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A Pharmacokinetic–Pharmacodynamic Model for Predicting the Impact of *CYP2C9* and *VKORC1* Polymorphisms on Fluindione and Acenocoumarol During Induction Therapy

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Abstract

Background and Objective: Vitamin K epoxide reductase complex, subunit 1 (*VKORC1*) and cytochrome P450 2C9 (*CYP2C9*) polymorphisms are taken into account when predicting a safe oral dose of coumarin anticoagulant therapy, but little is known about the effects of genetic predictors on the response to fluindione and acenocoumarol. The aims of this study were to characterize the relationship between fluindione and acenocoumarol concentrations and the international normalized ratio (INR) response, and to identify genetic predictors that are important for dose individualization.

Methods: Fluindione concentrations, *S*- and *R*-acenocoumarol concentrations, the INR and genotype data from healthy subjects were used to develop a population pharmacokinetic–pharmacodynamic model in Monolix software. Twenty-four White healthy subjects were enrolled in the pharmacogenetic study. The study was an open-label, randomized, two-period cross-over study. The subjects received two doses of an oral anticoagulant: 20 mg of fluindione (period A) or 4 mg of acenocoumarol (period B). The pharmacokinetics and pharmacodynamics were studied from day 2 to day 3.

Results: A two-compartment model with a first-order input model was selected as the base model for the two drugs. The pharmacodynamic response was best described by an indirect action model with *S*-acenocoumarol concentrations and fluindione concentrations as the only exposure predictors of the INR response. Three covariates (*CYP2C9* genotype, *VKORC1* genotype and body weight) were identified as important predictors for the pharmacokinetic–pharmacodynamic model of *S*-acenocoumarol, and four covariates (*CYP2C9* genotype, *VKORC1* genotype, CYP1A2 phenotype and body weight) were identified as predictors for the pharmacokinetic–pharmacodynamic model of fluindione. Because some previous studies have shown a dose–response relationship between smoking exposure and the CYP1A2 phenotype, it was also noted that smokers have greater CYP1A2 activity.

Conclusion: During initiation of therapy, *CYP2C9* and *VKORC1* genetic polymorphisms are important predictors of fluindione and acenocoumarol pharmacokinetic–pharmacodynamic responses. Our result suggests that it is important to take the CYP1A2 phenotype into account to improve individualization of fluindione therapy, in addition to genetic factors.

Background

Initiation of therapy with anticoagulant drugs protects against thrombosis, stroke and myocardial infarction, but is accompanied by a high incidence of adverse events.[1,2] Oral anticoagulant therapy is associated with an increased risk of major bleeding complications, particularly during the induction phase. The initial phase of treatment has been identified as the most critical and unstable, engendering risks of both over-anticoagulation (and hence of bleeding) and under-coagulation (and hence of thrombosis). These risks are caused by the inherent properties of oral anticoagulants, characterized by a narrow therapeutic index and a large interindividual variability in the response to dose. The latter situation makes it difficult to predict the daily maintenance dose, which for warfarin may range from as little as 0.5 mg to as much as 60 mg[3] and for acenocoumarol may range from 1 mg to 56 mg.[4] These large variations in dose requirements, influenced by pharmacokinetic and pharmacodynamic factors, are in turn determined by genetic and environmental factors, and demand frequent measurements of the international normalized ratio (INR) to evaluate the degree of anticoagulation and to assess the need for dosage alteration.

Warfarin is the main vitamin K antagonist prescribed in the UK and the USA, while acenocoumarol, the 4′-nitro analogue of warfarin, is widely used in European countries.[5] Fluindione, an indane-dione derivative, is the most widely prescribed oral vitamin K antagonist in France (65% of prescriptions).[5,6]

Elimination of the two coumarins depends entirely on their hepatic biotransformation by the cytochrome P450 (CYP) 2C9 enzyme. Two common single nucleotide polymorphisms (SNPs) have been described in *CYP2C9*; one in exon 3 (CGT> TGT; Arg144Cys) is denoted as *CYP2C9**2, while the SNP in exon 7 (ATT>CTT; Ile359Leu) is called *CYP2C9**3. Patients with one or two of these variant alleles (*CYP2C9**2 and *CYP2C9**3) require smaller doses of coumarins than carriers of the most common *CYP2C9**1 wild-type allele.[7] These allelic variants of *CYP2C9* code for enzymes with approximately 12% (*CYP2C9**2) and 5% (*CYP2C9**3) of the enzymatic activity of the wild-type genotype, *CYP2C9**1.[8,9] Moreover, patients with these variant alleles have a higher incidence of minor bleeding episodes,[1,10] even though a dose–response association for the *CYP2C9**2 variant allele has not been consistently found.[4,11] The *CYP2C9**3 variant allele is also associated with a reduced likelihood of achieving stability within the target INR range during the first 6 months of acenocoumarol and warfarin therapy, and is associated with an increased risk of over-anticoagulation (INR >6).[12,13]

Oral anticoagulants exert their effects by reducing the regeneration of vitamin K from its epoxide through inhibition of vitamin K epoxide reductase. This protein is coded by the vitamin K epoxide reductase complex subunit 1 gene (*VKORC1*).[14] Recently, we and others have retrospectively identified novel *VKORC1* SNPs that correlate with the potency of warfarin[15,16] and acenocoumarol.[17] *VKORC1* haplotype group A/A, as described by Rieder et al.,[18] is present in 37% of European-American subjects and is associated with lower levels of *VKORC1* messenger RNA expression and a need for lower warfarin maintenance doses, compared with the A/B or B/B haplotype groups. The *VKORC1* 1173C>T SNP (rs9934438) is used to tag the major *VKORC1* haplotype groups A and B used in the nomenclature proposed by Rieder et al.[18] The C wild-type allele of the C1173T *VKORC1* genetic polymorphism corresponds to the group B *VKORC1* haplotype and the T allele corresponds to the group A *VKORC1* haplotype. Other factors that are thought to affect warfarin and acenocoumarol dosage include age, body weight, smoking status, prior venous thromboembolism, race and certain medications such as amiodarone and antibacterials.[19] Several dosing algorithms that combine clinical and genetic parameters to predict therapeutic warfarin dosage have also been developed; by including *CYP2C9* and *VKORC1* genotypes, they could explain 53–54% of the variability.[15,20-23]

Even though the combined effect of both *VKORC1* and *CYP2C9* on coumarins has been fully investigated, less information is available on the fluindione response. Surprisingly, there are very few clinical studies; one of them involved patients and found that the optimal dose to attain the therapeutic index for each patient ranged from 5 mg to 40 mg,[24] confirming once again the importance of interpatient variability. Only one study estimated the pharmacokinetic and pharmacodynamic characteristics of fluindione in patients receiving long-term treatment.[25] Recently, we and others have suggested that *CYP2C9* genetic polymorphisms affect the fluindione response in patients, but so far no prospective study on such pharmacogenetic–pharmacokinetic response relationships is available.[26] Indeed, little is known about the pharmacokinetic disposition of fluindione, except that it has an intermediate half-life, which is close to the half-life of the more active enantiomer of warfarin.[25] Moreover, it has previously been described that coumarins are mainly metabolized by CYP2C9, but some other CYPs such as CYP3A4 and CYP1A2 are involved in a minor pathway.[27] Indeed, we hypothesized that CYP2C9 may not be the only CYP involved in the metabolism of fluindione and that CYP1A2 should be taken into account. Some previous studies have shown a dose–response relationship between smoking

exposure and CYP1A2 enzyme induction in the liver.[28] The paraxanthine/caffeine ratio determined in plasma is a more reliable index for assessment of CYP1A2 activity. In order to assess the impact of CYP1A2 activity on fluindione pharmacokinetics, we decided to phenotype for CYP1A2 activity in healthy subjects, using the caffeine test.[29]

In 2007, the US FDA updated the warfarin label to include information about genotypes for prescribers, and recently some algorithms have been published to optimize coumarin therapy.[15,21,30,31] Meanwhile, a fundamental question remains: whether genotyping could provide early information about maintenance doses of fluindione as well, and/or whether fluindione could be used in carriers of the *CYP2C9**3 allelic variant, who are known to be at higher risk of early over-anticoagulation with coumarins. We chose to investigate the initial phase of treatment, since the safe and effective dose for an individual patient is not known at that stage and is determined empirically, with an increased risk of over-anticoagulation.

Therefore, the purpose of this analysis was to develop a population model to describe the pharmacokinetics of both fluindione and acenocoumarol and the pharmacokinetic–pharmacodynamic relationship between drug concentrations and the pharmacodynamic response, including identification of important predictors for *a priori* dose individualization of both oral anticoagulants.

Table I. Demographic characteristics of healthy subjects [n = 24]

Characteristic	Genotype	
	*CYP2C9**1/*1 [n = 11][a]	*CYP2C9**3 allele carriers [n = 13][a]
Sex (n)		
Male	10	9
Female	1	4
Age (y)	28.5 ± 8.6	26.8 ± 8.0
BMI (kg/m^2)	23.6 ± 2.1	22.4 ± 2.6
Body weight (kg)	71.4 ± 10.9	67.8 ± 9.8
Baseline INR	1.13 ± 0.11	1.28 ± 0.24
VKORC1 C1173T genotype (n)		
CC	1	4
CT	6	6
TT	4	3
Smokers (n)	5	5
PAX/CAF		
In smokers	0.51 ± 0.25	0.64 ± 0.21
In non-smokers	0.40 ± 0.14	0.40 ± 0.12

a Results are expressed as mean ± standard deviation unless specified otherwise.

BMI = body mass index; ***CYP*** = cytochrome P450; **INR** = international normalized ratio; **PAX/CAF** = paraxanthine/caffeine ratio; ***VKORC1*** = vitamin K epoxide reductase complex, subunit 1.

Subjects, Materials and Methods

Subjects

In this study, 24 White healthy subjects, of whom 10 were smokers (defined as subjects consuming >3 cigarettes/day) were enrolled from a large database of pharmacogenetically characterized healthy subjects, who had been previously genotyped for different *CYP2C9* alleles (table I). Each subject's body mass index (BMI) had to be between 20.5 and 25.3 kg/m^2. This pharmacogenetic database was built for a previous study, which enrolled 263 healthy subjects aged between 18 and 65 years.[32] The subjects could not participate in the database if they had a personal or family history of thrombosis or bleeding; hepatic or renal disease; metabolic, endocrinal, cardiovascular or pulmonary disorders; or allergy. Subjects with abnormalities shown on biological clotting tests were excluded. Concomitant drug intake, including use of oral contraceptives or alcohol consumption (>50 g/day), was forbidden. Smoking was allowed. In our study, we aimed to include White subjects who were carriers of *CYP2C9**2 and/or *CYP2C9**3. Among all subjects in the database, the total number of potentially eligible White subjects was 230. These subjects were solicited to participate in the study, on the basis of their genotype. The subjects could not participate in this study if they were homozygous carriers of *CYP2C9**2 and *CYP2C9**3 and were not judged to be healthy on the basis of their medical history and physical examination.

The study was started before the *VKORC1* gene was identified in 2004,[14] thus we could not modify the methodological design of the study. Although subjects were recruited only on the basis of their *CYP2C9* genotype, not their *VKORC1* genotype, the Hardy-Weinberg equilibrium was respected. One week before and during the study, any drug intake (including oral contraceptives) or grapefruit consumption was strictly forbidden. All subjects refrained from taking coffee, tea, Coca-Cola, chocolate or any caffeine-containing beverage for at least 24 hours prior to the CYP1A2 phenotyping test and during each study period. This study took place at the Centre d'Investigation Clinique, Hôpital Saint Antoine (affiliated with the Institut National de la Santé et de la Recherche Médicale and Assistance Publique-Hôpitaux de Paris [Paris, France]), from February 2004 to September 2004.

The local ethics committee of the Hôpital Pitié-Salpétrière (Paris, France) approved the study protocol. After participants

had given their written informed consent, a screening evaluation was performed, including the medical history, physical examination and standard clinical biochemical and haematological tests.

Study Design

The study was an open-label, randomized, two-period crossover study. CYP1A2 phenotyping was conducted in the 2 weeks prior to drug intake. The subjects were asked whether during the preceding 24 hours they had adhered to the instructions to avoid consumption of caffeine-containing food and beverages. The two study periods were separated by a washout interval of at least 2 weeks. During one study period, subjects took their first oral dose of fluindione 20 mg (Previscan®; Procter Gamble Pharmaceuticals France, Neuilly-sur-Seine, France) [period A] or acenocoumarol 4 mg (Sintrom®; Novartis Pharma, Rueil-Malmaison, France) [period B] at 9:00am. On the morning of the second day, after an overnight fast, the subjects were hospitalized and the second dose of oral anticoagulant (fluindione 20 mg [period A] or acenocoumarol 4 mg [period B]) was administered with 150 mL of tap water in the morning. The pharmacodynamics of both oral anticoagulants were studied from day 2 (T_{24h}) to day 3 (T_{72h}). The pharmacokinetics of fluindione and *S*- and *R*-acenocoumarol were evaluated during the same period.

The major study endpoint was the INR measured 24 hours after administration of the second oral anticoagulant dose (INR_{T48h}), since it is usually the first INR control after the initiation of oral anticoagulant treatment.

Blood Sampling

Blood samples were collected immediately before administration of the second oral anticoagulant dose and at 0.5, 8, 12, 24 and 48 hours thereafter for determination of the INR. For acenocoumarol and fluindione plasma concentrations, blood samples were collected in heparinized tubes 0.5, 1, 2, 3, 4, 8, 12, 24 and 48 hours after the second dose. Plasma was separated within 1 hour after blood collection and stored at −80°C until further analysis.

Genotyping

*CYP2C9**2 and *CYP2C9**3 (rs numbers 1799853 and 1057910, respectively), as well as *VKORC1* genetic polymorphism for 1173 C>T (rs number 9934438), were determined using custom TaqMan® allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) as previously described.[17,33] Primers and fluorescent probes were designed for *VKORC1* genotyping using Primer Express® software from Applied Biosystems. The post-PCR-generated fluorescence intensity was quantified using ABI Prism® 7000 sequence detector system software version 1.2.3 (Applied Biosystems, Courtaboeuf, France).

Bioanalytical Methods

Plasma fluindione was assayed with the use of a Surveyor® high-performance liquid chromatography (HPLC)-UV system with ChromQuest™ software (Thermo Finnigan LLC, San Jose, CA, USA).[34] The UV spectrophotometer was set at a wavelength of 280 nm. The separation was achieved at 40°C temperature, with a reversed-phase 100X 4.6 mm internal diameter BetaBasic-8 column and 5 um particle size packing (Thermo Electron Corporation). The mobile phase composition was optimized to a 0.067 mol/L dibasic sodium phosphate buffer (adjusted to pH 6.3 with phosphoric acid) and acetonitrile (82/18, vol/vol) mixture. The flow rate was set at 1.5 mL/min. The following extraction procedure was used: 100 µL of plasma from human heparinized blood (spiked plasma used for calibration and controls; patients' plasma samples) was added to a 1.5 mL tube that contained 50 µL of 20 mg/L internal standard solution (warfarin) and 100 µL of acetonitrile. A 150 µL volume of supernatant was transferred to another tube that contained 200 µL of phosphate buffer; 25 µL of the mixture was injected into the HPLC system. The calibration curve was linear over the range 0.05–6 mg/L. The method was highly reproducible. The coefficient of variation was 6.1% for a fluindione concentration of 0.1 mg/L, 2.8% for a concentration of 0.5 mg/L, and 2.3% for a concentration of 4 mg/L (ten measurements for each concentration). The estimated lower limit of quantification was 0.1 mg/L under the conditions described above, with a signal-to-noise ratio of 3 and a coefficient of variation lower than 20%. Plasma *R*- and *S*-acenocoumarol concentrations were assayed by enantioselective HPLC after derivatization with *N*-carbobenzoyl-*L*-proline as previously described.[35,36] Determination of CYP1A2 activity was assessed by calculating the paraxanthine/caffeine ratio in plasma obtained 5 hours after intake of 140 mg caffeine (Nescafe, Nestlé). Caffeine and its metabolites were quantified by HPLC as previously described for CYP1A2 phenotyping.[29] The INR was determined using Thromborel® S (Dade Behring®, Marburg, Germany).

Statistical Analysis

The sample size was calculated to detect a 20% increase in the INR_{T48h} (the major study endpoint) in *CYP2C9**3 allelic carriers, assuming an INR_{T24h} of 1.5 ± 0.16 in healthy wild-type subjects during the acenocoumarol period,[12,17,37] with 80%

power at a two-tailed alpha of 0.05. Results are presented as means ± standard deviations (SDs).

NQuery Advisor® sample size software (version 7.0, GraphPad Software) was used to calculate the sample size.

Modelling Strategy and Population Pharmacokinetic–Pharmacodynamic Model

The Basic Pharmacokinetic Model

Data analysis was performed using Monolix nonlinear mixed-effects modelling software (version 3.1, release 2).[38] The stochastic approximation expectation maximization algorithm combined with the Markov chain Monte Carlo simulation procedure was used to estimate the maximum likelihood of the model. We explored various structural models, one- and two-compartment disposition with linear elimination, first-order oral absorption and absorption delay. Interindividual variability of pharmacokinetic parameters was considered to be log normal. Different error models (proportional, exponential and additive) were investigated to describe the residual variability of the model. Model selection was based on visual inspection of goodness-of-fit plots, the precision of parameter estimates and the likelihood ratio test based on the decrease in the objective function value, the Akaike information criterion (AIC) or the Bayesian information criterion (BIC). When comparing two nested models, the likelihood ratio test was used. For other model selection, AIC and/or BIC were performed.

The Basic Pharmacokinetic–Pharmacodynamic Model

Fluindione and acenocoumarol exert an anticoagulant effect by inhibiting the vitamin-K-dependent coagulant factor in the liver. A pharmacokinetic–pharmacodynamic model was built to associate the fluindione or acenocoumarol concentrations and the effect measured with the INR. Concentrations of *S*-acenocoumarol and *R*-acenocoumarol alone and a combination of the two enantiomers with an additive effect were tested as potential predictors of the INR response.[39] The delay for development of a response after the drug reaches its target was modelled by an indirect response model.[40] The INR, which increases during anticoagulant treatment and may be viewed as an inverse measure of coagulation activity, was considered as the outcome in the pharmacodynamic model. To maintain the coherence of the mechanistic hypothesis of the indirect response model, we used the inverse of the differential equation, as previously described by Mentré et al.[25] (equation 1):

$$\frac{d\left(\frac{1}{INR}\right)}{dt} = k_{in} \times \left(1 - \left(\frac{C_t^{\gamma}}{C_t^{\gamma} + C_{50}^{\gamma}}\right)\right) - k_{out} \times \frac{1}{INR}$$

$$\frac{1}{INR_0} = \frac{k_{in}}{k_{out}} \qquad \text{(Eq. 1)}$$

This pharmacokinetic–pharmacodynamic model involves k_{in}, the zero-order rate constant for production of the response; k_{out}, the first-order rate constant for loss of the response; γ, the shape parameter of the hyperbolic function; C_t, the concentration of fluindione or acenocoumarol; and C_{50}, the concentration of fluindione or acenocoumarol that reduces the 1/INR by 50%, respectively. In our model, we assumed that the maximal inhibitory effect is 100%, in accordance with previous results from Mentré et al.[25] Different error models (proportional, exponential and additive) were investigated to describe the residual variability of the model.

Assessment of the Effects of Covariates

For pharmacokinetic analyses, age, sex, body weight, BMI, CYP2C9 activity, CYP1A2 activity and smoking status were considered as covariates of interest on clearance (CL) and the central volume of distribution (V_1).

In the pharmacodynamic analyses, age, sex, body weight and *VKORC1* genotypes were tested as covariates on the parameter governing the effect of the drug, in accordance with the model selection criteria described above. The influences of continuous covariates (i.e. body weight, age, BMI and CYP1A2 activity) were modelled on the basis of the allometry, according to equation 2, using CL as an example:

$$TVCL = \theta_{CL} \times \left(\frac{BW}{BW_{median}}\right)^{\theta_{BW}} \qquad \text{(Eq. 2)}$$

where BW is the body weight of the subject, BW_{median} is the median body weight of the population, TVCL is the typical value of the population CL estimate, θ_{CL} is the value for a subject with the median covariate value, and θ_{BW} is the estimated influential exponent for body weight. Categorical covariates included sex, smoking status and genotyping effects of *CYP2C9* and *VKORC1*. Genotyping effects were classified into two categories: carriers of the wild-type allele and carriers of at least one allelic variant.

They were modelled according to equation 3 for a subject with the *CYP2C9**1/*1 genotype:

$$TVCL = \theta_{CL} \qquad \text{(Eq. 3)}$$

and equation 4 for a carrier of the *CYP2C9**3 allelic variant:

$$TVCL = \theta_{CL} \times \theta_{CYP2C9^*1/^*3} \qquad \text{(Eq. 4)}$$

The covariates were added to the model in accordance with the automated procedure described elsewhere.[41] This involved a forward inclusion step and a backward elimination step. In the forward step, parameter-covariate relations were added to the model in a stepwise manner until no further relation was statistically significant ($p < 0.05$). In the following backward step, the relations that were identified were excluded from the model,

in a similar stepwise manner, if they failed to achieve statistical significance at the $p < 0.01$ level. The outcome of this procedure was the final model.

Model Evaluation

The goodness of fit of the model was established by plotting the population predictions of the model versus observations, the individual predictions versus observations and the normalized prediction distribution error metrics for residuals versus time.[42] The standard errors (SEs) of all parameters were also calculated using a stochastic approximation of the Fisher information matrix.[43]

The visual predictive check (VPC) simulation technique was used as an internal validation.[44] The VPC was generated by simulating the parameters of the dataset 1000 times. The ability of the model to describe observations was evaluated by visual inspection of the distribution of simulated concentrations. Furthermore, the median parameter values and the 90% prediction interval of the VPC replicates were compared with the observations of the original dataset. The precision of population parameters was also calculated. The SEs of all parameters were also calculated using a stochastic approximation of the Fisher information matrix.[43]

Simulation of the International Normalized Ratio (INR) during Induction Therapy

INR versus time curves during fluindione and acenocoumarol therapy were simulated to visualize potential differences in the shape of the response curve for typical individuals with different combinations of covariates. All simulations were performed in Monolix software using the final pharmacokinetic–pharmacodynamic parameter that was previously estimated. To simulate steady-state conditions for acenocoumarol and fluindione plasma concentrations, 20 doses were simulated.

Results

Demographic Data

Twenty-four subjects were included and completed the whole study. The *VKORC1* genotype was determined after inclusion, and Hardy-Weinberg equilibrium was respected for *VKORC1* polymorphism ($p > 0.05$). These groups did not significantly differ concerning age (mean ± SD 27.6 ± 8.1 years) and body weight (mean ± SD 69.4 ± 10.3 kg). The mean ± SD BMI was 22.9 ± 2.4 kg/m^2. Thirteen were *CYP2C9**3 allele carriers, two of whom were double heterozygous (*CYP2C9**2/*3) and 11 of whom were *CYP2C9* homozygous wild type (*CYP2C9**1/*1). There were five smokers per genotype group, with a mean consumption of 14.7 cigarettes/day. The characteristics of the subjects are summarized in table I. No period–treatment interactions and no period effects were detected. Full-profile data for pharmacokinetic and pharmacodynamic analysis were obtained from 24 subjects.

Fluindione Pharmacokinetic–Pharmacodynamic Model Building

Fluindione pharmacokinetics were adequately described by a two-compartment, first-order input model with no lag time. With this model, CL of fluindione was estimated to be 0.17 L/h, with interpatient variability of 18%. The V_1 was 5.19 L. No covariance was found between the parameters CL and V_1. Parameter estimates from the final model, including information on interindividual variability and uncertainty in parameter estimates, are given in table II. The error model was described by a proportional type. Covariates contributing to interindividual variability in CL were the *CYP2C9* genotype, body weight and CYP1A2 phenotype. After taking into account these covariates, the unexplained interindividual variability in CL was reduced from 40.0% to 18.0%. Body weight had an effect on V_1 and decreased the interindividual variability from 27% to 20%.

The pharmacokinetic–pharmacodynamic model that was selected was an indirect response model with a coefficient of sigmoidicity. An additive residual error model was found to best describe the data. Interpatient variability could be estimated for the C_{50} and k_{in}, as shown in table II. The only covariate identified as contributing to interindividual variability was the *VKORC1* genotype on C_{50}, with a decrease from 30.5% to 18%.

The goodness-of-fit plots for the basic and covariate models of fluindione are presented in figure S-1 in the Supplemental Digital Content (SDC; available online at http://links.adisonline.com/CPZ/A22) and show no apparent bias in model predictions apart from some misfit in the absorption phase. The descriptive quality of the model is illustrated by the VPC in figure 1. The results of the VPC show that 95% of the observed pharmacokinetic data and 94% of the observed pharmacodynamic data were included in the 90% envelope of the simulated values, and demonstrate the good predictive properties of the model.

Acenocoumarol Pharmacokinetic–Pharmacodynamic Model Building

The pharmacokinetic models for *S*- and *R*-acenocoumarol were built separately. A two-compartment model with first-order

Table II. Summary of the population pharmacokinetic–pharmacodynamic parameters of fluindione

Parameter	Estimate	RSE (%)
Population parameters		
k_a (h^{-1})	1.83	26
CL (L/h) = $\theta_2 \bullet [BW/69]^{\theta 3} \bullet [PAX/CAF\ 0.42]^{\theta 4} \bullet [1 - \theta_5$ if *CYP2C9**3]		
θ_2	0.17	8
θ_3	0.75 FIX	
θ_4	0.617	21
θ_5	–0.363	26
V_1 (L) = $\theta_6 \bullet [BW/69]^{\theta 7}$		
θ_6	5.19	5
θ_7	1 FIX	
Q (L/h)	0.173	27
V_2 (L)	2.77	20
C_{50} (mg/L) = $\theta_{10} \bullet [1 - \theta_{11}$ if *VKORC1* CC or CT]		
θ_{10}	3.63	12
θ_{11}	0.3	46
k_{in} (h^{-1})	0.0385	11
k_{out} (h^{-1})	0.0391	11
γ	2.32	15
Interindividual variability parameters		
ω_{ka}	0.47	31
ω_{CL}	0.18	40
ω_{V1}	0.2	43
ω_{C50}	0.28	35
ω_{kin}	0.01	53
Residual error parameters		
$\sigma_{concentration}$	0.158	5
σ_{INR}	0.047	7

γ = shape parameter of the hyperbolic function; θ = parameter of interest; σ = standard deviation of a residual parameter; ω = standard deviation of an interindividual parameter; **BW** = body weight; $\mathbf{C_{50}}$ = concentration that reduces the 1/INR by 50%; **CL** = apparent total clearance; ***CYP*** = cytochrome P450; **INR** = international normalized ratio; $\mathbf{k_a}$ = absorption rate constant; $\mathbf{k_{in}}$ = zero-order rate constant for production of the response; $\mathbf{k_{out}}$ = first-order rate constant for loss of the response; **PAX**/**CAF** = paraxanthine/caffeine ratio; **Q** = inter-compartmental clearance; **RSE** = relative standard error; $\mathbf{V_1}$ = central volume of distribution; $\mathbf{V_2}$ = peripheral volume of distribution; ***VKORC1*** = vitamin K epoxide reductase complex, subunit 1.

absorption and elimination with lag time was fitted to the *S*-acenocoumarol data. The anticoagulant response to acenocoumarol was best described by a model involving the *S*-acenocoumarol concentration as the only exposure predictor of the INR response. With the final model, CL of *S*-acenocoumarol was estimated to be 15.3 L/h, with interpatient variability of 24.0%. The V_1 of *S*-acenocoumarol was 24.7 L (table III). Data for *R*-acenocoumarol are not shown. According to some previous results from Thijssen et al.,[36,45] the much higher potency of racaemic acenocoumarol in carriers of the genetic polymorphism (the *CYP2C9**3 allele) is caused particularly by increased exposure to the *S*-enantiomer.

Three covariates were identified as contributing to the interindividual variability in the pharmacokinetic–pharmacodynamic model of *S*-acenocoumarol. The *CYP2C9* genotype on CL reduced the interindividual variability from 42% to 24.7%. Body weight on V_1 decreased the interindividual variability from 66.7% to 53%.

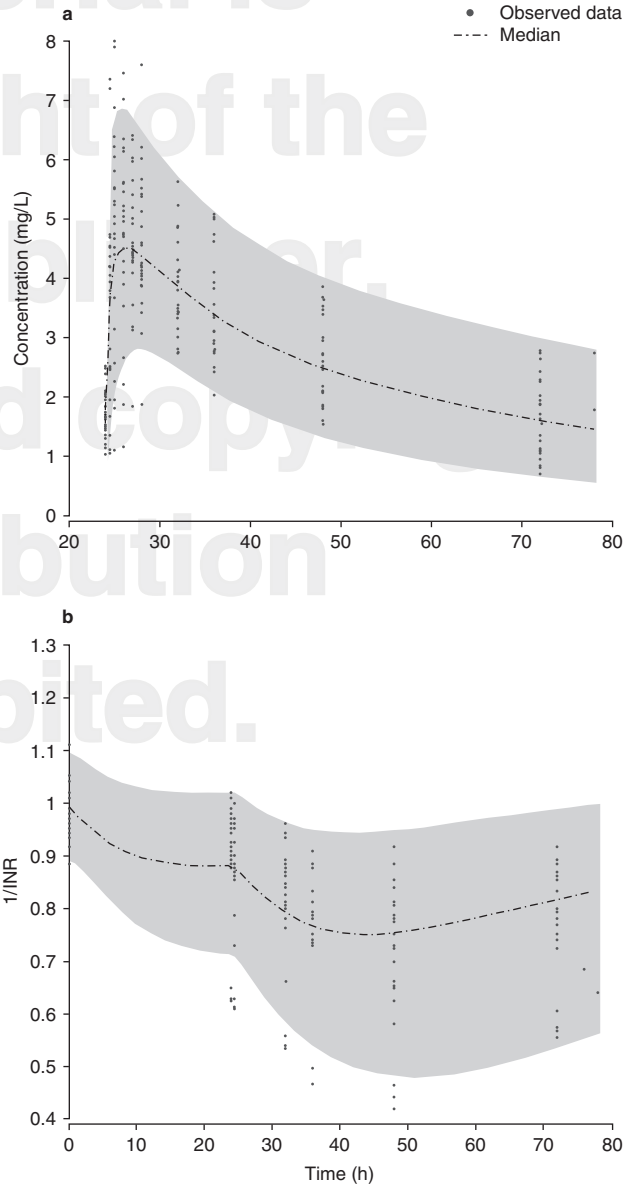

Fig. 1. Visual predictive check for (**a**) fluindione pharmacokinetics and (**b**) fluindione pharmacodynamics. The grey shaded area represents the 90% prediction interval. **INR** = international normalized ratio.

Table III. Summary of the population pharmacokinetic–pharmacodynamic parameters of *S*-acenocoumarol

Parameter	Estimate	RSE (%)
Population parameters		
Lag time	0.269	17
k_a (h^{-1})	0.936	14
CL (L/h) = θ_3 • [1 – θ_4 if *CYP2C9*3*]		
θ_3	15.3	9
θ_4	–0.3	41
V_1 (L) = θ_5 • [BW/69]$^{\theta 6}$		
θ_5	24.7	14
θ_6	1 FIX	
Q (L/h)	4.85	13
V_2 (L)	52.5	24
C_{50} (mg/L) = θ_9 • [1 – θ_{10} if *VKORC1* CC or CT]		
θ_9	0.0023	25
θ_{10}	1.29	47
k_{in} (h^{-1})	0.0269	12
k_{out} (h^{-1})	0.0268	12
γ	1.2	10
Interindividual variability parameters		
$\omega_{lag\ time}$	0.22	59
ω_{ka}	0.37	34
ω_{CL}	0.24	43
ω_{V1}	0.53	23
ω_{C50}	0.6	21
Residual error parameters		
$\sigma_{concentration}$	0.415	7
σ_{INR}	0.08	7

γ = shape parameter of the hyperbolic function; θ = parameter of interest; σ = standard deviation of a residual parameter; ω = standard deviation of an interindividual parameter; **BW** = body weight; C_{50} = concentration that reduces the 1/INR by 50%; **CL** = apparent total clearance; ***CYP*** = cytochrome P450; **INR** = international normalized ratio; k_a = absorption rate constant; k_{in} = zero-order rate constant for production of the response; k_{out} = first-order rate constant for loss of the response; **Q** = inter-compartmental clearance; **RSE** = relative standard error; V_1 = central volume of distribution; V_2 = peripheral volume of distribution; ***VKORC1*** = vitamin K epoxide reductase complex, subunit 1.

The only covariate identified as contributing to interindividual variability in the pharmacodynamic parameters was the *VKORC1* genotype on C_{50}, which decreased interindividual variability from 140% to 108%. The residual variability was best described using an additive error model. The final parameters are summarized in table III.

The goodness-of-fit plots for the basic and covariate models of acenocoumarol are presented in figure S-2 in the SDC, and show no apparent bias in model predictions. The final model was evaluated by a VPC (1000 replicates). The results of the VPC showed that 93% of the observed pharmacokinetic data and 91% of the observed pharmacodynamic data were included in the 90% envelope of the simulated values, and demonstrate the good predictive properties of the model (figure 2).

Simulation of the INR Response during the Induction Phase

For fluindione, the simulation of the INR response following administration of the same daily dose to a set of typical individuals is illustrated in figure 3. Figure 3a illustrates the

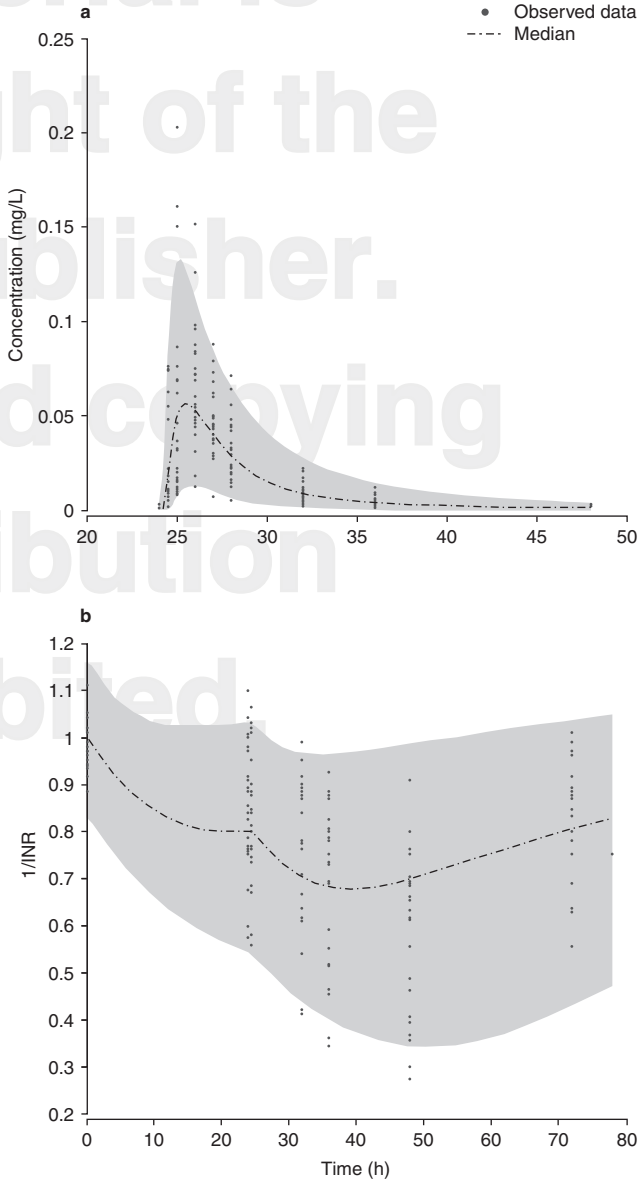

Fig. 2. Visual predictive check for (**a**) acenocoumarol pharmacokinetics and (**b**) acenocoumarol pharmacodynamics. The grey shaded area represents the 90% prediction interval. **INR** = international normalized ratio.

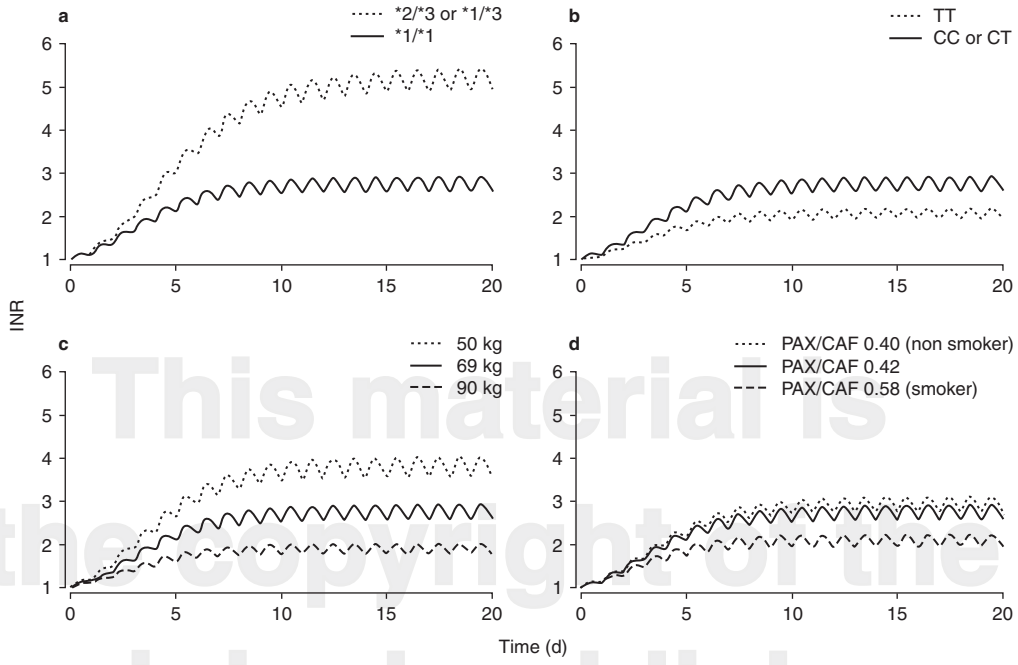

Fig. 3. Predicted INR response during the induction phase following administration of fluindione at the same dose in typical individuals with different combinations of (**a**) *CYP2C9* genotype; (**b**) *VKORC1* genotype; (**c**) bodyweight; and (**d**) CYP1A2 phenotype and smoking status. ***CYP*** = cytochrome P450; **INR** = international normalized ratio; **PAX/CAF** = paraxanthine/caffeine ratio; ***VKORC1*** = vitamin K epoxide reductase complex, subunit 1.

effect of the *CYP2C9* genotype alone and figure 3b shows the effect of the *VKORC1* genotype alone. Figure 3c illustrates the body weight effect and figure 3d shows the CYP1A2 phenotype effect. From these figures, we describe two major predictors of the fluindione dose requirement in an individual patient: *CYP2C9**3 and body weight.

For acenocoumarol, figure 4a illustrates the predicted INR response during the induction phase following administration of the same daily dose to a set of typical individuals and the effect of the *CYP2C9* genotype alone. Figure 4b and figure 4c illustrate the effect of *VKORC1* and body weight alone, respectively, and figure 4d shows the combined effect of *CYP2C9* and *VKORC1* genotypes. From these figures, the most important predictor of the acenocoumarol dose requirement in an individual patient is the *CYP2C9**3 genotype, whereas body weight seems to have a rather modest effect.

The impact of different covariates on the simulation of the higher INR at steady state (INR_{max}) is compared in table IV. For each drug, the higher INR depended on the covariates *CYP2C9**1/*3 or *2/*3 and *VKORC1* TT, but only fluindione was influenced by the smoking status.

Discussion

Traditionally, pharmacokinetic–pharmacodynamic studies of anticoagulant drugs have focused mainly on warfarin, whereas fluindione and acenocoumarol are currently the most commonly used anticoagulants in France. Most of the algorithms currently available with genetic and clinical parameters were built for warfarin dosing.[21-23] Until now, the pharmacokinetic–pharmacodynamic model was less frequently used. Concerning coumarins, only one model has evaluated the impact of age, the *CYP2C9* genotype and the *VKORC1* genotype on the individualization of warfarin therapy.[46] The other model was about acenocoumarol by our group, but did not assess the pharmacogenetic effect.[47]

This study was planned and performed to evaluate the effect of *CYP2C9* genetic polymorphisms on fluindione and acenocoumarol pharmacokinetics and pharmacodynamics during the induction of treatment.

The concentrations of fluindione and acenocoumarol were adequately described by an oral, two-compartment model with a first-order input model, generated using complete pharmacokinetic data. A previous study of acenocoumarol with single-dose administration described the same model.[47] This is the first time that a fluindione pharmacokinetic model has been described after two doses in healthy subjects and with a complete pharmacokinetic profile over 48 hours. In a previous study, Mentré et al.[25] were the first to describe a pharmacokinetic model of fluindione, with a one-compartment model using trough concentrations after six doses.

This is the first exploratory study specifically focussing on the induction period and using an indirect response model of

acenocoumarol and fluindione in healthy subjects. To date, only a few studies have analysed fluindione pharmacokinetics and pharmacodynamics.[25,48,49] Mentré et al.[25] were the first to publish this model, estimating the pharmacokinetic–pharmacodynamic parameters of multiple fluindione doses, based on sparse individual data in a group of 49 patients. Our study was based on a complete individual dataset for pharmacokinetics and pharmacodynamics measured over 48 hours after the last intake.

A significant proportion of the observed variability in pharmacokinetics is caused by variability in acenocoumarol and fluindione CL. The *CYP2C9* genetic polymorphism had a significant effect on *S*-acenocoumarol CL in our analysis. Our results are in concordance with those of previous studies showing that the *CYP2C9* genotype influences the pharmacokinetics and pharmacodynamics of acenocoumarol.[50,51] Interestingly, for fluindione, three covariates were observed: body weight, *CYP2C9* genotype and CYP1A2 phenotype. We have shown that hepatic metabolism of fluindione appears to be mediated by CYP2C9 and probably CYP1A2, which could have a major influence on fluindione pharmacokinetics and pharmacodynamics. Previous studies have shown that smoking induces CYP1A2 via the aryl hydrocarbon receptor.[28] Kalow and Tang[28] showed a dose–response relationship between smoking exposure and enzyme induction in the liver. Furthermore, our group demonstrated that the paraxanthine/caffeine ratio was significantly higher in young smokers than in young non-smokers (0.78 ± 0.19 vs 0.41 ± 0.19; $p = 0.004$).[29] Our results suggest that in heavy smokers, CYP1A2 activity could influence the dose response, but further study is needed.

Moreover, this is the first study to take into account genetic polymorphisms of *CYP2C9* and *VKORC1* in a pharmacodynamic–pharmacokinetic population model. The influence of acenocoumarol therapy on pharmacodynamics and pharmacokinetics was included in the model in the form of the *S*-acenocoumarol concentration only, in accordance with a previous study showing that *S*-acenocoumarol is mainly metabolized by CYP2C9 and is the major active molecule.[36,51] Only three covariates (*CYP2C9* genotype, *VKORC1* genotype and body weight) were identified as important predictors of the *S*-acenocoumarol response in the pharmacokinetic–pharmacodynamic model, whereas four covariates (*CYP2C9* genotype, *VKORC1* genotype, CYP1A2 phenotype and body weight) were identified as predictors of the fluindione response. However, as shown in figures 3 and 4, when we compared the predicted INR response during the induction phase following administration of fluindione and acenocoumarol to typical individuals, we observed that the impact of a *CYP2C9* genetic polymorphism was stronger for fluindione than for acenocoumarol. Moreover, when we compared the simulations using the

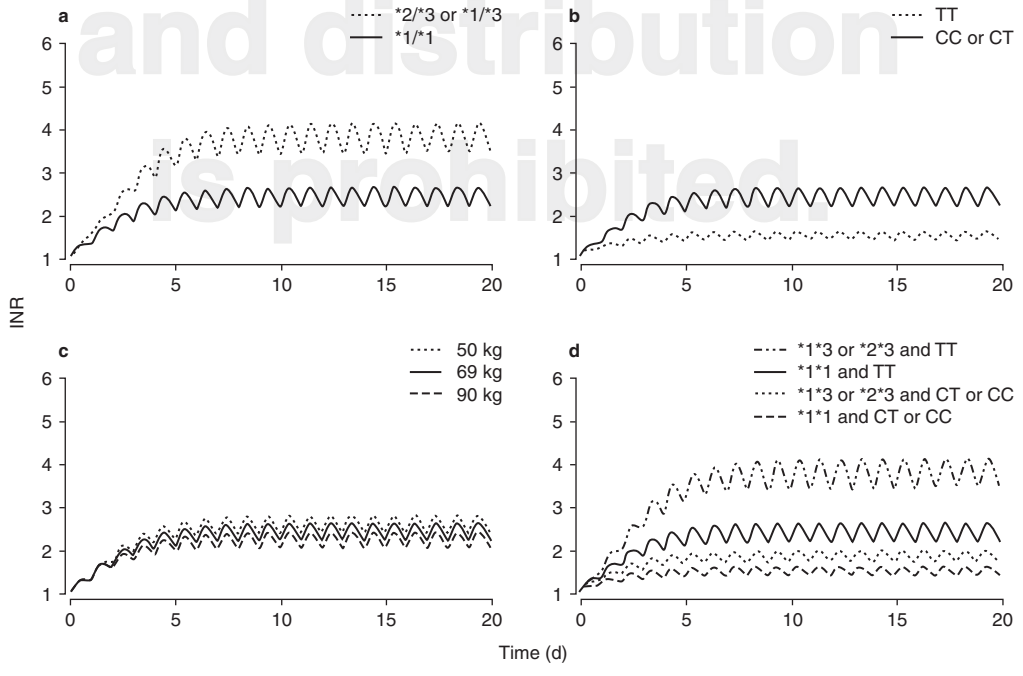

Fig. 4. Predicted INR response during the induction phase following administration of acenocoumarol at the same dose in typical individuals with different combinations of (**a**) *CYP2C9* genotype; (**b**) *VKORC1* genotype; (**c**) bodyweight; and (**d**) *CYP2C9* and *VKORC1* genotype. ***CYP*** = cytochrome P450; **INR** = international normalized ratio; ***VKORC1*** = vitamin K epoxide reductase complex, subunit 1.

Table IV. Impact of covariates on the INR_{max}

Covariates	INR_{max}	
	Fluindione	Acenocoumarol
*CYP2C9**1*1 Body weight 69 kg PAX/CAF 0.42 *VKORC1* TT	2.9	2.6
*CYP2C9**1*3 and *VKORC1* TT	2.2	1.6
*CYP2C9**1*3 or *2*3 and *VKORC1* TT	5.4	4.1
*CYP2C9**1*3 or *2*3 and *VKORC1* CT or CC	3.7	1.9
PAX/CAF		
In smokers	2.2	2.6
In non-smokers	3.1	2.6
Body weight		
50 kg	4.0	2.8
90 kg	2.0	2.4

CYP = cytochrome P450; **INR_{max}** = international normalized ratio at steady state; **PAX/CAF** = paraxanthine/caffeine ratio; ***VKORC1*** = vitamin K epoxide reductase complex, subunit 1.

higher INR at steady state, the impact of the combination of *CYP2C9**1/*3 or *2/*3 and the *VKORC1* TT genotype remained important for the two molecules. Our results describe for the first time the influence of the *VKORC1* genetic polymorphism on the fluindione pharmacodynamic response during the induction of therapy, confirming the strong predictive value of the *VKORC1* genotype for all oral anticoagulants. This result is also supported by our previous findings in which we demonstrated the influence of the *VKORC1* genotype on the acenocoumarol pharmacodynamic response in healthy subjects after a single dose.[17] Moreover, our findings support the assessment of patient genotypes for *CYP2C9* and *VKORC1* genetic polymorphisms preceding the initiation of oral anticoagulant therapy with fluindione.

The model also suggests differences in the shape of the response curve for subjects with different sets of covariates during the induction of treatment. In accordance with our previous results, we confirmed in this study that *CYP2C9* and *VKORC1* genotypes are important predictors of the acenocoumarol response in the pharmacokinetic–pharmacodynamic model. Moreover, recent papers[15] have identified the *VKORC1* genotype as the single most important factor, explaining up to 33% of the overall variability in warfarin maintenance doses within a population. The results of our study do not contradict this, since acenocoumarol is a coumarin drug, but the data still suggest that the single most important predictor in an individual subject is the *CYP2C9* genotype, with differences in the INR response of approximately 1.6- and 1.9-fold between typical individuals with the same *VKORC1* TT genotype, for acenocoumarol and fluindione, respectively.

Some limitations of our study have to be considered. The major study end point was the INR_{T48h}, after two doses of acenocoumarol or fluindione, in agreement with standard clinical practice at the induction of oral anticoagulant treatment. The pharmacokinetics of fluindione were evaluated only over 48 hours after drug intake, though our results are in accordance with those of previous pharmacokinetics studies.[6,25,52] However, it is important to outline that the young age of subjects in our study and the two administered doses do not represent the usual framework for the use of oral anticoagulants. We cannot exclude that at steady state, *CYP2C9* genetic polymorphisms could have a different effect on the fluindione pharmacokinetic–pharmacodynamic response, particularly in elderly patients with hepatic and renal impairment.

Conclusion

The results of this study provide new insights into the contribution of pharmacogenetic factors to the variability in fluindione and acenocoumarol pharmacokinetic–pharmacodynamic responses. We have shown effects of *CYP2C9* and *VKORC1* genetic polymorphisms on fluindione and acenocoumarol pharmacokinetics and pharmacodynamics, whereas the CYP1A2 phenotype and smoking status appeared to influence only the fluindione response. Genotyping prior to fluindione initiation may hold promise for detecting patients at high risk of fluindione- and acenocoumarol-related over-anticoagulation and bleeding. However, future clinical studies are needed to confirm the effects of CYP2C9, CYP1A2 and VKORC1 activity on fluindione pharmacodynamics and pharmacokinetics at steady state in patients.

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