Accepted Article
This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/jth.12007
© 2012 International Society on Thrombosis and Haemostasis

Received Date : 22-Aug-2012
Accepted Date : 17-Sep-2012
Article type : Letter - to the Editor

Long-term anticoagulant effects of CYP2C9 and VKORC1 genotypes in phenprocoumon users

T.I. Verhoef*, W. K. Redekop†, H. Hegazy*, A. de Boer*, A.H. Maitland-van der Zee* on behalf of the EU-PACT group**

*Utrecht Institute of Pharmaceutical Sciences, division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, Utrecht, The Netherlands.

Author for correspondence:
Anke-Hilse Maitland-van der Zee
Utrecht University, Faculty of Science
Division of Pharmacoepidemiology & Clinical Pharmacology
PO Box 80 082, 3508 TB Utrecht, The Netherlands
Tel: +31-622 736 715
Fax: +31-30 253 9166
E-mail: a.h.maitland@uu.nl

**The members of the EU-PACT group are:
Anticoagulant treatment with phenprocoumon is challenging because of the narrow therapeutic range and the wide inter- and intra-patient variability in dose response. Frequent monitoring of the international normalized ratio (INR) is therefore required. Polymorphisms in two genes, \textit{CYP2C9} and \textit{VKORC1} explain approximately one third of the variation in dose requirements \cite{1-3}. \textit{CYP2C9} encodes the main metabolizing enzyme of coumarins, the cytochrome P450 2C9 enzyme (CYP2C9), while \textit{VKORC1} encodes the pharmacodynamic target enzyme for coumarins, vitamin K epoxide reductase multiprotein complex 1 (VKORC1).

Earlier this year, we found that in the first month of acenocoumarol therapy, the risk of underdosing is highest in patients with a \textit{VKORC1} wild-type \cite{4}. This increased risk of a subtherapeutic
INR was also seen in months 2 and 3, but not after the third month of coumarin treatment. In addition, the risk of overdosing was highest in patients with a VKORC1 TT genotype in the first 6 months. The effect of CYP2C9 genotype on under- or overdosing of acenocoumarol was smaller than the effect of VKORC1 and this effect was only found in the first month of therapy and not after the initiation period [4]. This has not been investigated for phenprocoumon yet. The aim of this study was therefore to examine the association of CYP2C9 and VKORC1 polymorphisms with the risk of over- and underanticoagulation after the initiation period of phenprocoumon.

To investigate this, we looked at data from two different studies, the pre-EU-PACT study [5] and the study of Schalekamp et al. [6]. The study protocols of both studies were approved by a Medical Ethics Committee (Leiden University Medical Center, Leiden for pre-EU-PACT, Utrecht Medical Centre, Utrecht for the study of Schalekamp) and patients provided informed consent before study inclusion. All procedures were conducted in accordance with the Helsinki Declaration. More details about the design and data collection in both studies can be found elsewhere [4].

We examined the occurrence of at least one INR <2, >3.5 or >6.0 in several time periods up to 1.5 years after treatment initiation and tested for differences among the genotypes with chi-square analysis. The time periods we used were: 0-1 month (day 1-30), 1-3 months (day 31-90), 3-6 months (day 91-180), 6-9 months (day 181-270), 9-12 months (day 271-360), 12-15 months (day 361-450) and 15-18 months (day 451-540) after treatment initiation. We also looked at the time within, below and above the therapeutic range, since this method is more robust when the frequency of INR measurements differs between patients. All analyses were performed using SPSS 18.0.

In total, 794 phenprocoumon users from the two studies [5,6] were eligible for analyses in this study. Patient characteristics and genotypes of all 794 patients are shown in the Supplement Table 1. Data on height and weight were only available in the Pre-EU-PACT study (n=486). The most frequent indication for phenprocoumon treatment was atrial fibrillation. The average number of INR measurements per time period ranged from 3.4 to 5.5. Only data of patients using phenprocoumon during
the entire time period were included in the analysis of that period. The maximum follow-up in the Schalekamp study was six months (n=308).

Significant differences in out-of-range INR values between the genotypes were only found during the first month of phenprocoumon therapy. In the first month, 89% of the patients with a VKORC1 wild-type had at least one subtherapeutic INR. This frequency was significantly lower among patients with CT (76%, p<0.001) and TT (50%, p<0.001). Supratherapeutic INR values occurred in 33% of the VKORC1 wild-type patients, versus 48% (p<0.001) and 66% (p<0.001) in patients with a CT or TT genotype, respectively. Of the wild-type patients, 3% had at least one INR>6. This percentage was increased in patients with a TT genotype (17%, p<0.001), but there was no statistically significant difference for patients with a CT genotype (6%, p=0.12).

Occurrences of subtherapeutic INR values or INR values >6 were not significantly different among the CYP2C9 genotypes. However, INR values >3.5 occurred more often in carriers of a CYP2C9*3 allele (62%, p<0.001) or a CYP2C9*2 allele (52%, p=0.01) than in wild-type patients (40%). For both VKORC1 and CYP2C9 genotypes, no significant differences in out-of-range INRs were found after the first month. The risk of out-of-range INRs for the different periods and genotypes are shown in the Supplement (Supplement Figures 1-4).

Similar results were obtained in the analyses of time within, below and above therapeutic INR range (see Figure 1). In the first month, time below therapeutic INR range was longest in VKORC1 and CYP2C9 wild-type patients (up to 33%) and time above therapeutic INR range was longest in VKORC1–TT and CYP2C9*3 carriers (up to 37%). The risk of having at least one INR<2 did not vary significantly among the CYP2C9 genotypes, but the time spent below therapeutic INR range was significantly shorter in *2 carriers (19%) and *3 carriers (14%) than in wild-type patients (26%, p<0.001). No significant differences were found after month 1 of the treatment.

Our study demonstrated that in the first month of phenprocoumon therapy, the risk of underdosing is highest in patients with VKORC1 and CYP2C9 wild-types. In addition, the risk of overdosing was highest in patients with a VKORC1 TT genotype or carriers of a CYP2C9 variant allele.
These results correspond with the results we have seen for acenocoumarol users, as described in a previous article [4]. However, the results beyond the first month of treatment are not similar. Specifically, while there were no differences in the risk of out-of-range INRs between the different genotypes after the first month of phenprocoumon therapy, there were differences in risk between the VKORC1 genotypes up to the sixth month of acenocoumarol treatment.

A limitation of this study is the fact that the Pre-EU-PACT study contained retrospective data [5]. The data of Schalekamp et al., however, was collected prospectively [6]. Data for a specific time period was only used in the analysis if the patient used phenprocoumon for this entire period. Because very unstable patients are expected to stop the therapy early, this patient group might be underrepresented in our study.

Information about the patient’s genotype can be used to predict the right dose of phenprocoumon [5]. Carriers of a VKORC1 or CYP2C9 variant allele require a lower dose and have an increased risk of supratherapeutic INR values. If these patients are genotyped before treatment initiation, they could be treated with a lower dose, thereby decreasing the risk of overanticoagulation. In both this study and our previous study on acenocoumarol, we also found an increased risk of a subtherapeutic INR in VKORC1 and CYP2C9 wild-type patients during the first month. Information about the patient’s genotype could therefore also be used to identify patients need a higher dose to decrease the risk of complications from underdosing. In this way, genetic information could be used to improve the safety and efficacy of anticoagulation treatment in both wild-type patients and variant carriers. The relevance of pharmacogenetic information for phenprocoumon users, however, seems to be limited to the first month of treatment.

Phenprocoumon has a longer elimination half life than acenocoumarol (110-130 hours versus 6-8 hours) [3,7]. Treatment with phenprocoumon is therefore somewhat more stable and patients on phenprocoumon spend more time within the therapeutic INR range than patients on acenocoumarol [8]. This might be a reason why only acenocoumarol users—and not phenprocoumon users—show differences between the genotypes in the risk of out-of-range INRs after the first month of treatment.
The results of this study suggest that pharmacogenetic information might help to prevent subtherapeutic or supratherapeutic INRs in the first month of phenprocoumon therapy and thereby reduce the risk of adverse events. The value of this information after the first month of phenprocoumon treatment appears to be limited. Currently, clinical trials are underway to investigate the effectiveness and cost-effectiveness of a genotype-guided dosing regimen versus a standard dosing regimen [9,10].

Acknowledgements

We would like to thank the Anticoagulation Clinic Leiden, F.J.M. van der Meer, T. Schalekamp, S. le Cessie and R.M.F. van Schie for their support in data collection and analysis.

Conflict of Interest/Disclosure

This project is funded by the European Community's Seventh Framework Programme under grant agreement HEALTH-F2-2009-223062. The department of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute of Pharmaceutical Sciences, employing authors Talitha I. Verhoef, Hoda Hegazy, Anthonius de Boer and Anke-Hilse Maitland-van der Zee, has received unrestricted research funding from the Netherlands Organisation for Health Research and Development (ZonMW), the Dutch Health Care Insurance Board (CVZ), the Royal Dutch Pharmacists Association (KNMP), the private-public funded Top Institute Pharma (www.tipharma.nl, includes co-funding from universities, government, and industry), the EU Innovative Medicines Initiative (IMI), EU 7th Framework Program (FP7), the Dutch Medicines Evaluation Board, the Dutch Ministry of Health and industry (including GlaxoSmithKline, Pfizer, and others). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.
References


© 2012 International Society on Thrombosis and Haemostasis


Figure Legends

**Figure 1.** Percentage time in different INR ranges during the first month of phenprocoumon use. A: *VKORC1* genotypes B: *CYP2C9* genotypes