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ORIGINAL PAPER

Acenocoumarol sensitivity and pharmacokinetic characterization of *CYP2C9 *5/*8,*8/*11,*9/*11* and *VKORC1*2* in black African healthy Beninese subjects

Aurel Constant Allabi · Yves Horsmans · Jean-Claude Alvarez · André Bigot · Roger K. Verbeeck · Umit Yasar · Jean-Luc Gala

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Abstract This study aimed at investigating the contribution of *CYP2C9* and *VKORC1* genetic polymorphisms to inter-individual variability of acenocoumarol pharmacokinetics and pharmacodynamics in Black Africans from Benin. Fifty-one healthy volunteers were genotyped for *VKORC1* 1173C>T polymorphism. All of the subjects had previously been genotyped for *CYP2C9*5*, *CYP2C9*6*, *CYP2C9*8*, *CYP2C9*9* and *CYP2C9*11* alleles. Thirty-six subjects were phenotyped with a single 8 mg oral dose of acenocoumarol by measuring plasma concentrations of (*R*)- and (*S*)-acenocoumarol 8 and 24 h after the administration using chiral liquid-chromatography tandem mass-spectrometry. International normalized ratio (INR) values were determined prior to and 24 h after the drug intake. The allele frequency of *VKORC1* variant (1173C>T) was 1.96% (95% CI 0.0–4.65%). The INR values did not show statistically significant difference between the *CYP2C9* genotypes, but were correlated with body mass index and age at 24 h post-dosing ($P < 0.05$). At 8 h post dose, the (*S*)-acenocoumarol concentrations in the *CYP2C9*5/*8* and *CYP2C9*9/*11* genotypes were about 1.9 and 5.1 fold higher compared with the *CYP2C9*1/*1* genotype and 2.2- and 6.0-fold higher compared with the *CYP2C9*1/*9* group, respectively. The results indicated that pharmacodynamic response to acenocoumarol is highly variable between the subjects. This variability seems to be associated with *CYP2C9*5/*8* and **9/*11* variant and demographic factors (age and weight) in Beninese subjects. Significant association between plasma (*S*)-acenocoumarol concentration and *CYP2C9* genotypes suggested the use of (*S*)-acenocoumarol for the phenotyping purpose. Larger number of subjects is needed to study the effect of *VKORC1* 1173C>T variant due to its low frequency in Beninese population.

Keywords *CYP2C9* · *VKORC1* · Acenocoumarol · INR · Benin · African

A. C. Allabi (✉)
Unité de Pharmacologie, Faculté des Sciences de la Santé de Cotonou, Université d'Abomey-Calavi, Campus du Champ de Foire, 01 BP 188 Cotonou, Benin
e-mail: acallabi@hotmail.com

Y. Horsmans
Clinical Pharmacology Unit, UCL, Brussels, Belgium

J.-C. Alvarez
Laboratory of Pharmacology-Toxicology, Garches Hospital, AP-HP, Université Versailles Saint-Quentin, 92380 Garches, France

A. Bigot
Unité d'Immunologie, Faculté des Sciences de la Santé de Cotonou, UAC, Cotonou, Benin

R. K. Verbeeck
Ecole de Pharmacie, UCL, Brussels, Belgium

R. K. Verbeeck
Faculty of Pharmacy, Rhodes University, Grahamstown, South Africa

U. Yasar
Department of Pharmacology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

J.-L. Gala
Laboratory of Applied Molecular Technology, UCL, Brussels, Belgium

1 Introduction

Vitamin K antagonists (VKAs), such as warfarin, acenocoumarol and phenprocoumon, are life-saving drugs, but

have a narrow therapeutic index and are associated with a high risk of major bleeding side effect especially during the initial phase of treatment, in connection with intercurrent disease, or due to drug–drug interactions (Ufer 2005). Moreover, there is substantial inter-individual variability in response to VKAs caused by several factors including variations in the *CYP2C9* and *VKORC1* genes (Belle and Singh 2008). Frequent measurements of the International Normalized Ratio (INR) of the prothrombin time and dosage adjustments are needed during the therapy with VKAs (Ufer 2005). Acenocoumarol (4′-nitrowarfarin) is the most commonly prescribed VKA in Benin for the prevention and treatment of venous thromboembolism. It is also used for the prevention of systemic embolism in atrial fibrillation and for the prevention of myocardial infarction in high-risk patients.

Acenocoumarol is commercially available as a racemic mixture of roughly equal amounts of (*R*)- and (*S*)-enantiomers. The plasma clearance of (*S*)-acenocoumarol, the more potent enantiomer, is approximately tenfold higher than that of (*R*)-acenocoumarol. Thus, the terminal elimination half-life of (*S*)-acenocoumarol is substantially shorter (1.8 h) than that of the R-enantiomer (6.6 h), resulting in much higher plasma concentrations of the latter. As a consequence, (*R*)-acenocoumarol is mostly responsible for the anticoagulant effect despite the higher intrinsic anticoagulant activity of the (*S*)-enantiomer. The CYP2C9 isoform of cytochrome P450 is mainly involved in the metabolic clearance of VKAs including acenocoumarol (Ufer 2005).

In Caucasians, the most important alleles of the *CYP2C9* gene are *CYP2C9*2* (Arg144Cys) and *CYP2C9*3* (Ile359Leu), which are associated with a reduction in metabolic activity of about 30 and 80%, respectively. The pharmacokinetics of acenocoumarol in healthy volunteers with different genotypes of CYP2C9 demonstrates that mean oral clearance of (*S*)-acenocoumarol is about 50% lower in the *CYP2C9*1*3* genotypes (10.9 vs. 19.8 L/h). Plasma half-life has been reported to be prolonged from up to 2.0 h (Thijssen and Ritzen 2003). In vitro enzyme kinetics study demonstrates a similar reduced (85%) intrinsic activity of the *CYP2C9*3* enzyme to catalyse (*S*)-acenocoumarol to hydroxy metabolites. The activity of the *CYP2C9*2* enzyme was about 50% of the *CYP2C9*1* (Thijssen and Ritzen 2003). In black Africans, on the other hand, *CYP2C9*5* (Asp360Glu), *CYP2C9*6* (818 delA), *CYP2C9*11* (Arg335Met) and infrequently *CYP2C9*8* (Arg150His) are important alleles responsible for the reduced metabolic activity (Scordo et al. 2001; Xie et al. 2002; Allabi et al. 2003; Rettie et al. 2006; Yasar et al. 2002).

Vitamin K antagonists are coumarin derivatives and exhibit their anticoagulant activity by inhibition of vitamin K epoxide reductase (VKOR). The vitamin K epoxide reductase complex subunit 1 gene (*VKORC1*) codes for the VKOR enzyme (Rost et al. 2004; Li et al. 2006). Several SNPs of the *VKORC1* leading to an altered metabolic activity have been identified so far (Rieder et al. 2005). Frequencies of SNPs in different populations show significant heterogeneity between races and ethnic groups. The most common *VKORC1* polymorphism in Caucasians is the −1639/3673G>A polymorphism, which is associated with an increased bleeding risk and lower acenocoumarol dose requirements (Schalekamp et al. 2006; Spreafico et al. 2008; Rettie et al. 2006; Montes et al. 2006; Markatos et al. 2008; Geisen et al. 2005; D'Andrea et al. 2005). *VKORC1* 1173C>T (or 6484C>T, rs9934438) variant is another common polymorphism in Caucasians and related to VKAs (Rieder et al. 2005). Spreafico et al. (2008) report that patients carrying homozygous haplotypes of *VKORC1*3* and **4* (means of 16.0 and 15.8 mg, respectively at the week 1) require about 60% higher doses of acenocoumarol compared with those of **2* (mean of 9.5 mg).

Several dose algorithms incorporating pharmacogenetic and demographic/clinical patient information have been proposed mainly for warfarin in Caucasian and Asian populations (Sconce et al. 2005; Gage et al. 2008; van Schie et al. 2011). Such algorithms are useful for the prediction of warfarin dose; however, the considerable inter-ethnic and inter-racial genetic variability does not allow the use of a single algorithm across the various populations. Moreover, compared with warfarin, even less information is available on the impact of pharmacogenetics on acenocoumarol dose requirements (Van Geest-Daalderop et al. 2009; van Leeuwen et al. 2008). In particular, for black African patients, published information on polymorphisms of the *CYP2C9* and *VKORC1* genes is still very limited or lacking. The aim of this study was to characterize the influence of genetic polymorphisms of *VKORC1* and *CYP2C9* on the pharmacokinetics and pharmacodynamics of acenocoumarol in relation to demographic characteristics such as age and body mass index (BMI) among healthy black Africans from Benin.

2 Methods

2.1 Subjects and study design

Fifty-one healthy subjects who were previously genotyped for *CYP2C9* in previous studies (Allabi et al. 2003, 2005) participated in *VKORC1* genotyping in the present study. Among them, 36 subjects (35 men and 1 woman), aged 25–54 years (mean ± SD 33.2 ± 6.7 years) participated in the phenotyping study with acenocoumarol on the basis of medical history, physical examination and laboratory tests,

including complete blood cell count, coagulation parameters, kidney and liver functions. All subjects were in good health, as judged by physical examination and according to the results of the laboratory tests.

A personal or family history of thrombosis or bleeding; hepatic or renal diseases; metabolic, endocrine, cardiovascular, or pulmonary disorders; or allergy were exclusion criteria for the study. Subjects with abnormalities shown on biologic clotting tests and women in their menstrual periods were also excluded. The study was approved by the Ethics Review Committee of the Faculty of Health Sciences (FSS-UAC), Université d'Abomey-Calavi, BENIN, and was conducted in accordance with the principles of Good Clinical Practice (GCP). Written informed consent was obtained from each study subject before enrolment.

2.2 Phenotyping test with acenocoumarol

During the week preceding the acenocoumarol phenotyping test, any concomitant drug intake and alcohol consumption was not allowed. Twenty-four hours before and during the phenotyping test, vegetable intake in the diet was not allowed, to limit vitamin K absorption. For safety reasons, all subjects were hospitalized during the phenotyping test. In the morning, after an overnight fast, subjects came to the Clinical Investigation Center at the hospital. The first blood sample was collected for INR measurement and *VKORC1* genotyping immediately before oral ingestion of an 8 mg acenocoumarol tablet (Sintrom, Novartis Pharma GmbH, Nuremberg, Germany) with 200 ml of water. Eight hours later, a second blood sample was drawn for acenocoumarol plasma concentration measurement. Twenty-four hours later, the last blood sample was drawn for the determinations of INR and acenocoumarol trough plasma levels. An 8 and 24 h blood sampling for the analysis of acenocoumarol has been chosen in the present study due to practical reasons and because half-life of the drug has been reported to be about 10 h.

All subjects received a 10 mg Vitamin K pill (Vitamin K1; F. Hoffman-La Roche Ltd, Basel, Switzerland) after the last blood sampling. Body weight, height, sex and age of the study subjects were recorded.

2.3 Genotyping of *VKORC1* and *CYP2C9*

The *CYP2C9* genotype of the study subjects had been carried out in previous studies (Allabi et al. 2004, 2005). *VKORC1* genotyping was carried out as follows: Genomic DNA was extracted from peripheral blood lymphocytes obtained during the first sampling. The 1173C>T polymorphism of the *VKORC1* gene was determined. Identification of polymorphisms in *VKORC1* was performed by a PCR followed by pyrosequencing. 5′-GAGAGGGGA GGATAGGGTCA-3′ and 5′-CCACCTGGGCTATCCTC TGT-3′ were used as forward and reverse primers, respectively (QIAGENE GmbH, Hilden, Germany), to amplify a 329-bp fragment in the following reaction mixture: 5 μL buffer (10 mM Tris hydrochloride, and 50 mM potassium hydrochloride, pH 8.3), 3 mM $MgCl_2$, 1U Taq Gold polymerase, 200 μM of each deoxynucleotide triphosphate (dNTP), 10 μM of each primer and 250 ng of genomic DNA in a final volume of 50 μL. The cycling conditions were 95°C for 5 min, followed by 40 cycles at 95°C for 40 s, 60°C for 40 s and 72°C for 80 s, with a final extension step at 72°C for 7 min.

A sequencing primer (5′-CCAGGAGATCATCGAC-3′) was further hybridized to the PCR product and incubated with reagents containing enzymes and substrates. One deoxynucleotide triphosphate (dNTP) was added to the reaction mixture at a time and was incorporated into the DNA strand if it was complementary to the base in the template strand. This incorporation drived the conversion of substrate luciferin to oxyluciferin.

2.4 Drug assay

(*R*)- and (*S*)-acenocoumarol concentrations were determined in the plasma samples using a validated chiral liquid chromatography tandem mass spectrometry (LC/MS/MS, Vantage, ThermoFischer, Les Ulis, France) method. Briefly, both acenocoumarol enantiomers were extracted in acidic conditions with methyl tert-butyl ether (MTBE) using R- and S-warfarin as internal standards (IS). Chromatography was performed by isocratic elution with formiate buffer/acetonitrile on a chiral column [(S, S) whelk-O-5/100 Kromasil, 15 × 2.1, Régis, USA]. Acenocoumarol was ionized by electrospray in the positive mode, and selected reaction monitoring was used as scanning mode, measuring three specific transitions for each enantiomer and IS. The method was linear between 1.2 ng/mL (limit of quantification) and 250 ng/mL. Intraday and interday precisions and accuracy were evaluated at three quality control levels, 7.5, 37.5 and 100 ng/mL. For both enantiomers and at the three levels, the intraday precision ranged from 2.0 to 4.8% and the interday precision ranged from 0.5 to 5.2% with an accuracy ranging from 103.7 to 106.0%.

2.5 Determination of INR

Venous blood was collected in tubes containing 0.105 M sodium citrate. Plasma and cells were separated by centrifugation at 4,000*g* for 30 min. The INRs were performed using the same reagent/instrument combination, namely

human recombinant thromborel S (Dade Behring, Marburg Germany), and an Electra 1600 coagulometer.

The parameter 'INR change' was determined using the following formula:

INR change = [(INR 24 h after acenocoumarol intake − INR before acenocoumarol intake)/INR before acenocoumarol intake] × 100.

2.6 Statistics

The non-parametric Kruskal–Wallis test was used to compare the pharmacodynamic (INR 24 h, INR change) and pharmacokinetic data [Racemic (*R*)- and (*S*)-acenocoumarol plasma concentrations at 8 and 24 h] between the *CYP2C9* genotypes. The 95% confidence interval (CI) was used for the relevant data. The influence of demographic parameters (weight, BMI and age) on the INR was assessed using non-parametric correlation analysis. The relationship between concentrations of (*R*)- and (*S*)-acenocoumarol was determined by correlation analysis. GraphPad Prism for windows 5.01 was used for the statistical analysis and $P < 0.05$ was set as statistically significant.

3 Results

Among the 51 healthy subjects who participated in the genotyping study, 36 voluntarily agreed to participate in the acenocoumarol phenotyping test. Demographic and genotype characteristics of the study population are summarized in Table 1.

3.1 *VKORC1* genotyping

Of the 51 Beninese subjects, 49 were homozygous for 1173C/C, the wild-type *VKORC1*. Only two subjects (3.92%) were heterozygous 1173C/T and the allele frequency of 1173T was 1.96% (95% CI 0.0–4.65). One of these subjects was also heterozygous for the *CYP2C9*9* variant (Table 1). This volunteer demonstrated second highest INR value at 24th hour of acenocoumarol intake among all of the participants in the phenotyping study; on the other hand, our data did not allow us to test the statistical significance due to the low number of subjects with 1173T variant (Table 2).

3.2 INR and *CYP2C9* genotypes

No significant differences between the various *CYP2C9* genotypes were found for the INR at 24 h and the change in INR values (Table 2).

3.3 INR and BMI/age

We evaluated whether body weight was a factor influencing the INR at 24 h. In view of the foregoing, we consistently observed a statistically significant correlation between BMI and INR at 24 h ($P = 0.0003$, R 0.54). Furthermore, age was significantly associated with INR variation at 24 h compared with the baseline ($P < 0.05$).

Table 1 *CYP2C9* and *VKORC1* genotypes and demographic characteristics of the 36 healthy volunteers participating in the phenotyping study

Genotype		Number of subjects (%)	Age		BMI	
CYP2C9	*VKORC1*		Mean ± SD	95% CI	Mean ± SD	95% CI
**1/*1*	*CC*	12 (33.3)	32.8 ± 5.3	[29.4–36.2]	22.9 ± 3.6	[20.7–25.2]
**1/*1*	*CT*	1 (2.8)	39		20.0	
**1/*9*	*CC*	6 (16.7)	31.8 ± 4.9	[26.7–37.0]	23.7 ± 2.2	[21.3–26.0]
**1/*9*	*CT*	1 (2.8)	27		22.6	
**1/*8*	*CC*	6 (16.7)	37.3 ± 10.1	[26.8–47.9]	22.5 ± 1.7	[20.7–24.4]
**8/*9*	*CC*	3 (8.3)	31.7 ± 6.7	[15.1–48.2]	24.3 ± 5.0	[12.0–36.7]
**6/*9*	*CC*	2 (5.6)	38.0 ± 0.0		20.5 ± 1.0	[11.7–29.3]
**1/*6*	*CC*	1 (2.8)	39		23.2	
**8/*11*	*CC*	1 (2.8)	35		23.2	
**9/*11*	*CC*	1 (2.8)	51		23.8	
**1/*11*	*CC*	1 (2.8)	39		25.1	
**5/*8*	*CC*	1 (2.8)	33		23.9	
Total		36 (100)				

Age and BMI were not significantly different between the various genotypes. Data for age and body mass index (BMI) were presented as mean ± standard deviation (SD) and 95% confidence interval (CI)

Table 2 INR values at the 24th hour of acenocoumarol intake and the change of INR compared with the baseline in 36 volunteers with different *CYP2C9* and *VKORC1* genotypes

Genotype			INR 24 h		Change of INR	
CYP2C9	*VCORC1*	*N*	Mean ± SD	95% CI	Mean ± SD	95% CI
**1/*1*	*CC*	12	1.65 ± 0.34	[1.41–1.88]	0.55 ± 0.31	[0.34–0.75]
**1/*1*	*TC*	1	1.30		0.30	
**1/*9*	*CC*	6	1.52 ± 0.22	[1.28–1.75]	0.45 ± 0.20	[0.24–0.66]
**1/*9*	*TC*	1	2.1		1.0	
**1/*8*	*CC*	6	1.48 ± 0.20	[1.27–1.70]	0.47 ± 0.18	[0.28–0.65]
**8/*9*	*CC*	3	1.83 ± 0.46	[0.69–2.98]	0.73 ± 0.46	[0.0–1.88]
**6/*9*	*CC*	2	1.50 ± 0.56	[0.0–6.58]	0.4 ± 0.57	[0.0–5.48]
**1/*6*	*CC*	1	1.6		0.6	
**8/*11*	*CC*	1	1.3		0.3	
**9/*11*	*CC*	1	1.5		0.4	
**1/*11*	*CC*	1	1.4		0.3	
**5/*8*	*CC*	1	1.8		0.7	

INR and the change of INR were not significantly different between the various genotypes. INR at 24 h and change of INR were presented as mean ± standard deviation (SD) and 95% confidence interval (CI)

3.4 Plasma acenocoumarol levels and *CYP2C9* genotypes

Figure 1 shows the total plasma acenocoumarol concentrations of each subject at 8th and 24th hour of 8 mg acenocoumarol administration. The plasma average concentrations of (*S*)-acenocoumarol at 8 and at 24 h in the subjects with different *CYP2C9* genotypes did not significantly differ (Fig. 2, $P > 0.05$). Subjects with the *CYP2C9*9/*11* and *CYP2C9*5/*8* genotypes, however, showed plasma concentrations of S-acenocoumarol at 8 h which are largely outside the 95% confidence interval for this parameter observed in the *CYP2C9*1/*1* and *CYP2C9*1/*9* genotypes. The concentration of (*S*)-acenocoumarol was the highest in the subject with the genotype *CYP2C9*5/*8*. These subjects with the *CYP2C9*9/*11* and *CYP2C9*5/*8* genotypes appeared to have reduced enzyme activity (Fig. 2).

The average concentrations of (*R*)-acenocoumarol at neither 8 nor 24 h differ significantly in the different groups of *CYP2C9* (Fig. 3, $P > 0.05$). Likewise, the average total concentrations of acenocoumarol (both at 8 and 24 h) in different groups of *CYP2C9* genotype were not significantly different ($P > 0.05$).

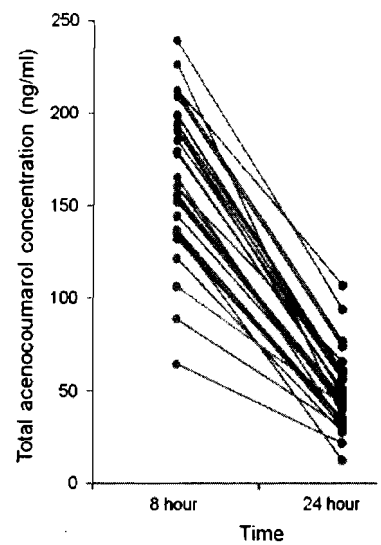

Fig. 1 Total acenocoumarol concentrations (ng/ml) of each individual at 8th and 24th hour of oral ingestion of 8 mg acenocoumarol tablet

4 Discussion

The anticoagulant activity of acenocoumarol due to inhibition of VKOR affects the synthesis of several clotting factors. Recently, different polymorphisms of *VKORC1* were described as having potential functional consequences (Rost et al. 2004; Rieder et al. 2005; Bodin et al. 2005; Aquilante et al. 2006; Markatos et al. 2008; Li et al. 2006). Rieder et al. (2005) have identified five major haplotypes (H1, H2, H7, H8 and H9) based on 10 SNPs of *VKORC1*. It has been shown that in subjects with H1 or H2 haplotypes, lower warfarin doses are required compared with those with haplotypes H7, H8 or H9. Geisen et al. Geisen et al. (2005) described three haplotypes (*VKORC1*2*, *VKORC1*3* and *VKORC1*4*) covering over 99% of the genetic variability in Europeans: The *VKORC1*2* haplotype (corresponding to H1 and H2) was strongly associated with an increased coumarin

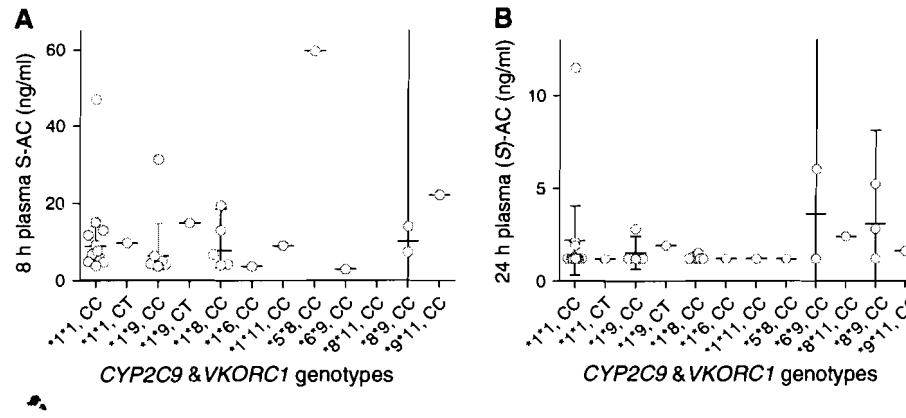

Fig. 2 (*S*)-acenocoumarol concentrations (ng/ml) at 8th (**a**) and 24th (**b**) hour of the drug intake in different genotypes of *CYP2C9* and *VKORC1* ($P > 0.05$). Mean and 95% confidence intervals are indicated for each genotype group

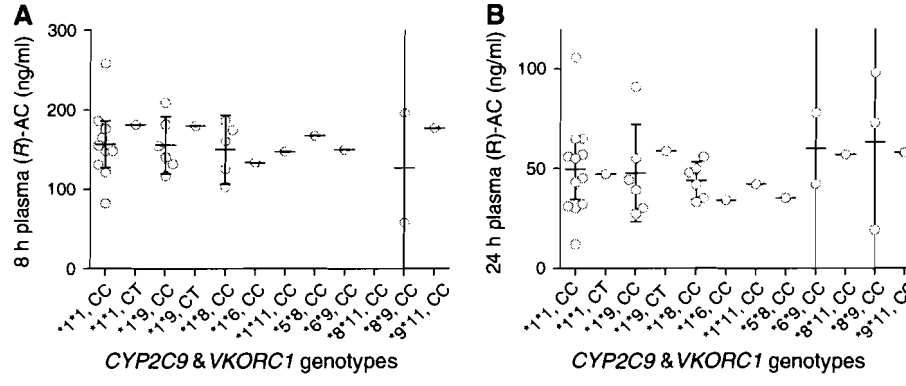

Fig. 3 (*R*)-acenocoumarol concentrations (ng/ml) at 8th (**a**) and 24th (**b**) hour of the drug intake in different genotypes of *CYP2C9* and *VKORC1* ($P > 0.05$). Mean and 95% confidence intervals are indicated for each genotype group

sensitivity whereas *VKORC1*3* and *VKORC1*4* (both included in the groups H7–H9) were associated with partial resistance.

In the present study, we evaluated the frequency of variant 1173 C>T (also named as: M17 variant or 6484C>T, a SNP in *VKORC1*2* haplotype, dbSNP: rs9934438) in 51 subjects previously genotyped for the *CYP2C9* polymorphisms. The allele frequency of 1.96% found in the present population from Benin was considerably lower than that observed in a German population (41.5%) (Geisen et al. 2005). Asians have an even higher frequency: a frequency of 91% was found in China (Yuan et al. 2005). Our results appear to be more in concordance with earlier published allele frequencies among African Americans (13%) (Geisen et al. 2005) and are in line with the notion that the black race has lower frequencies for the variants M17, M18 (6853G>C, a SNP in *VKORC1*2*, dbSNP: rs8050894) and M19 (7566C>T, a SNP in *VKORC1*2*, dbSNP: rs2359612) (Geisen et al. 2005). However the frequency of 25% obtained for M18 among Nigerians appears to be higher compared with 2 and 13% observed for M17 among Beninese and African–Americans. M17, M18 and M19 were previously found to be strongly linked (Geisen et al. 2005). Because only two subjects heterozygous for M17 of *VKORC1* were found in our sample, we could not study the effects of this gene variant on the pharmacodynamics of acenocoumarol. In addition, our data do not permit detecting any possible link between *VKORC1*2* allele and other *CYP2C9* alleles. The frequencies of *CYP2C9* variants presented in this study are not representative of the population because the rare genotypes were selected from a previous clinical trial and were invited to this study. Considering the small size and homogeneity of our study, further studies are warranted to confirm these findings.

Our data confirm that age, weight and BMI contribute significantly to the variability of INR as previously observed (Hillman et al. 2004; Kamali et al. 2004; Takahashi et al. 2006). Patient characteristics, co-administered drugs should also be considered to develop a dosage algorithm for acenocoumarol. For example, co-administration of proton pump inhibitors, especially esomeprazole and lansoprazole, has increased the risk for over anticoagulation due to the use of acenocoumarol in 2,755 patients from the Rotterdam Study (Teichert et al. 2011). Different algorithms have also been developed in different populations and more specifically among Caucasians and Asians (Sconce et al. 2005; Gage et al. 2008; van Schie et al. 2011). There are no algorithms proposed for sub-Saharan black patients published in the literature.

Table 2 INR values at the 24th hour of acenocoumarol intake and the change of INR compared with the baseline in 36 volunteers with different *CYP2C9* and *VKORC1* genotypes

Genotype			INR 24 h		Change of INR	
CYP2C9	*VCORC1*	*N*	Mean ± SD	95% CI	Mean ± SD	95% CI
**1/*1*	*CC*	12	1.65 ± 0.34	[1.41–1.88]	0.55 ± 0.31	[0.34–0.75]
**1/*1*	*TC*	1	1.30		0.30	
**1/*9*	*CC*	6	1.52 ± 0.22	[1.28–1.75]	0.45 ± 0.20	[0.24–0.66]
**1/*9*	*TC*	1	2.1		1.0	
**1/*8*	*CC*	6	1.48 ± 0.20	[1.27–1.70]	0.47 ± 0.18	[0.28–0.65]
**8/*9*	*CC*	3	1.83 ± 0.46	[0.69–2.98]	0.73 ± 0.46	[0.0–1.88]
**6/*9*	*CC*	2	1.50 ± 0.56	[0.0–6.58]	0.4 ± 0.57	[0.0–5.48]
**1/*6*	*CC*	1	1.6		0.6	
**8/*11*	*CC*	1	1.3		0.3	
**9/*11*	*CC*	1	1.5		0.4	
**1/*11*	*CC*	1	1.4		0.3	
**5/*8*	*CC*	1	1.8		0.7	

INR and the change of INR were not significantly different between the various genotypes. INR at 24 h and change of INR were presented as mean ± standard deviation (SD) and 95% confidence interval (CI)

3.4 Plasma acenocoumarol levels and *CYP2C9* genotypes

Figure 1 shows the total plasma acenocoumarol concentrations of each subject at 8th and 24th hour of 8 mg acenocoumarol administration. The plasma average concentrations of (*S*)-acenocoumarol at 8 and at 24 h in the subjects with different *CYP2C9* genotypes did not significantly differ (Fig. 2, $P > 0.05$). Subjects with the *CYP2C9*9/*11* and *CYP2C9*5/*8* genotypes, however, showed plasma concentrations of S-acenocoumarol at 8 h which are largely outside the 95% confidence interval for this parameter observed in the *CYP2C9*1/*1* and *CYP2C9*1/*9* genotypes. The concentration of (*S*)-acenocoumarol was the highest in the subject with the genotype *CYP2C9*5/*8*. These subjects with the *CYP2C9*9/*11* and *CYP2C9*5/*8* genotypes appeared to have reduced enzyme activity (Fig. 2).

The average concentrations of (*R*)-acenocoumarol at neither 8 nor 24 h differ significantly in the different groups of *CYP2C9* (Fig. 3, $P > 0.05$). Likewise, the average total concentrations of acenocoumarol (both at 8 and 24 h) in different groups of *CYP2C9* genotype were not significantly different ($P > 0.05$).

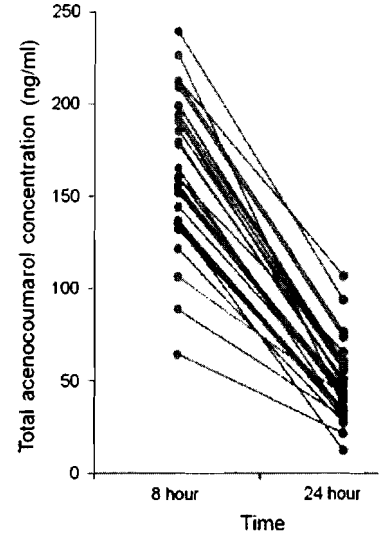

Fig. 1 Total acenocoumarol concentrations (ng/ml) of each individual at 8th and 24th hour of oral ingestion of 8 mg acenocoumarol tablet

4 Discussion

The anticoagulant activity of acenocoumarol due to inhibition of VKOR affects the synthesis of several clotting factors. Recently, different polymorphisms of *VKORC1* were described as having potential functional consequences (Rost et al. 2004; Rieder et al. 2005; Bodin et al. 2005; Aquilante et al. 2006; Markatos et al. 2008; Li et al. 2006). Rieder et al. (2005) have identified five major haplotypes (H1, H2, H7, H8 and H9) based on 10 SNPs of *VKORC1*. It has been shown that in subjects with H1 or H2 haplotypes, lower warfarin doses are required compared with those with haplotypes H7, H8 or H9. Geisen et al. Geisen et al. (2005) described three haplotypes (*VKORC1*2*, *VKORC1*3* and *VKORC1*4*) covering over 99% of the genetic variability in Europeans: The *VKORC1*2* haplotype (corresponding to H1 and H2) was strongly associated with an increased coumarin

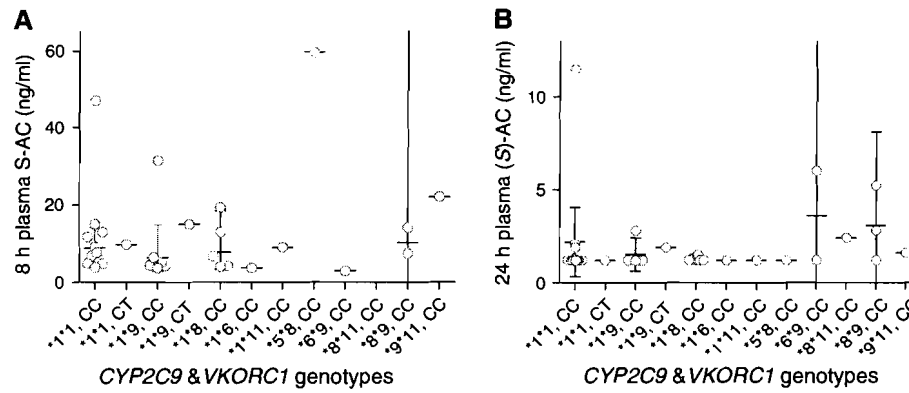

Fig. 2 (*S*)-acenocoumarol concentrations (ng/ml) at 8th (**a**) and 24th (**b**) hour of the drug intake in different genotypes of *CYP2C9* and *VKORC1* ($P > 0.05$). Mean and 95% confidence intervals are indicated for each genotype group

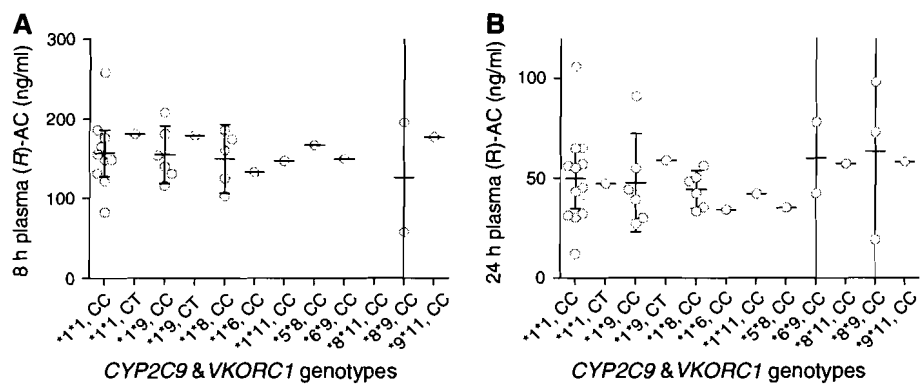

Fig. 3 (*R*)-acenocoumarol concentrations (ng/ml) at 8th (**a**) and 24th (**b**) hour of the drug intake in different genotypes of *CYP2C9* and *VKORC1* ($P > 0.05$). Mean and 95% confidence intervals are indicated for each genotype group

sensitivity whereas *VKORC1*3* and *VKORC1*4* (both included in the groups H7–H9) were associated with partial resistance.

In the present study, we evaluated the frequency of variant 1173 C>T (also named as: M17 variant or 6484C>T, a SNP in *VKORC1*2* haplotype, dbSNP: rs9934438) in 51 subjects previously genotyped for the *CYP2C9* polymorphisms. The allele frequency of 1.96% found in the present population from Benin was considerably lower than that observed in a German population (41.5%) (Geisen et al. 2005). Asians have an even higher frequency: a frequency of 91% was found in China (Yuan et al. 2005). Our results appear to be more in concordance with earlier published allele frequencies among African Americans (13%) (Geisen et al. 2005) and are in line with the notion that the black race has lower frequencies for the variants M17, M18 (6853G>C, a SNP in *VKORC1*2*, dbSNP: rs8050894) and M19 (7566C>T, a SNP in *VKORC1*2*, dbSNP: rs2359612) (Geisen et al. 2005). However the frequency of 25% obtained for M18 among Nigerians appears to be higher compared with 2 and 13% observed for M17 among Beninese and African–Americans. M17, M18 and M19 were previously found to be strongly linked (Geisen et al. 2005). Because only two subjects heterozygous for M17 of *VKORC1* were found in our sample, we could not study the effects of this gene variant on the pharmacodynamics of acenocoumarol. In addition, our data do not permit detecting any possible link between *VKORC1*2* allele and other *CYP2C9* alleles. The frequencies of *CYP2C9* variants presented in this study are not representative of the population because the rare genotypes were selected from a previous clinical trial and were invited to this study. Considering the small size and homogeneity of our study, further studies are warranted to confirm these findings.

Our data confirm that age, weight and BMI contribute significantly to the variability of INR as previously observed (Hillman et al. 2004; Kamali et al. 2004; Takahashi et al. 2006). Patient characteristics, co-administered drugs should also be considered to develop a dosage algorithm for acenocoumarol. For example, co-administration of proton pump inhibitors, especially esomeprazole and lansoprazole, has increased the risk for over anticoagulation due to the use of acenocoumarol in 2,755 patients from the Rotterdam Study (Teichert et al. 2011). Different algorithms have also been developed in different populations and more specifically among Caucasians and Asians (Sconce et al. 2005; Gage et al. 2008; van Schie et al. 2011). There are no algorithms proposed for sub-Saharan black patients published in the literature.

The acenocoumarol is a racemic mixture of two enantiomers, (*R*)- and (*S*)-acenocoumarol. It has been proven that the (*R*)-enantiomer is inherently more potent than the (*S*)-enantiomer (Meinertz et al. 1978). The plasma concentration of (*R*)-acenocoumarol found here is on average 10–20 times higher than that of (*S*)-acenocoumarol both 8 and 24 h after dosing due to the higher clearance of (*S*)-acenocoumarol (Thijssen et al. 2001). The subject with *CYP2C9*9/*11* genotype demonstrated also an increased plasma concentration of (*S*)-acenocoumarol at the 8 h (22.1 ng/ml), lying outside the 95% CI values of subjects with wild type genotype. The *CYP2C9*9/*11* seems also to be associated with reduced activity compared with wild-type genotype. In contrast, *CYP2C9*1/*9* subjects had concentrations of (*S*)-acenocoumarol at 8 h which were not significantly different from levels obtained in the *CYP2C9*1/*1*. These results are in line with earlier observations that the variant *CYP2C9*9* behaves like the wild-type allele (Blaisdell et al. 2004; Allabi et al. 2005). Our observation in a single subject confirms also the findings from previous studies that *CYP2C9*5* exhibits reduced metabolic activity (Dickmann et al. 2001; Allabi et al. 2004, 2005).

Furthermore, we found a positive and significant correlation between plasma concentrations of (*S*)- and (*R*)-acenocoumarol at 8 and 24 h as also previously reported between clearances of (*S*)-warfarin and (*R*)-warfarin (Takahashi et al. 2006). This could indicate that CYP2C9 is the major metabolic pathway for both enantiomers despite the fact that (*R*)-acenocoumarol may also follow alternative pathways such as CYP1A2 and CYP3A4 (Thijssen et al. 2000; Ufer 2005). Therefore, (*S*)-acenocoumarol concentrations 8 h after the first drug intake could be used for the phenotyping of CYP2C9 in patients. This strategy could be incorporated into a dosing algorithm for acenocoumarol.

No significant association was found in the present study between INR and the different *CYP2C9* genotypes. A similar finding has been reported in 25 Russian patients who use acenocoumarol due to high risk of thromboembolism (Sychev et al. 2011); *CYP2C9* genetic variants were not associated with the development of severe hypocoagulation episodes in those patients (Sychev et al. 2011). This could be due to the limited numbers of observations. In a previous study conducted in healthy volunteers, there were two black heterozygous subjects carrying *CYP2C9*5*. The INRs at 24 h in these subjects did not differ from that of *CYP2C9*1/*1* (Morin et al. 2004). In a recent study, Cavallari et al. (2010) could explain 36% of the inter-individual variation in INR by integrating into the model variants *CYP2C9*5*, *CYP2C9*6*, *CYP2C9*8*, *CYP2C9*11* and demographic factors such as age and weight.

Combining the observations of the present study with earlier published data, it can be concluded that subjects with the *CYP2C9*5*, **8* and **11* alleles would require significantly lower doses of acenocoumarol and might be exposed to an increased risk of over-anticoagulation, particularly those who are compound heterozygotes (e.g. *CYP2C9*5/*8*) and homozygous for the variants. The detection of these alleles might be useful to select the most sensitive patients exposed to a higher risk of over anticoagulation.

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