How polymorphisms of the cytochrome P450 genes affect ibuprofen and diclofenac metabolism and toxicity

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Interindividual variability in drug metabolism is an important cause of adverse drug reactions and variability in drug efficiency. Polymorphisms of cytochrome P450 (CYPs) genes have a significant effect on drug metabolism and toxicity. This review brings an update about how genetic polymorphisms of CYP2C8 and CYP2C9 enzymes affect the disposition and clinical outcomes of ibuprofen and diclofenac, two of the most common pain relievers. The most common side effects associated with the influence of CYP2C8*3 and CYP2C9*2*3 variants on ibuprofen and diclofenac pharmacokinetics are hepatotoxicity and gastrointestinal bleeding. CYP genotyping may therefore identify patients at increased risk of these adverse reactions, and these patients could have their doses adjusted or start receiving another NSAID that does not share the same metabolic pathways with ibuprofen or diclofenac. However, before genotyping is introduced into regular clinical practice, more research is needed to evaluate the effectiveness of this strategy in improving treatment with ibuprofen and diclofenac.

KEY WORDS: adverse effects; allelic variants; CYP2C8; CYP2C9; drug metabolism; gastrointestinal bleeding; genotyping; hepatotoxicity; pharmacogenetics; pharmacogenomics; pharmacokinetics

Since its completion, the Human Genome Project has given a strong boost to pharmacogenetic and pharmacogenomic research of variability in drug effects based on individual genetic make-up (1). Genetic factors affect the pharmacokinetic profile of drugs and change their efficacy and toxicity properties (2). The final goal of pharmacogenomics is to make use of genetic testing to optimise pharmacotherapy and adjust it to individual needs (3).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications in developed countries. According to the American Gastroenterological Association, more than 30 million Americans use NSAIDs every day (data available at http://www.webmd.com/arthritis/features/making-decision-on-nsaids).

NSAIDs inhibit the ability of cyclooxygenase (COX) to produce prostaglandins from arachidonic acid, which in turn suppresses inflammation in most patients (4, 5). NSAIDs are prescribed as analgesics, anti-inflammatory, and antipyretic agents for a number of indications. Ibuprofen and diclofenac are often prescribed as NSAIDs of choice in managing rheumatoid arthritis, post-operative pain, and chronic pain associated with cancer. Both are metabolised by polymorphic phase I metabolic enzymes, predominantly cytochromes P450 (CYPs) and by phase II UDP-glucuronosyltransferases (6). Phase III polymorphic drug transporters have also been evidenced to modulate their toxicity and efficacy (7, 8).

This review brings an update about the association between genetic polymorphisms of CYP enzymes and individual differences in ibuprofen and diclofenac disposition and clinical outcomes.

Ibuprofen metabolism

Ibuprofen is a racemic mixture of the (S)-(+) and (R)-(−) enantiomers administered through several pharmaceutical formulations. (S)-(+) ibuprofen is the active enantiomer, both in vitro and in vivo (9). Nearly 65% of R-(−)-ibuprofen is inverted to the (S)-(+) enantiomer in the liver and some of it is pre-systemically inverted in the gut in the presence of acylCoA thioester, with alpha-methylacylcoenzyme A racemase (AMACR) acting as the catalyst (9, 10).

(S)-(+) and (R)-(−)-ibuprofen are promptly metabolised by phase I detoxification enzymes in human liver (10). The metabolic pathways of its enantiomers differ significantly.
Diclofenac metabolism

Diclofenac is rapidly and completely absorbed from human intestines after oral administration and is detoxified through hydroxylation and glucuronidation (18). Its oxidation to 4'- and 5'-hydroxylated derivatives is catalysed by CYP2C9 and CYP3A4 (19), whereas glucuronidation is catalysed by UGT2B7 (20-22). The oxidised metabolites are mainly excreted through the kidney (65 % of diclofenac), while the rest (35 %) as acyl glucuronide is excreted through the bile (23, 24). Oral bioavailability of diclofenac drops to 50-60 % of the applied dose as a result of first-pass metabolism and low enterohepatic circulation. After excretion into the intestines, diclofenac acyl glucuronide undergoes hydrolysis by intestinal bacteria enzymes called β-glucuronidases. As a result of its reabsorption, enterohepatic recirculation of diclofenac is high (25). Acyl glucuronides of diclofenac are chemically unstable compounds that can undergo epimerisation through acyl migration to the 2-, 3-, or 4-O-glucuronide, particularly in the alkaline medium of the bile. These isomers are believed to resist deconjugation by β-glucuronidases (26).

Transports may play an important role in the drug’s fate in human organism. Using a mice model, Lagas et al. (27) have shown that multidrug resistance proteins 2 (MRP2/ABCC2) and 3 (MRP3/ABCC3) as well as breast cancer resistance protein (BCRP/ABCG2) have a significant role in the pharmacokinetics of diclofenac glucuronides. In their study, MRP2/ABCC2 and BCRP significantly changed biliary excretion of diclofenac glucuronides, while MRP3/ABCC3 was the main efflux transporter from the liver to blood. Concurrent loss of function of MRP2/ABCC2, MRP3/ABCC3, and BCRP/ABCG2 resulted in significant accumulation of reactive diclofenac glucuronides in the mice liver and acute but mild toxicity.

Acquired or hereditary deficiency of ABCC2, known as Dubin-Johnson syndrome in humans, can cause an increased concentration of bilirubin glucuronides (28). As diclofenac shares the same ABCC2 transporter pathway, it should be prescribed to these individuals with caution to avoid adverse reactions to diclofenac, hepatotoxicity in particular.

Genetic polymorphisms of CYP enzymes

Interindividual variability as a consequence of polymorphisms in CYP genes is highly associated with the level of drug toxicity (29, 30). Among over fifty CYP enzymes, the human CYP2C family accounts for 18-30 % of the total content of CYP450 enzymes in the human liver and is responsible for the metabolism of nearly one fifth of the commonly prescribed drugs such angiotensin-II antagonists, NSAIDs, oral antidiabetics, antiepileptics, oral anticoagulants, psychotropic drugs, and some alkylating antineoplasic drugs (2). In addition, CYP2C9 metabolises endogenous substrates such as arachidonic and linolenic acid. Updated information regarding the list of allelic variants of CYP2C isoforms is available online at http://www.imm.ki.se/CYPalleles/.

CYP2C8

The CYP2C8 subfamily accounts for nearly 35 % of the total human CYP2C-coded enzymes in the liver and has a role in the metabolism of different drugs and endogenous compounds (31). Whereas Agúndez et al. (32) claim that CYP2C8 is directly involved in the oxidative metabolism of NSAIDs such as diclofenac and ibuprofen, Totah et al. (33) suggest that CYP2C8 has only a minor or intermediate contribution in their metabolism.

The CYP2C8 gene is located on chromosome 10q24, spans 31 kb, and shares 74 % of the sequence with the CYP2C9 gene (34). According to a recent report (35), at the locus of the CYP2C8 gene there are 16 allelic variants. These variants are responsible for interindividual and interethnic variability in drug response (36), since their frequencies vary significantly between races and population groups. Clinically the most important variants are CYP2C8*2 to *5 (37, 38). The frequencies of the alleles *2, *3, and *4 are 0.3, 10.9, and 5.9 in Caucasians and 15.9, 0.0, 0.41 in Blacks, respectively, while in the Asians these variants have not been found, or are extremely low (39, 40). Speed et al. (41) reported that 89 % of the CYP2C8*3 carriers are also the carriers of the CYP2C9*2 variant. These authors also suggested that due to a strong linkage disequilibrium between these variants, it would be difficult to distinguish between associations with CYP2C8 or CYP2C9. Although the metabolism of most NSAIDs is associated with CYP2C9, CYP2C8 variants may also define interindividual differences in the pharmacokinetics of some NSAIDs, including ibuprofen and diclofenac (42, 43).
The relationship between the CYP2C8 genotype and the pharmacokinetics and clinical outcomes of ibuprofen and diclofenac therapy

Ibuprofen

A Spanish group of scientists (43) reported results of an investigation performed with 355 healthy participants who received a single dose of 400 mg ibuprofen. Considering that the CYP2C9 genotype could confound the association with the pharmacokinetics of ibuprofen, participants with the low-activity CYP2C9*3*3 genotype were excluded from the study. The clearance of (R)-ibuprofen was 40% and 37.1% lower in the participants carrying the CYP2C8*3*3 and CYP2C8*1*3 alleles, respectively compared to individuals carrying the CYP2C8*1*1 genotype (wild type) (p=0.03). This study showed that the half-life of (R)-ibuprofen was significantly longer in the CYP2C8*3*3 and CYP2C8*1*3 genotype carriers compared to wild-type carriers (9 and 4.2 h, respectively, vs. 2 h, p<0.025). Considering that the CYP2C8*3 variant was associated with the presence of one or two copies of CYP2C9*2 variant in 13 of 16 participants, it is difficult to distinguish the effects of either variant on the R-ibuprofen metabolism. According to Garcia-Martin et al. (44), the clearance of both R- and S-ibuprofen clearance in CYP2C8*3 homozygotes was nearly one ninth of the clearance in the wild-type homozygotes. The CYP2C9*2 variant affected ibuprofen pharmacokinetics only in the participants who were also carriers of the CYP2C8*3 variant. The authors observed only a limited genotype effect on the enantiospecific clearance of ibuprofen.

According to Agúndez et al. (32), carriers of the CYP2C8*3 and CYP2C9*2 or CYP2C9*3 variants manifested an increased risk of gastrointestinal bleeding after administration of different NSAIDs, including ibuprofen and diclofenac, but could not tell whether it was the parent drug or metabolite resulting from alternative metabolic pathways to have caused the bleeding.

There is evidence that NSAIDs lower the risk of colorectal cancer. The Colorectal Cancer Study Group tested the hypothesis that the CYP2C8 and CYP2C9 variants could change the protective effect of NSAIDs against colorectal cancer in 478 patients with colorectal cancer and 733 controls (45). While the use of NSAIDs, including ibuprofen and aspirin, was confirmed as beneficial in reducing the colorectal cancer risk, no variant modified their protective effects.

Diclofenac

Dorado et al. (46) investigated the effects of CYP2C8 polymorphisms on diclofenac metabolism (after a single 50 mg dose) in 142 healthy Spanish volunteers. The participants were genotyped for CYP2C8 and 2C9 variants, and the variants analysed for association with the concentration of diclofenac and its metabolites. CYP2C8*3 and 2C8*4 carriers had a higher urinary concentration ratio of diclofenac / 5-hydroxy-diclofenac compared to wild-type carriers. The authors pointed to the significant overlap between the CYP2C8 and CYP2C9 variant allele carriers and concluded that it was difficult to estimate the separate effects of CYP2C8 polymorphisms on diclofenac metabolism. Having in mind that 65% of diclofenac and its metabolites are excreted through the kidneys and 35% are excreted through the liver (47), other polymorphisms (such as those of some drug transporters like ABCC2) and non-genetic factors (like comedication and comorbidities) may affect the metabolism of diclofenac and its excretion.

Reports of hepatotoxic effects of diclofenac (36, 48, 49) have raised the question whether the gene variants CYP2C8*3, CYP2C9*2, *3 or UGT2B7*2, which code for low-activity enzymes, could worsen these effects. Aithal et al. (50) found no such association with CYP2C9*2 or *3 variants, but Daily et al. (36) did. They investigated the contribution of the CYP2C8 and UGT2B7 gene variants coding for metabolic enzymes (responsible for the formation of reactive diclofenac metabolites) and the contribution of the variant ABCC2-24C>T of the MRP2 drug transporter (responsible for the biliary excretion of the reactive metabolites). Patients who suffered diclofenac-induced hepatotoxicity were more frequent carriers of the gene variants predisposing for low-activity proteins in comparison to the patients who had not developed diclofenac-induced hepatotoxicity. They explained that increased levels of reactive metabolites may result in higher levels of protein-diclofenac adducts and consequently in hepatotoxicity. They also observed that the CYP2C8 variants seemed to contribute less to diclofenac-induced liver injury than the allelic variants of the other two genes.

CYP2C9

Gene coding for the CYP2C9 enzyme is located on the long arm of chromosome 10 in the region that also contains genes for the CYP2C8, 2C18, and 2C19 enzymes (48). The enzyme contains 490 amino acids, weighs 55.6 kDa, and plays a key role in the metabolism of nearly one hundred drugs. So far, 67 allelic variants of the CYP2C9 gene have been identified (51).

The relationship between the CYP2C9 genotype and the pharmacokinetics and clinical outcomes of ibuprofen and diclofenac therapy

Ibuprofen

Several investigations tested if the CYP2C9 *2, *3, and CYP2C8*3 allelic variants could reduce ibuprofen metabolism and/or clearance (40, 42, 52), predisposing the carriers of both allelic variants to a higher risk of adverse drug reaction.
Kirchheiner et al. (52) were among the first to study the kinetics of an oral 600 mg dose of ibuprofen racemate in 21 healthy carriers of all combinations of the CYP2C9 variants *2 and *3. Data were evaluated using a population pharmacokinetic model that integrated pharmacogenetic information. According to their results, only the 2C9*3 variant affected the pharmacokinetics of the racemic and S-ibuprofen. Mean S-ibuprofen clearances were 3.25 L h⁻¹, 3.82 L h⁻¹, and 1.52 L h⁻¹ in the carriers of the CYP2C9 allele combinations *1/*1, *1/*3, and *3/*3, respectively. They did not find any significant effects of the CYP2C9*2 variant. In the next step, the authors tested the association between the CYP2C9 polymorphisms and the formation of thromboxane B₂, which is the stable product of thromboxane A₂ hydrolysis (a potent vasoconstrictor and stimulus of platelet aggregation) reflecting cyclooxygenase type 1 inhibition. The obtained results suggested that more beneficial effects, that is to say, higher inhibition of thromboxane formation was observed in the carriers of the alelic variants CYP2C9 *1/*3, *2/*3, and *3/*3 than in the wild-type carriers (*1/*1). A similar trend was observed for prostaglandin E₂ (important inflammatory mediator), reflecting cyclooxygenase type 2 inhibition. Subjects with prolonged ibuprofen availability (poor CYP2C9-mediated metabolism) may run a higher risk of adverse drug reactions but, on the other hand, they can have better pharmacodynamic responses, which can be clinically relevant.

López-Rodríguez et al. (42) studied the effects of the CYP2C9 variants on the metabolism of both ibuprofen enantiomers in healthy volunteers and found lower metabolism of racemic ibuprofen in the 2C9*3 variant carriers, resulting in significantly higher area under the curve (AUC) and lower clearance than in the 2C9*1 allele carriers (p<0.05). As for S-ibuprofen, the clearance was 45% lower in the 2C9*3 allele carriers, AUC 87% higher, and half-life 47% longer than in the 2C9*1 allele carriers. R-Ibuprofen clearance was also lower in the 2C9*3 variant carriers by 30%. In terms of safety, the CYP2C9*3 carriers had fewer adverse events, which was explained with lower expression of inducible nitric oxide synthase.

García-Martín et al. (44) analysed genetic factors responsible for interindividual differences in the pharmacokinetics of ibuprofen and its enantiomers in subjects who received a single 400 mg oral dose of racemic ibuprofen. The CYP2C9*3 and CYP2C8*3 variants lowered the clearance of S-(+)-ibuprofen, whereas the 2C8*3 allele was responsible for lower clearance of the R-(−)-enantiomer. The lowering effect of the CYP2C9*2 variant on ibuprofen clearance was observed only in combination with 2C8*3. Compared to the wild-type carriers, participants with the 2C9*1/*2 and 2C8*1/*3 variant combinations (19% of the participants) showed a 65% clearance whereas the 2C9*3 and 2C8*3 homozygotes and double heterozygotes had only 7-27% of the wild-type ibuprofen clearance (p<0.001). Karauzniewicz-Lada et al. (53) looked for correlations between the CYP2C9 and CYP2C8 variants and the concentrations of ibuprofen and its metabolites in the plasma and urine of healthy volunteers who received a single 400 mg dose of racemic ibuprofen. The 2C9*2 and *3 and 2C8*3 variant carriers showed lower ibuprofen metabolism than other genotypes, and the lowest metabolism was observed in a carrier of the CYP2C9*1/*2 and CYP2C8*1/*3 variant combination. Variant allele carriers maintained S-ibuprofen plasma levels for much longer, which led to a 40% higher AUC. They also differed from the wild-type carriers in the plasma levels of ibuprofen metabolites (C₄₋₈₆=1.53 vs 2.71 mg L⁻¹ for IBP-OH and 1.66 vs 4.52 mg L⁻¹ for IBP-COOH, respectively). Reduced clearance and longer half-life were also observed.

Patent ductus arteriosus (PDA) affects as many as 31% of infants whose birth weight is between 501 and 1500 g (54). Ibuprofen is the first-line therapy for PDA with the aim to close the ducts through a mechanism that is most likely based on prostaglandin inhibition. Durrmeyer et al. (55) studied the association between CYP2C9 and CYP2C8 polymorphisms and response to ibuprofen therapy (ductus closure) in extremely preterm neonates diagnosed with haemodynamically significant PDA. In multivariate analysis the only two factors significantly associated with the response to ibuprofen were higher gestational age and non Caucasian ethnicity but not CYP2C polymorphism. This can be explained by the developmental influence: CYP enzymes in the liver of neonates, especially preterm neonates, are still maturing, and polymorphisms at that age do not have the same impact as in adults. Besides, CYP variant frequencies differ significantly between races and populations, which can also explain why ethnicity was found to be a significant predictor of ibuprofen efficacy. Other factors like heterogeneity of studied population and sample size could also have affected response to ibuprofen and could have masked the effects of CYP polymorphisms.

Diclofenac

Zi et al. (56) investigated in vitro the effects of the CYP2C9*2 and CYP2C9*13 variants expressed in yeast (and corresponding to the most common gene variants in the Chinese population) on the kinetics of diclofenac 4'-hydroxylation. The in vitro data suggest that these variants could lower the clearance of oral diclofenac. They also tested the effects of these enzymes on diclofenac interactions with nine drugs that inhibit diclofenac 4'-hydroxylation. The CYP2C9*13 enzyme significantly weakened the inhibitory potencies of sulphaphenazole, fluvasatin, fluvoxamine, and tranylcypromine. These findings can help in co-administration of diclofenac with other drugs in individuals carrying the CYP2C9 low-activity alleles.

Another group (57) investigated the impact of CYP2C9 *2 and *3 variants on diclofenac metabolism in vitro and in vivo but did not find significant differences in 4'-hydroxylation of diclofenac between the genotypes.
Moreover, the absence of correlation between diclofenac hydroxylation and losartan (also a substrate of CYP2C9) oxidation in vivo fuelled the scepticism regarding the benefits of using diclofenac as a predictor of CYP2C9 metabolic activity.

Pilotto et al. (58) investigated the effects of the CYP2C9 genotypes on gastroduodenal toxicity related to diclofenac. They studied several NSAIDs, including diclofenac, ibuprofen, celecoxib, and naproxen in 26 patients with gastroduodenal bleeding who were using NSAIDs and 52 matched controls. Gastroduodenal bleeding was strongly associated with CYP2C9*3 carriers as opposed to non-carriers (adjusted odds ratio 7.3).

Exploring the risk factors of NSAID-induced small intestinal injuries, including diaphragm disease, Ishihara et al. (59) tested the role of the CYP2C9*2, *3, and *13 alleles. Multivariate analysis indicated that the use of oxicams or diclofenac and the presence of comorbidities were associated with an increased risk of NSAID-induced small intestinal injury (adjusted odds ratio 2.97, p=0.041), but other factors including age, sex, concomitant use of proton pump inhibitors, indications for NSAID use, duration of NSAID use, and the CYP2C9*2, *3 and *13 single nucleotide polymorphisms were unrelated. However, the use of meloxicam and the CYP2C9*3 variant were significantly associated with an increased risk of diaphragm disease.

CONCLUSION

Examples provided in this review article suggest that knowing which enzymes are involved in ibuprofen and diclofenac metabolism may help to predict their bioavailability and behaviour.

In this respect, CYP2C9 and CYP2C8 genotyping may identify subpopulations of patients who run a higher risk of overexposure to the two NSAIDs with adverse consequences. Besides CYP enzymes, relevant are phase II metabolic pathways, especially UGT enzymes (mainly UGT2B7) and drug transporters. Some of the ABC members (like ABCC2) can modulate the hepatobiliary as well as renal transport/excretion. Since both pathways are involved in the metabolism of arachidonic acid to biologically active epoxyeicosetrenoic acids (EETs) (60), which have potent vasodilator and anti-inflammatory properties (61), CYP2C enzymes have also been recognised as physiologically relevant in the generation of reactive oxygen species (ROS) in vascular endothelial cells, affecting thus the vascular tone and homeostasis (62).

Once the relevant genotypes are established, the dose can be better predicted and adjusted or another NSAID chosen that does not share the same metabolic pathways. However, before genotyping is introduced into regular clinical practice, more research is needed to answer how effective would genotyping be for adjusting doses to the individual needs of NSAID users. Current guidelines on translating pharmacogenomics into clinical practice seem to bring some promise (3). It is important, however, to bear in mind that pharmacogenetics/pharmacogenomics is just a tool that has to be assessed with other relevant factors that may affect drug behaviour, including age, gender, comorbidities, and other concomitant drugs (63). This approach will improve treatment with NSAIDs while avoiding serious adverse effects.

Competing interests

All authors have completed the Unified Competing Interest form available at www.icmje.org/coi_disclosure.pdf and declare that they have received no support from any organisation for the submitted work; have no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; and have no other relationships or activities that could appear to have influenced the submitted work.

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Kako polimorfizmi gena citokroma P450 utječu na metabolizam i toksičnost ibuprofena i diklofenaka

Interindvidualne razlike u metabolizmu mogu biti važan čimbenik nastanka nuspojava te varijabilnosti u učinkovitosti lijeka. Polimorfizmi gena koji kodiraju metaboličke enzime citokrome P450 (CYP) mogu imati značajan učinak na metabolizam lijeka i toksičnost. Ovaj pregled donosi spoznaje o tome kako polimorfizam enzima CYP2C8 i CYP2C9 utječe na bioraspoloživost i kliničke ishode lijekova. Hepatotoksičnost i gastrointestinalno krvarenje najčešće su nuspojave povezane s utjecajem varijanti CYP2C8*3 i CYP2C9*2*3 na farmakokinetiku ibuprofena i diklofenaka. Na osnovi rezultata genotipizacije CYP-a mogu biti prepoznati pacijenti koji imaju povećan rizik od razvoja nuspojava te im je nužno prilagoditi dozu lijeka ili odabrati drugi lijek koji ne dijeli isti metabolički put. Osim CYP, značajan utjecaj imaju i polimorfizmi gena koji kodiraju fazu II metabolizma, osobito enzimi UGT, te transporteri, poput ABCC2, koji mogu modulirati ne samo transport na barijeri jatre i žuči nego i izlučivanje bubrezima. Stoga je u budućim istraživanjima nužan poligenski pristup. Prije uvođenja genotipizacije u redovitu kliničku praksu potrebno je provesti daljnja istraživanja koja će uključivati veće fenotipski dobro definirane skupine ispitanika za procjenu učinkovitosti ove strategije u poboljšanju lijecenja ibuprofena i diklofenaka. Zbog značajne međuetničke razlike u učestalosti polimorfizama gena CYP istraživanja treba provesti među različitim rasama i populacijama.

KLJUČNE RIJEČI: CYP2C8; CYP2C9; genotipizacija; hepatotoksičnost; farmakogenetika; farmakogenomika; farmakokinetika; nuspojave lijeka