Cytochrome P450 2C9 polymorphism and acenocoumarol therapy

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Abstract

Background: Oral anticoagulants, in Hungary acenocoumarol being the one exclusively used, have a low therapeutic index and a high bleeding complication rate. The cytochrome P450 2C9 enzyme plays an important role in their metabolism.

Aim: To investigate the influence of CYP2C9 polymorphism on the occurrence of bleeding complications related to acenocoumarol therapy.

Methods: Genotyping of 421 patients (183 men and 238 women, mean age 66.2±11.8 years), who had been taking acenocoumarol for at least 6 months, was performed. Based on patient history and laboratory data, the correlations between genotype and acenocoumarol dose and bleeding complications were retrospectively analysed.

Results: In 145 patients bearing alleles with reduced activity (CYP2C9*2 and/or *3), the optimal dose of acenocoumarol was significantly ($p<0.001$) lower than in patients with the wild type allele (2.12±0.96 mg/day and 2.90±1.46 mg/day, respectively). In comparison with wild type allele patients, the mean daily acenocoumarol dose was lower in the CYP2C9*2 group, and the lowest in *3 bearers. Although the occurrence of minor bleeding complications in patients with the variant allele was significantly ($p <0.005$) higher (OR=1.99 [CI: 1.20–3.33]) than in other patients, there was no difference in major bleeding complications.

Conclusions: Patients bearing CYP2C9 alleles with reduced enzymatic activity have a lower acenocoumarol requirement. In patients with CYP2C9*2 and *3 alleles the frequency of minor bleeding complications and the occurrence of high INR values were significantly higher, but there was no difference in the rate of major bleedings.

Key words: oral anticoagulants, acenocoumarol, cytochrome P450

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Introduction

Coumarins, acenocoumarol in Hungary, are widely used for the primary and secondary prevention of thrombotic events. A low therapeutic index and frequent bleeding complications are characteristic for these drugs. Determining the proper therapeutic dose is complicated by a variety of conditions, such as co-morbidities, age, other drugs used, diet and pharmacogenetic factors. One of the latter is polymorphism of the cytochrome P450 CYP2C9 enzyme, which metabolises a wide range of cardiovascular drugs, including oral anticoagulants and statins [1, 2].

The enzyme CYP2C9 has three common alleles (CYP2C9*1, CYP2C9*2 and CYP2C9*3) and six phenotypes. The variants CYP2C9*4, CYP2C9*5 and CYP2C9*6 have been described in non-European subjects. The wild type CYP2C9*1 allele has normal (100%) enzymatic activity (assessed for warfarin metabolism), while the CYP2C9*2 (Arg144→Cys) and CYP2C9*3 (Ile359→Leu) alleles have only 12% and 5% of this activity, respectively [3, 4]. The rate of bleeding complications is relatively high. The occurrence of minor complications is 7.6-16.5%, while that of major ones varies between 1.3% and 2.7% per hundred patient-years [5, 6].

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The effects of genetic factors on the metabolism of acenocoumarol are substantially less known than those of warfarin [7]. It has been shown that the pharmacokinetics of R-acenocoumarol did not differ between the genotypes, but the S-acenocoumarol plasma concentration was significantly higher in patients who had variant *2 and *3 alleles [8]. In the work of Tassies et al., the presence of variant alleles was associated with a lower acenocoumarol dose and a higher frequency of over-anticoagulation at the initiation of therapy, but the occurrence of bleeding complications was not significantly higher [9].

In this study, we investigated the influence of CYP2C9 polymorphism on acenocoumarol dosing and the occurrence of bleeding complications related to this therapy during everyday clinical practice.

Methods

Patients

We studied 421 patients (mean age 67.2±11.8 years) receiving oral anticoagulant therapy, in whom INR was controlled in the Central Laboratory of our hospital. The patients had been taking acenocoumarol for at least 6 months (the mean follow-up time was 56.3±62.8 months, the maximum 361 months, Table I). There was no difference between the age of patients with wild type versus variant alleles (66.6±12.0 *vs* 68.3±11.2, p=0.164). The indications for the use of oral anticoagulant therapy were as follows: 206 patients – atrial fibrillation; 60 – artificial heart valve; 17 – cardiomyopathy; 69 – deep vein thrombosis; 33 – pulmonary embolism; and 36 – history of multiple thromboembolic events. Written informed consent was obtained from patients for participation in the study, genetic investigation and processing of their data. The frequency of bleedings was determined by interrogation and revision of clinical and laboratory data. Epistaxis, macroscopic haematuria, and gastrointestinal and gynaecological bleeding causing a significant (>3 g/l) haemoglobin decrease were considered major bleeding complications, while microscopic haematuria, fecatest positivity, and epistaxis causing insignificant change in haemoglobin level were considered minor bleeding complications. The latter three and the maximal and minimal INR values, as well as present and previous maximal and minimal doses of acenocoumarol were registered.

Table I. Demographic characteristics of the studied patients

	Men	Women
Number	183	238
Mean age (years ± SD)	67.6±11.2	66.8±12.2
Follow-up time (months ± SD)	52.7±52.1	59.1±69.9

Laboratory investigations

The analysis of haemoglobin was performed with the CELL-DYN 3500 SL (Abbott) haematology analyser using the modified haemiglobincyanide method. INR values were determined by the STA Compact automated coagulometer using the Neoplastine CI Plus kit (Diagnostica Stago, Ansieres, France). Creatinine concentrations (Jaffe method) were analysed by the Olympus AU 2700 automated clinical chemistry analyser, using Olympus System Reagents (Olympus Diagnostica GmbH, Hamburg, Germany).

Genotyping was performed by Real-Time Fluorescence PCR using the LightCycler CYP2C9 Mutation Detection Kit (Roche Molecular Biochemicals, Mannheim, Germany) on the Roche LightCycler according to the manufacturer's instructions. We used a commercial kit, which is fully characterised and documented, and fulfils the requirements of the in vitro Diagnostic Medical Device (IVD) Directive of the EC (98/79/EC). Details on this kit are also available on the homepage of the company: http://www.rochediagnostics.com/ba_rmd/rmd_products_genomics_06.html and http://www.roche-applied-science.com/sis/rtpcr/lightcycler/system.jsp?id=010.

Based on medical history and laboratory data, the correlations between genotype and acenocoumarol dose and bleeding complications were retrospectively analysed. Blood and urine tests and fecatest were performed at the time of the study in all the patients, but during the anticoagulation process these were not routinely done.

Statistical analysis

Results are presented as mean ± standard deviation or numbers and percentages. The analysed parameters were compared using Student's paired t-test, variance and correlation analysis, and Kruskal-Wallis and Pearson χ^2 tests where appropriate (SPSS statistical program). A p value < 0.05 was considered statistically significant.

Results

The frequency distribution for the CYP2C9*1, CYP2C9*2, and CYP2C9*3 alleles was found to be 0.814, 0.110, and 0.076, respectively. The frequencies of the six genotypes in the 421 patients are presented in Table II. The occurrence of wild type homozygosity was 65.6% and at least one mutant allele was found in 34.4% of patients. The mean acenocoumarol dose in the whole studied group was 2.63±1.37 mg/day. In the 145 patients bearing alleles with reduced activity

(CYP2C9*2 and/or CYP2C9*3), the optimal dose was significantly (p <0.001) lower than in patients with the wild type allele (2.12±0.96 mg/day and 2.90±1.46 mg/day, respectively; Figure 1). Evaluating separately the mean daily dose in the patients with variant alleles, it proved to be 2.10±0.91 in CYP2C9*2 and 1.91±1.05 in CYP2C9*3 bearers, both significantly lower than that in CYP2C9*1/*1 wild type patients (p <0.001).

In 180 out of all studied patients the creatinine level was above the normal value. There was a moderate, but significant, negative correlation between acenocoumarol dose and age (–0.257) as well as creatinine level (–0.178).

Table III presents the number of patients with the occurrence of INR higher than 6 in various genotype groups. The incidence of high INR was significantly more frequent in the presence of variant alleles (p=0.021).

The frequency of minor bleeding complications was 17.3% (38 in 276 wild type, 35 in 145 patients with variant alleles), and of major ones 3.3% (8 and 6 patients, respectively). Although the occurrence of minor bleeding complications in patients bearing variant alleles was significantly (p <0.005) higher (Odds ratio /OR/=1.99 [Confidence intervals /CI/: 1.20–3.33]), there was no statistically significant difference in major bleeding complications. Comparing separately the frequency of minor bleeding in wild type (14%) and CYP2C9*2 (27%) or CYP2C9*3 (22%) patients, a significant difference was found between CYP2C9*1/*1 (wild type) and CYP2C9*2 bearers (p <0.01).

Age was significantly higher in patients with INR higher than 6 (69.6±11.9 *vs* 66.1±12.0; p <0.005). Since no difference was found between the age of patients with wild type and variant alleles, we believe that age could not explain differences in the acenocoumarol requirement. There was no significant correlation between age or renal function and bleeding complications according to the different allele frequencies.

Table II. The frequency of Cyp2C9 genotypes in the 421 patients

Genotype	1/1	1/2	1/3	2/2	2/3	3/3
Patients (n)	276	78	55	3	9	0
%	65.6	18.5	13.1	0.7	2.1	0

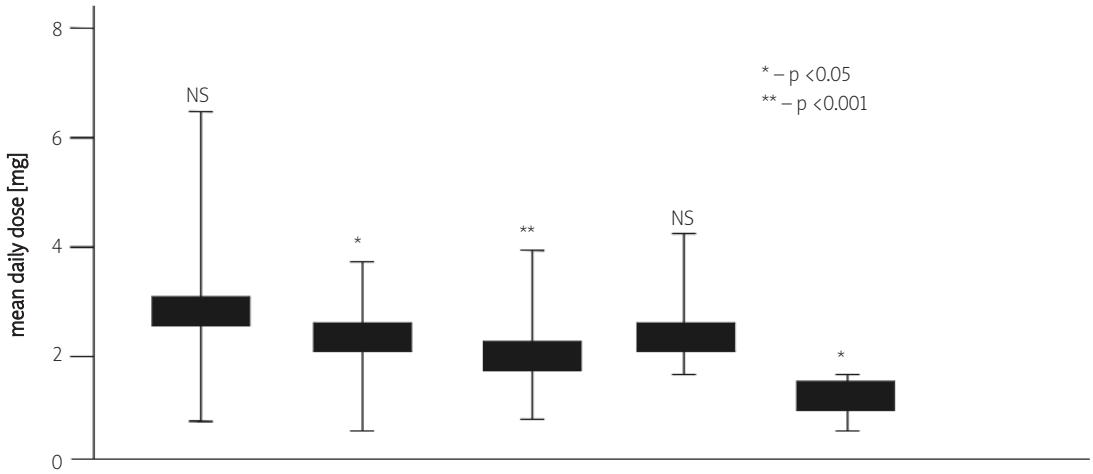
Table III. The number of patients with INR > 6 in various CYP2C9 genotype groups

Genotype	1/1	1/2	1/3	2/2	2/3	total
Total number of patients	276	78	55	3	9	421
Number of patients with INR >6	79	23	24	0	6	132
% of patients with INR >6	29	29	44	0	67	

In the whole study group, 24 patients were taking aspirin, 2 were taking other thrombocyte aggregation inhibitors, 8 non-steroid anti-inflammatory drugs, and 4 steroid hormones. There was no correlation between the administration of these drugs and bleeding complications.

Discussion

The frequency of usage of oral anticoagulation therapy is growing continuously. Because of the relatively low therapeutic index and the frequent



Cyp2C9 genotypes	1/1	1/2	1/3	2/2	2/3	3/3
number of patients	276	78	55	3	9	0
Daily dose (mg ±SD)	2.90±1.45	2.27±0.96	2.01±1.06	2.55±1.27	1.31±0.74	–

Figure 1. CYP2C9 genotypes and the acenocoumarol dose

occurrence of bleeding complications, careful monitoring of treatment is required. The widespread use of pharmacogenetic examinations raises the question of whether knowing the genotype could improve the quality and accuracy of anticoagulation.

In this study, the CYP 2C9 genotype of 421 patients taking acenocoumarol was determined and correlations of the genotype with clinical data were analysed. The frequency distribution of the CYP2C9 alleles in the studied group was similar to that reported by others [3]. In our study, the acenocoumarol dose of patients bearing alleles with reduced enzymatic activity (CYP2C9*2 and*3) was significantly lower, which proves that genotype is an important determinant of the optimal dose, similarly as in the case of warfarin. However, in patients with a daily dose <2 mg the occurrence of INR values >6 was high. We also found a correlation between genotype and minor rather than major bleeding complications.

Taking all these findings into consideration, we believe that the usual method of accurate anticoagulation monitoring might be sufficient; however, a prospective, large, randomised study is required to establish whether routine genotyping before the initiation of oral anticoagulant therapy would be cost-effective and could provide a considerable benefit in the prevention of significant bleeding. The demonstrated association between minor bleeding and over-anticoagulation raises the issue of the need for a cost-effectiveness analysis in this field. The negative correlation with age and serum creatinine directs particular attention to older people and patients with impaired renal function. Tassies et al. stated that in patients having other than homozygous wild type genotype, the mean acenocoumarol dose was lower, but the frequency of bleeding complications was not higher [9].

Verstuyft et al. initially stressed the important role of routine genotyping before the beginning of anticoagulant treatment [10]. However, as time passed, their view changed and in 2003 the same team believed that in the case of over-anticoagulation environmental factors played a more important role than genetic ones [11].

Hermida et al. showed that the metabolism of aconocoumarol depends less on genotype than does that of warfarin. Thus, in cases with unstable warfarin treatment, genotyping seems justified (12). In the work of Laporte et al., there was no difference in the effectiveness of anticoagulation in the case of administering acenocoumarol or warfarin [13].

Evaluating the correlation between acenocoumarol pharmacokinetics and genetic polymorphism, Thijssen and Ritzen documented that the metabolism of S-acenocoumarol is hardly influenced by the presence of variant alleles, which is why routine genotyping is not necessary. INR determination is a sensitive marker, and in the case of overdose, even in the presence of allele *3, the omission of the drug results in rapid elimination. These authors believe that determination of the genotype could help in special conditions only [8].

Similarly to our data, Visser et al. showed that patients with one or more variant allele (s) required a significantly lower dose of acenocoumarol compared to wild-type allele patients [14]. Among 996 outpatients attending an anticoagulation clinic, an increased risk of bleeding was found in those having a variant allele of CYP2C9. This risk was not present during the initiation phase of the therapy [15]. Trying to investigate to what extent CYP P450 2C9 genetic variability contributes to the acenocoumarol pharmacodynamic response, Morin et al. stated that this accounts for 14% of inter-individual variability [16].

Study limitations

The major disadvantage of our study is that the collection of data from medical history was retrospective, based on the existing medical records and patient history (the mean follow-up time was around 56 months, the maximum about 30 years).

Conclusions

Based on the data of our 421 patients and surveying the literature, the following conclusions can be proposed:

1. The frequency distribution of the CYP2C9 alleles was as reported by others.
2. The CYP2C9 polymorphism is the major determinant of the acenocoumarol dose requirement.
3. The dose requirement decreases with age and renal function impairment.
4. In patients bearing variant alleles, the occurrence of minor bleeding complications and an INR value >6 is significantly more frequent than in other patients.
5. In patients with a lower acenocoumarol requirement, caution is needed when initiating treatment.
6. Large, prospective, randomised trials are required to answer the question of whether genotyping before the initiation of anticoagulation therapy is cost-effective and clinically valuable.

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Polimorfizm cytochromu P450 2C9 i leczenie acenokumarolem

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Streszczenie

Wstęp: Doustne leki przeciwzakrzepowe, w tym acenokumarol, który jest jedynym antykoagulantem stosowanym na Węgrzech, charakteryzują się niskim współczynnikiem terapeutycznym i wysokim ryzykiem powikłań krwotocznych. Układ cytochromu P450 (CYP2C9) bierze istotny udział w metabolizmie antykoagulantów doustnych.

Cel pracy: Ocena wpływu polimorfizmu CYP2C9 na częstość występowania powikłań krwotocznych związanych ze stosowaniem acenokumarolu.

Metodyka: Grupa badana składała się z 421 chorych (183 mężczyzn, 238 kobiet, średni wiek 66,2±11,8 lat), u których stosowano acenokumarol przez okres co najmniej 6 mies. przed przeprowadzeniem niniejszej analizy. Na podstawie wywiadu, danych z historii choroby, badań laboratoryjnych i genetycznych dokonano retrospektywnej oceny zależności pomiędzy genotypem a stosowaną dawką acenokumarolu i występowaniem powikłań krwotocznych.

Wyniki: U 145 chorych, u których występowały allele o zmniejszonej aktywności (CYP2C9*2 lub *3) optymalna dawka acenokumarolu była istotnie niższa niż u chorych z *dzikim* typem allelu (odpowiednio 2,12±0,96 mg/dobę *vs* 2,90±1,46 mg/dobę, p <0,001). Dawka dobowa acenokumarolu była najwyższa u chorych z *dzikim* typem allelu, pośrednia u chorych z CYP2C9*2, a najniższa u nosicieli genu *3. Wartości INR >6 stwierdzano istotnie częściej u chorych, u których stwierdzano allele o zmniejszonej aktywności (p=0,021). Częstość występowania małych powikłań krwotocznych była istotnie wyższa u chorych, u których stwierdzano allele o zmniejszonej aktywności (OR=1,99; CI=1,20–3,33, p <0,005), natomiast częstość występowania dużych powikłań krwotocznych była podobna we wszystkich badanych podgrupach. Stwierdzono również umiarkowaną, ale istotną statystycznie ujemną korelację pomiędzy dawką acenokumarolu a wiekiem (r=–0,257) i stężeniem kreatyniny (r=–0,178).

Wnioski: Chorzy z obniżoną aktywnością enzymatyczną CYP2C9 wymagają mniejszych dawek acenokumarolu. U chorych z allelami CYP2C9*2 i *3 częstość występowania małych powikłań krwotocznych i przypadki bardzo wysokich wartości INR były istotnie wyższe niż u pozostałych chorych, natomiast częstość występowania istotnych krwawień nie różniła się pomiędzy grupami.

Słowa kluczowe: doustne antykoagulanty, acenokumarol, cytochrom P450

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