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Research Article

Influence of CYP2C9 and VKORC1 polymorphisms on the time required to reach the therapeutic INR

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Abstract. Oral anticoagulation (OAC) is characterized by a narrow therapeutic index and a high interindividual variability, both in terms of pharmacokinetics and pharmacodynamics. We have considered useful and interesting to research the factors that could play a role in determining the time required for Acenocoumarol to achieve its optimal therapeutic response. Material and method: The present research is a cross analytic observational study. We included 105 patients treated with an initial dose of 4 mg Acenocoumarol, for one or more of the following clinical situations: 1. Deep venous thrombosis of the lower limbs (DVT) ± pulmonary thromboembolism (PTE); 2. Permanent atrial fibrillation (AF); 3. Prosthetic heart valve. Results and conclusions: The presence of CYP2C9*2 and CYP2C9*3 alleles did not affect the time required to reach a therapeutic INR. The c.-1639G>A polymorphism of the VKORC1 gene significantly and statistically influenced the time to reach the target INR. The existence of a supratherapeutic INR during the initial phase of anticoagulant treatment causes a 35% lower probability of reaching a therapeutic INR on the fifth day of anticoagulant treatment.

Key Words: oral anticoagulation, CYP2C9*2, CYP2C9*3, VKORC1, therapeutic INR.

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Introduction

Oral anticoagulation (OAC) is characterized by a narrow therapeutic index and a high interindividual variability, both in terms of pharmacokinetics and pharmacodynamics (Sareva *et al* 2007). Besides environmental factors, there are genetic factors with important contribution to interindividual variability. Frequency of CYP2C9 gene polymorphisms varies by race, Caucasian, African or Asian. Mutant enzymes resulting from the existence of these polymorphisms are less active than normal enzymes, leading to a decreased metabolism of coumarin derivatives (Kirchheiner & Brockmöller 2005). Highlighting VKORC1 gene polymorphisms has resuled in a significant contribution to understanding the interindividual variability of the response to Acenocoumarol therapy (Limei *et al* 2010).

Given that the response to Acenocoumarol therapy is not uniform and that the time required to reach the therapeutic INR varies from patient to patient, we have considered it useful and interesting to research the factors that could play a role in determining the time required for Acenocoumarol to achieve its optimal therapeutic response.

Material and method

The present research is a cross analytic observational study. We included 105 patients treated with an initial dose of 4 mg Acenocoumarol, for one or more of the following clinical situations: 1. Deep venous thrombosis of the lower limbs (DVT) ± pulmonary thromboembolism (PTE); 2. Permanent atrial fibrillation (AF); 3. Prosthetic heart valve. All patients signed the informed consent for participating in the study and in the genetic determinations. The following variables were recorded for each patient: demographic data, time to reach the therapeutic INR (expressed in number of days, considering the number of days as necessary in order to achieve a constant value of INR on three consecutive determinations for the same dose of Acenocoumarol); concomitant medication, INR values, CYP2C9*2, CYP2C9 * 3, VKORC1 polymorphisms.

Genotyping for the CYP2C9*2, CYP2C9*3, and VKORC1 -1693 G>A alleles was performed using the PCR-RFLP method (polymerase chain reaction - restriction fragment length polymorphism) (Aynacioglu *et al* 1999; Chen *et al* 2008). Data recording was performed using Microsoft Excel XP. Statistical analysis was performed with Medcalc version12.3.

Results

Table 1 shows the characteristics of the patient group considered for the study. CYP2C9 genotype distribution in the study group is shown in table 2. VKORC1 polymorphisms were present in different percentages in the study group, as shown in table 3.

Patient age had a normal distribution in the study (Kolmogorov-Smirnov test). The minimum age was 22, the maximum was 91, the mean was 63.7 ± 13.5. The average time to reach the therapeutic INR was 5 days, at least 1 day and up to a maximum of 15 days, with a non-normal distribution (Kolmogorov-Smirnov test). We applied Spearman correlation in order to study a possible

association between patient age and the time required to reach the therapeutic INR, but we found no statistically significant correlation (r=0.017, p=0.86). We didn't find any statistically significant difference between men and women regarding the median time to INR (Mann-Whitney test, p=0.43). Not even the area (urban or rural) influenced the time to reach the therapeutic INR (Mann-Whitney test, p=0.78). Concomitant statin, amiodarone, proton pump inhibitors (PPI) or spironolactone therapy did not affect the time required to reach the therapeutic target (Mann-Whitney test, p>0.05). Neither the presence of CYP2C9*2 and CYP2C9*3 allele affected the time needed to reach the therapeutic INR (Mann-Whitney test, p=0.26 and p=0.87).

Table 1. Characteristics of the patient group

Variable	No. (%) of patients
Men	54 (51.4%)
Women	51 (48.6%)
Rural area	58 (55.2%)
Urban area	47 (44.8%)
DVT	81 (81.0%)
AF	25 (23.8%)
Prosthetic heart valve	4 (3.8%)
Amiodarone therapy	2 (1.9%)
PPI therapy	5 (4.8%)
Spironolactone therapy	11 (10.5%)
Statin therapy	54 (51.4%)

Table 2. CYP2C9 genotype distribution

CYP2C9 genotype	No. of patients	% Patients
***1/*1**	64	61%
***1/*2**	22	21%
***1/*3**	16	15.20%
***2/*3**	3	2.90%

Table 3. Distribution of VKORC1 polymorphisms

c.–1639 G>A polymorphism of the VKORC1 gene	No. of patients	% patients
GG	31	29.50%
GA	60	57.10%
AA	14	13.30%

The c.-1639G>A polymorphism of the VKORC1 gene (Kruskal-Wallis test, p=0.03) and the supratherapeutic INR (Mann-Whitney test, p=0.003) during exploratory dosing, significantly statistically influenced the time to reach the target INR.

Cox regression was used to assess the independent influence of each parameter studied on the probability of reaching the therapeutic INR depending on the time required for this. We set as the dependent event the fifth day of anticoagulant treatment. Compared to the presence of GG genotype, the presence of AA genotype of the c-1639G>A polymorphism of the VKORC1*2 gene, shows a 1.9-fold increase (hazard ratio) in the probability that the patient will reach a therapeutic INR until the fifth day of anticoagulant therapy. The existence of a supratherapeutic INR during the beginning of the anticoagulant therapy causes a 35% lower probability for a patient to reach a therapeutic INR on the fifth day of anticoagulant therapy.

Table 4. Cox regression. Time required to reach the therapeutic INR

Variable	p	HR	95% CI	
			min	max
Age	0.704	1.005	0.98	1.03
Gender	0.313	1.156	0.872	1.532
Statin	0.999	1	0.748	1.336
Amiodarone	0.411	1.535	0.553	4.263
PPI	0.595	0.84	0.441	1.599
Spironolactone	0.499	0.833	0.49	1.416
CYP2C9*2	0.389	1.162	0.825	1.636
CYP2C9*3	0.449	1.138	0.815	1.588
VKORC1*2(GA)	0.071	0.709	0.487	1.03
VKORC1*2 (AA)	0.021	1.916	1.103	3.329
Acenocumarol (mg/week)	0.608	0.99	0.954	1.028
Supratherapeutic INR	0.007	0.649	0.474	0.888

Discussions

OACs are ideal drugs to test the paradigm of personalized medicine, being among the most prescribed drugs, but also a major cause of adverse reactions (Pirmohamed 2006; Wester *et al* 2008). Achieving a balance between the need for effective anticoagulation and the reduction in the time needed to reach the therapeutic INR, without increasing the risk of side effects, is important not only for patients, but also for the health system, taking into account the economic aspects of oral anticoagulation therapy.

In this research, we studied the influence of several factors on the time needed to reach the therapeutic INR, considering it as one of the phenotypic expressions of the response to Acenocoumarol therapy.

The results of our research on socio-demographic factors or concomitant therapy and their influence on the time needed to reach the therapeutic target are in agreement with the data published by Limdi *et al* (2009) or Higashi *et al* (2002), who did not find statistical significance regarding this influence.

Our study shows no influence of the presence of CYP2C9*2 and CYP2C9*3 alleles on the time to achieve the therapeutic INR. In a study on 185 patients treated with warfarin, most of them for atrial fibrillation, Higashi *et al* (2002) found no difference, in terms of time to reach the therapeutic INR, between the wild-type group of patients and the group with one of the two alleles: CYP2C9*2 and CYP2C9*3. Note that between this study and our research, there is a difference in defining the time to INR, as we defined it as the time until obtaining three consecutive INR values between 2 and 3, whereas in Higashi's study, it was defined as the time required to reach the first therapeutic INR value. The research conducted by Ozer *et al* (2010) on 100 patients treated with warfarin showed that wild-type CYP2C9 patients experienced a statistically significant longer

time to reach the therapeutic INR, compared with CYP2C9*2 and CYP2C9*3 subtypes .

The difference between the studies might be given by the heterogeneity of the group created by Ozer *et al* (2002) - Caucasians, Asians, African-Americans. It is well known that the allele frequency of CYP2C9 gene varies by race. Our results are consistent with those obtained by Schwarz *et al* (2008), which showed that CYP2C9 genotype did not affect the time to reach the therapeutic INR . As the new data reported in our study, the research conducted by Gong *et al* (2011) also did not show any influence of CYP2C9 genotype on the time to the first therapeutic INR.

After analyzing the data, a statistical significance was observed regarding the influence of c-1639G>A polymorphism of the VKORC1 gene on the time to reach the therapeutic INR. Patients carrying A allele require less time to achieve the therapeutic INR. Literature data shows different results regarding the influence of VKORC1 genotype on the time required to reach the therapeutic INR. The study conducted by Gong *et al* (2011) did not find VKORC1 genotype as factor influencing the time to INR105. Instead, just as with our results, Schwarz *et al* (2008) showed that VKORC1 haplotype has a significant effect on the time required to reach the first therapeutic INR. Patients with one or two alleles of VKORC1 haplotype have a shorter time to reach the terapeutic INR104. Schalekamp *et al* (2006) conducted a study that included patients who received treatment with Acenocoumarol. In contrast to our research, the results of these authors indicate that the patients carrying VKORC1 polymorphisms do not differ from those with wild-type VKORC1 genotype in terms of the time needed to reach a therapeutic INR. In the study conducted by Limdi *et al* (2010) on patients in initial phase of warfarin therapy, the therapeutic INR was reached much faster in patients with VKORC1 polymorphisms alone or with CYP2C9 and VKORC1 variants, compared with those with only one CYP2C9 variant or without CYP2C9 and VKORC1 polymorphisms.

Note that by applying Cox regression we have shown that there is a supratherapeutic INR during the initial phase of anticoagulant therapy causing a 35% lower probability that a patient will not reach a therapeutic INR on the fifth day of anticoagulant treatment. The presence of supratherapeutic INR may be due to the existence of CYP2C9 and/or VKORC1 genetic polymorphisms, as shown by the results of numerous pharmacogenetic studies of oral anticoagulants (Higashi *et al* 2002; Schalekamp *et al* 2006; Ozer *et al* 2010; Gong *et al* 2011).

Conclusions

The presence of CYP2C9*2 and CYP2C9*3 alleles did not affect the time required to reach a therapeutic INR. The c.-1639G>A polymorphism of the VKORC1 gene significantly and statistically influenced the time to reach the target INR. The presence of AA genotype of c.-1639G>A polymorphism of VKORC1*2 gene determined a 1.9-fold increase (hazard ratio) in the probability that the patient will reach a therapeutic INR until the fifth day of anticoagulant treatment. The existence of a supratherapeutic INR during the initial phase of anticoagulant treatment causes a 35% lower probability of reaching a therapeutic INR on the fifth day of anticoagulant treatment.

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