrow therapeutic index. Due to interaction between genotype and drug response, there is > 10 fold interindividual variability in the dose required to achieve a therapeutic response. Warfarin is a racemic mixture of R and S enantiomers. S is 5 times potent than R and is transformed into inactive metabolites by cytochrome P 450 enzyme – Cyp2C9. Vitamin K Epoxide Reductase (VKOR) is the key enzyme in vitamin K cycle and molecular target of Warfarin. Warfarin inhibits C 1 subunit of VKOR enzyme. In the liver, reduced vitamin K is essential for gamma carboxylation of factors II, VII, IX, X to make them functionally active coagulation factors. Due to inhibition of VKOR 1, reduced vitamin K is not available which affects synthesis of functional clotting factors. That is how Warfarin acts as an anti-coagulant. Thus gene for CYP2C9, a metabolic enzyme and gene for VKOR 1, a target enzyme of Warfarin are very important. Pharmacogenetic analysis of these two has an influence on the maintenance dose of Warfarin.6-5

The rate of metabolism of Warfarin is genetically determined and varies by ethnicity and race. CYP2C9 *1 is the wild type whereas *2 and *3 are the variant alleles of CYP2C9. The CYP2C9*3 allele is defined by an A-to-C nucleotide substitution that leads to an exchange of leucine by isoleucine at amino acid position 359. The distribution of CYP2C9*2 and *3 mutant alleles in our population (0.04 and 0.08) is less than that of the Caucasians (0.12 and 0.08) but more than that of the Orientals (0.00 and 0.03). Both these *2 and *3 are functionally defective. They are associated with significantly reduced enzyme activity and they metabolize Warfarin slowly. So carriers of these variant alleles of CYP2C9 are associated with increased sensitivity to Warfarin and require lower maintenance dose. The *2 allele and *3 allele variants can cause in vitro reduction in enzymatic activity by 30 and 80% respectively and this leads to increased anticoagulant efficacy of warfarin, thereby decreasing the required warfarin dosage for therapeutic range maintenance. Amongst both these, *3 allele reduces metabolism of S-Warfarin by 80% and so average dose of Warfarin is reduced more with *3 than with *2. Dose is lowest in patients who are homozygous for *3. Our patient had homozygous mutant of CYP2C9*3 and therefore the dose was < 2 mg.

VKORC 1 genotype predicts 25% variability in dose. Alleles of VKORC 1 are associated with altered response to Warfarin. There are 3 haplotypes of VKORC 1 namely AA, AB and BB; all of which have effect on Warfarin dosage. BB is the wild type. Patients with haplotype B require a higher maintenance dose of warfarin than those with haplotype A. Patients with AA require a lower maintenance dose and with variant CYP2C9, the dose is reduced further. Due to sensitivity, doses of < 1 mg /day are sufficient to achieve therapeutic anticoagulation. Resistance necessitates dose in excess of 15 mg /day.

Mutations in the coding region in heterozygous patients lead to Warfarin resistance. A common promoter mutation (1639 C-A) predicts warfarin sensitivity and so average daily dose of Warfarin is reduced. This was observed in our patient.

Asians belong to low dose phenotype. Incidence of CYP2C9 *3 is 2-5% in Asians and that of VKORC 1 sensitivity haplotypes is 89% in Asians.

Combinations of mutations reduce the dose of Warfarin further. CYP2C9 genotype is a strong predictor of the time to reach first INR of > 4. Initial variability in INR response is strongly associated with variability in VKORC 1 than that with CYP2C9. Patients with AA haplotypes of VKORC 1 leads to a more rapid achievement of therapeutic INR but also a shorter time to reach an INR of more than four which is associated with bleeding.

**Conclusion**

Our patient had double mutations - homozygous for CYP2C9*3 and heterozygous for VKORC 1. She was not able to maintain a stable target INR needing too many dose alterations and hospital visits. She had to be given transfusions for excessive bleeding which could have been due to Warfarin toxicity. Thus achieving a target INR and balancing benefits Vs risks of Warfarin were problems in our case. Her pharmacogenomics of Warfarin revealed sensitivity to Warfarin explaining her fluctuation in INR and helped optimize drug efficacy (personalized medicine approach) while minimizing toxicity. It would appear therefore that one dose may not suit all!

**References**

4. Redman AR. Implications of cytochrome P450 2C9 polymorphism on warfarin metabolism and dosing. Pharmacotherapy 2001;21:235-