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REVIEW ARTICLE

Evidence for a pharmacogenetic adapted dose of oral anticoagulant in routine medical practice

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Abstract Oral anticoagulants (OA) are a leading cause of fatal haemorrhagic adverse events in relation with an important interindividual variability of response to these drugs. Besides several clinical factors, this interindividual variability of response to OA has a pharmacogenetic basis. Carriers of cytochrome P450 2C9 (*CYP2C9*)-deficient alleles have a reduced clearance of warfarin and are exposed to dramatic overdoses in the first weeks of treatment. Genetic polymorphisms of vitamin K epoxide reductase (*VKORC1*), the target of OA, identify patients with a high sensitivity to OA who are at risk of early overdose. Most pharmacogenetic evidence is presently restricted to warfarin. Several warfarin dosing algorithms have been constructed, adapted on *CYP2C9* and *VKORC1* genotypes and clinical factors, to predict the best dose for each patient. Carriers of one of allelic variant need a 20–30% reduction of warfarin dose. However, definite evidence concerning the usefulness of these algorithms in terms of reducing the frequency of major bleeding episodes is still lacking. Ongoing prospective randomised trials will ascertain definitive answer over the coming years.

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Introduction

Oral anticoagulants (OA) have proven to be highly effective antithrombotic drugs for the treatment or prevention of deep venous thrombosis, pulmonary embolism, stroke and myocardial infarction for more than 50 years. Besides these well-demonstrated clinical benefits, OA are associated with a high risk of major bleeding, which unfortunately limits their use, particularly in the elderly [1]. In France, OA are associated with 17,000 hospitalisations for major bleeding annually. In the United States, more than 20 million patients are treated with OAs and 29,000 visits for bleeding complications are observed each year. Warfarin (WA) represents the first cause of lethal adverse drug reaction in the United States [2]. Several risk factors of bleeding have been identified, such as age, female gender, drug interactions, and previous bleeding [2]. Besides these classical risk factors, pharmacogenetic risk factors have been identified in the past decade and were introduced in the summarised product characteristics of WA by the US Food and Drug Administration (FDA) in 2007, indicating that lower initiation doses should be considered for patients carrying allelic variants of *CYP2C9* and vitamin K epoxide reductase (*VKORC1*) [3]. Additional genetic variants of other genes have been identified (*CYP4F2*, *GGCX*, *APOE*, *PROC* and clotting factors [4–7]), but they play a minor role in OA pharmacodynamics and are out of the scope of this review considering practical applications of pharmacogenetics tests.

Table 1 Pharmacokinetic profile of vitamin K antagonists: partly based on Ufer et al. [10]

	Mean daily dose	Excretion as unchanged drug	CYP2C9 metabolism	Influence of *CYP2C9* allelic variants	Phase 2 enzymes	Half life
Warfarin (enantiomeric)	6 mg	<5%	Intensive	*CYP2C9*2* *CYP2C9*3*	No	S-W=30 h R-W=40 h
Phenprocoumon (enantiomeric)	2–3 mg	40%	Medium	*CYP2C9*3*	Yes	S-P=120 h R-P=120 h
Acenocoumarol (enantiomeric)	4 mg	1%	Intensive	*CYP2C9*3*	No	S-AC=2 h R-AC=7 h
Fluindione	15 mg	Below 10%	Medium	(*CYP2C9*3* ?)	No	31 h

S-W S-warfarin, *R-W* R-warfarin, *S-P* S-phenprocoumon, *R-P* R- phenprocoumon, *S-AC* S-acenocoumarol, *R-AC* R-acenocoumarol

Different oral anticoagulant drugs

WA is available all over the world and represents the most prescribed OA in North America, Asia, and England, whereas acenocoumarol (AC), phenprocoumon, and fluindione are more commonly used in several European countries [8, 9]. WA, AC and phenprocoumon are administered as racemate, and their S-enantiomers retain the highest anticoagulant activity. There are some significant differences in the pharmacokinetic profile of the different OA [10], which can be classified as short- or long-acting drugs based on their terminal half-life (Table 1). They all act by decreasing vitamin-K-dependant clotting factors (II, VII, IX and X) via the inhibition of vitamin K epoxide reductase (VKOR), which recycles reduced vitamin K (inactive) into active oxidised vitamin K (Fig. 1). All OA have a similar tolerance profile, except for fluindione, an indanedione derivative, which is associated to more frequent immunoallergic adverse drug reaction such as hepatitis and nephritis [11].

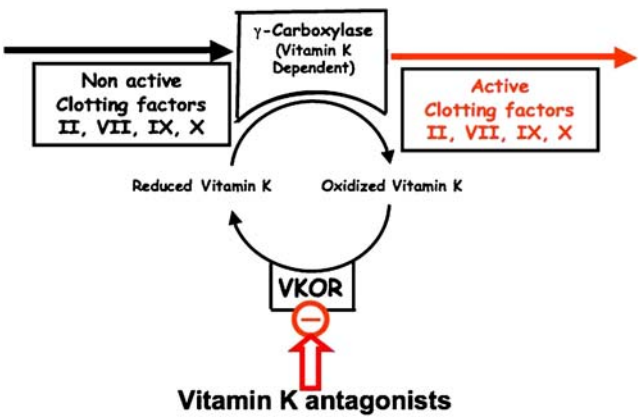

Fig. 1 Mechanism of action of vitamin-K antagonists. *VKOR* vitamin K epoxide reductase

CYP2C9 and oral anticoagulants metabolism

Cytochrome P4502C9 (CYP2C9) represents quantitatively the second CYP isoform expressed in the liver [12] and is involved in the metabolic clearance of different drugs such as oral hypoglycemic agents, nonsteroidal anti-inflammatory drugs, angiotensin II blockers, anticonvulsivants and antidepressant drugs [13]. Its role in the metabolism of WA was highlighted at the end of last century [14–16].

In vitro data concerning CYP2C9-dependant metabolism of oral anticoagulants

CYP2C9 metabolises S-warfarin, the most active enantiomer, whereas CYP3A4, CYP2C19 and CYP1A2 are involved in R-warfarin metabolism [17–19]. Concerning acenocoumarol, CYP2C9 seems to be the most potent isoform involved in the metabolism of both enantiomers [20, 21]. Acenocoumarol CYP2C9-dependant intrinsic clearance is far higher compared with WA, which explains its shorter half-life in humans. The role of CYP2C9 in phenprocoumon metabolic clearance seems to be less pronounced compared with the two previous OA, with a similar involvement of CYP3A4 and CYP2C9 [10, 22]. Furtheremore, phenprocoumon is also extensively conjugated, implicating phase-2 enzymes in its metabolic clearance. No data are available for the metabolism of fluindione, exept that it is mainly cleared as hydroxylated and conjugated metabolites that have never been identified.

Two main functional *CYP2C9*-deficient allelic variants were identified in the early 1990s: *CYP2C9*2* (Arg $_{144}$ into Cys) and *CYP2C9*3* (Ile $_{359}$ to Leu) [23]. Both polymorphisms were rapidly shown to be associated with decreased S-warfarin hepatic clearance and, as a consequence, to need lower steady-state daily doses to achieve a usual therapeutic target [International Normalised Ratio (INR) 2–3] [14, 16, 24]. The influence of *CYP2C9* genetic

polymorphism has also been observed for AC [25] and phenprocoumon, whereas no data are available for fluindione. Compared with WA, the influence of the *CYP2C9*-deficient allele *CYP2C9*2* seems absent [26, 27] or less important for acenoucoumarol [10]. Furthermore, the contribution of CYP2C9-dependant clearance to the overall oral clearance of phenprocoumon seems to be lower than that observed with WA [10, 28]. The importance of *CYP2C9* genetic polymorphism is probably higher among patients of Caucasian origin, in whom the frequencies of the *CYP2C9*-deficient alleles is almost the double compared with other ethnic groups. Indeed, the allelic frequencies of *CYP2C9*2* and *CYP2C9*3* are 0.1 and 0.08, 0.04 and 0.02 and 0.01 and 0.04 among subjects of Caucasian, African and Asiatic origins [29–31]. The *CYP2C9*-dependant pharmacogenetic effect observed for OA has three major clinical consequences, which are described below.

CYP2C9 and daily dose requirements

In 1999, Aithal et al. described for the first time that patients carrying the *CYP2C9*2* and/or *CYP2C9*3* allele needed much lower doses of WA at steady state [24] compared with noncarriers. These results were further confirmed by many different independent groups [32, 33]. A similar relationship between CYP2C9 genetic polymorphism and AC daily dose requirement was observed [26, 34, 35]. Concerning phenprocoumon, slightly lower dose requirements were described for carriers of *CYP2C9*-deficient alleles [36, 37], but this was not observed by all authors [38], probably in relation with the lower impact of CYP2C9-dependant metabolism of phenprocoumon compared with WA and acenocoumarol.

CYP2C9 and time to reach stable anticoagulation

Aithal et al. were the first to detect that patients treated with WA and carrying one *CYP2C9*-deficient allele had more difficulties reaching stable anticoagulation [24]. This observation was also confirmed by other groups [33] and with the other related OA, AC [34, 39] and phenprocoumon [36].

CYP2C9 and risk of bleeding

As a consequence of this difficulty finding the right dose and to achieve stable anticoagulation, it has been shown that carriers of *CYP2C9* allelic variants have a 3.68 increased risk of major bleeding with WA [24]. The CYP2C9-dependant pharmacogenetic risk of bleeding is maximal during the initiation phase of the anticoagulant treatment. Increased risk of major haemorrhage with WA conferred by *CYP2C9* allelic variants has been estimated to be 5.3 before stabilisation of therapy and 2.2 after stabilisation [32, 33]. A similar increased number of overanticoagulation episodes (INR>6) and bleeding events has been described for carriers of *CYP2C9* variants treated with AC [39, 40]. More conflicting data exist concerning the CYP2C9-dependant risk of bleeding with phenprocoumon [36, 40, 41], which is probably smaller than that observed with WA and AC due to a smaller involvement of CYP2C9 in phenprocoumon oral clearance [28].

VKORC, the target of oral anticoagulants

The gene that encodes VKOR (*VKORC1*), the target enzyme for OA (Fig. 1), was identified in 2004 [42, 43]. Rost et al. identified the *VKORC1* gene from four independent families whose members were considered resistant to WA. One patient needed more than 17 mg/day of WA to achieve adequate anticoagulation, two others about 40 mg/d and the fourth did not respond to any dose of WA. They carried rare mutations of *VKORC1* of amino acids 29 (Val/Leu), 45 (Val/Ala), 58 (Arg/Gly) and 128 (Leu/Arg) [42]. Several months later, other groups described that common *VKORC1* single nucleotide polymorphisms (SNPs) were strongly associated with OA sensitivity [44–48]. *VKORC1* genetic polymorphism has the same influence for all OA [37, 45, 49].

Several SNPs in complete linkage disequilibrium identify a haplotype combination called A [32, 48], which is associated with OA hypersensitivity. Two of these SNPs (−1639G>A, rs9934438 or 1173C>T, rs17878363) are usually used to identify (tag) this *VKORC1* A haplotype group, which is associated with lower levels of hepatic VKORC expression [48]. Therefore, patients carrying the *VKORC1* A haplotype have lower levels of hepatic VKOR and as a consequence are more sensible to OA. The allelic frequency of this *VKORC1* A haplotype is rather low among subjects from African origin (0.15), high in Caucasians (0.42) and extremely frequent among Asians [32, 45, 47, 50]. As observed for *CYP2C9,* VKORC1 genetic polymorphism has three major clinical consequences on the pharmacodynamics of OA, which are described below.

VKORC1 and daily dose requirements

Since *VKORC1* genetic polymorphism modulates WA sensitivity, it also influences the dose of WA at steady state to reach stable anticoagulation [48, 51]. Swartz et al. observed a clear gene–dose effect with *VKORC1* A associated with a higher INR response, requiring lower doses of WA [51]. From a large cohort of patients treated with WA, it has been estimated that the dose should be reduced by 28% for each *VKORC1* A allele [52]. Similar

Table 2 Consequences of *CYP2C9* and *VKORC1* polymorphisms on OAs pharmacokinetics and pharmacodynamics

	CYP2C9				*VKORC1*			
	PK	Daily dose	Time to stable INR	Overdose and bleeding risk	PK	Daily dose	Time to stable INR	Overdose and bleeding risk
Warfarin	Yes (for S-warfarin) [64, 65]	Yes [14, 24, 33, 51, 57, 64, 66]	Yes [33, 59, 60, 67]	Yes [24, 33]	No	Yes [44, 51, 68]	Yes [32, 69, 70]	Yes [32]
Phenprocoumon	Yes (Minor) [10, 28]	Yes (minor) [36, 37, 40, 41]	Yes (minor) [36, 41, 37]	Yes (minor) [36, 37, 40]	No	Yes [37, 53]	No data	Yes [37, 53]
Acenocoumarol	Yes [25, 27, 71]	Yes [26, 34, 35, 39, 54]	Yes [34, 39]	Yes [38–40]	No	Yes [53, 54, 72]	Yes [39]	Yes [54, 55]
Fluindione	No data	No data	No data	Inconclusive [73]	No	No data	No data	Yes [55]

INR International Normalised Ratio

findings have been obtained for other OA, such as AC [53, 54] and phenprocoumon [37, 53]. *VKORC1* genetic polymorphism also mainly explains why steady-state doses of OA differ between patients of different ethnic origins: *VKORC1* A (linked to OA sensitivity) is rare among blacks, who need higher doses of OA; the same A haplotype is present in 90% of patients of Asian origin, who need the smallest doses to achieve stable anticoagulation.

VKORC1 and time to reach stable anticoagulation

WA-treated patients homozygous for the *VKORC1* A/A haplotype (sensitive to OA) achieve their first INR in the target range 2.4 sooner compared with others and reach an INR above 4, 2.5 sooner [51]. They also spend twice as much time above the INR upper limit of 4 compared with other patients [51] and are thus more difficult to equilibrate.

VKORC1 and risk of bleeding

As a consequence of this difficulty to obtain of stable INR and a stable dose, *VKORC1* genetic polymorphism has been rapidly recognised as an important risk for bleeding in patients treated with OA. This higher frequency of WA overdose among *VKORC1* A/A patients was mainly observed during the first month following the introduction of the OA [51]. A similar increased risk of bleeding linked to *VKORC1* genetic polymorphism has been observed with phenprocoumon [37, 53] and AC [54, 55].

Pharmacogenetic-based dosing algorithms

Different algorithms have been developed to determine the right dose for the right patient [56]. The first algorithms, which were employed for a long time, were based on clinical variables such as INR target, patient age, history of previous bleeding, reason for prescription (atrial fibrillation or venous thrombosis), associated diseases and concomitant drugs prescribed. As it has been estimated that *CYP2C9* and *VKORC1* genetic polymorphisms explain 35–50% of the interindividual variability of OA response during the initiation phase of the treatment, dosing algorithms rapidly integrated genotype information. One of the first of these pharmacogenetic-based dosing normograms including *VKORC1* and *CYP2C9* genotypes was proposed by Sconce

Table 3 Predicted steady-state warfarin daily dose requirements based on pharmacogenetic-based algorithms

	VKORC1 non-A/non-A	*VKORC1* non-A/ A	*VKORC1* A/ A	*VKORC1* non-A/non-A	*VKORC1* non-A/non A
	*CYP2C9 *1/*1*	*CYP2C9 *1/*1*	*CYP2C9 *1/*1*	*CYP2C9 *1/*3*	*CYP2C9 *3/*3*
Sconce et al. [57]	5 mg/d	4 mg/d	3 mg/d	3 mg/d	2 mg/d
Gage et al. [52]	6 mg/d	4 mg/d	3 mg/d	4 mg/d	3 mg/d

Additional information introduced in the algorithm of Sconce et al.: age 70 years, height 1.7 m. Additional information introduced in the algorithm of Gage et al.: age 70 years, height 1.7 meters, weight 75 kg, target INR 2.5, white origin, nonstatin and no concomitant amiodarone, nonsmoker, male gender

VKORC1 vitamin K epoxide reductase

et al. [57], which allowed in a replication cohort to explain about 80% of the interindividual variability of the WA dose needed to achieve an INR between 2 and 3. Different other pharmacogenetic-based normograms have been developed [58] for Caucasian and non-Caucasian ethnic groups [59]. At the time, one of the best validated dosing algorithm was proposed by Gage et al. [52] and includes not only *CYPC2C9* and *VKORC1* genetic data but also numerous clinically relevant items such as associated drugs, smoking status and ethnic origin. It explains 53–54% of the variability in the WA dose and was prospectively validated in a replication cohort. It was obtained from one of the largest collaborative cohorts (1,015 patients) and was made available online at www.warfarindosing.org. Tables 2 and 3 compare the dose of WA that should be introduced in a 70-year-old white nonsmoking man without concomitant drugs (statins or amiodarone) of 75 kg and 1.70 m tall based on two different dosing normograms. Both algorithms predict relatively similar doses. However, Gage et al.'s algorithm allows the dose to be adapted to much more relevant clinical factors. Pharmacogenetic-based algorithms for OA other than WA are missing at this time.

Pharmacogenetic prospective randomised clinical trials

Unfortunately, only three. small-scale prospective randomised trials were performed to determine whether an initial pharmacogenetic-adapted dose could improve the time to reach stable anticoagulation and the frequency of bleeding episodes during initiation of OA therapy [60–62]. The first study, which included only 38 patients, was just a pilot trial testing the feasibility of *CYP2C9* genotyping before introducing WA; no differences could be observed between the *CYP2C9*-adapted and nonadapted patients [62]. The second study was aimed to decrease by 50% the time spent out of the INR range during the initiation of WA treatment [60]. The authors used a WA loading dose during the first 2 days of treatment and randomised the patients in two arms: one according to a standard clinical titration (99) and the other based on a *CYP2C9*- and *VKORC1*- adapted algorithm (101). The authors could not confirm their primary hypothesis in relation to a lower frequency of the clinical event compared with what was expected in the control group and a too ambitious planned difference between both groups (underpowered study for the main outcome). However, they could detect that patients in the pharmacogenetic-guided arm needed less INR controls and had less dose modifications. No differences could be observed concerning the bleeding events. The last trial was planned to reduce the time to reach the first therapeutic INR range and the time to reach stable anticoagulation [61]. Patients were randomised in two arms: one with a standardised dose of WA (142), and the other (141) with a pharmacogenetic-adapted dose of WA, which included only *CYPC9* genotype in its normogram. Both study endpoints were reached sooner (2.23 days and 18.1 days, respectively) in the pharmacogenetic-adapted arm. Furthermore, in the pharmacogenetic-adapted arm, patients spent more time within the INR therapeutic range (80.4% vs 63.4%) and experienced less minor bleeding (3.2% vs 12.5%).

One might be surprised by the low number of randomised trials in this field despite strong scientific evidence indicating that *CYP2C9* and *VKORC1* explain about 50% of the interindividual variability of WA response. Unfortunately, this is a common problem in the field of pharmacogenetics, which lacks randomised trials. One reason is the recent discovery in 2005 of the influence of *VKORC1* polymorphisms on OA response. Several randomised trials, some including a larger number of patients, are ongoing and are available at www.clinicaltrials.gov. The results of these trials will be available in the coming years and will hopefully definitively demonstrate the usefulness of a pharmacogenetically adapted dose of WA. The next explanation for so few randomised trials in the field is that the ultimate objective of such a study is to reduce the frequency of major bleeding episodes (the most clinically relevant endpoint), which occur in clinical trials in 1% of the study population per year [63]. Such an event frequency implicates inclusion of at least 1,000 patients. Another difficulty in performing such randomised trials is the need for rapid pharmacogenetic tests, the results of which must be sent to the physician within a few hours. Such rapid genotyping is for the moment restricted to several university hospitals, but this situation should change rapidly with the development of validated diagnostic kits, some of which have been approved by the FDA.

Future prospective randomised trials will also bring extremely important information concerning the frequency of recurrent thrombotic events. All pharmacogenetic efforts have focused on reducing OA overdose and the risk of bleeding. But difficulties attaining stable anticoagulation also includes INR episodes below 2 and possibly increased frequency of thrombotic recurrence. Furthermore, a pharmacogenetically adapted dose might limit the bleeding episodes but at the same time increase the early recurrence of thrombotic events, which might totally blunt the benefit of the pharmacogenetically adapted dosing. Therefore, attention must be paid to precisely evaluate the real risk/benefit ratio of a pharmacogenetically adapted dosing.

No randomised trials have been performed with OA other than WA. A prospective randomised trial is planned in Europe to compare the utility of a pharmacogenetic-based prescribing between WA, AC and phenprocoumon.

Conclusion

A large number of studies have demonstrated that the genetic polymorphisms of *CYP2C9* and *VKORC1* significantly influence OA pharmacokinetics and pharmacodynamics. However, most of the evidence is restricted to WA, and even for this drug, a definite demonstration of the utility of systematic genotyping to adapt its dose is still lacking. The benefit of such pharmacogenetic testing remains to be validated in terms of costs with a pharmacoeconomic approach.

The definitive answer concerning the usefulness of the pharmacogenetically based WA dosing will be available in the coming years. This process will probably take some time for the OA used in Europe. Meanwhile, physicians can benefit from the www.warfarindosing.org normogram, which has the advantage of including the first INR values and a delayed genotype.

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