

CYP2C9 polymorphisms: Considerations in NSAID therapy

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*The increased focus on safety in clinical trials represents a formidable hurdle regarding the availability of marketed drugs. The lengthy experimental process of ensuring the safety of a drug creates a need for faster, more efficient identification of drug toxicities. Profiling for individual genetic variability could be an essential screening process for potential adverse effects, especially within different ethnic populations. The identification of such variants should improve the management of patient care by, for example, identifying which patients should avoid a specific drug and which patients should be administered a modified dose. A suitable approach in implementing such a strategy could potentially reduce medical costs and improve the overall process and success of drug therapy. For example, polymorphisms in cytochrome P450 (CYP) 2C9, an enzyme involved in a variety of drug metabolisms, should be considered during future drug development of novel non-steroidal anti-inflammatory drugs (NSAIDs) because individuals with several variant alleles (eg, CYP2C9*2 and CYP2C9*3) have demonstrated decreased metabolic clearance compared with individuals with the wild-type enzyme (CYP2C9*1). The widespread use of NSAIDs, along with an increase in the occurrence of inflammatory diseases (such as arthritis) in aging populations, creates an incentive to consider CYP polymorphisms in treatment strategies.*

Keywords Adverse drug reaction, COX-2, cytochrome P450 2C9, genetic variability, MDL QSAR, Metadrag, NSAID, pharmacodynamic, pharmacogenetic, polymorphism

Abbreviations

CL_{int} clearance interval (V_{max}/K_m), **CYP** cytochrome P450, **NSAID** non-steroidal anti-inflammatory drug, **QSAR** quantitative structure-activity relationship.

Introduction

The family of cytochrome P450 (CYP) enzymes plays a major role in the metabolism of several drugs, with the CYP2C9 isoform being considered a key enzyme within this family [1-4]. The CYP2C9 isoform is second only to CYP3A4 in terms of total human liver microsomal P450 content, with average microsomal levels as high as 89 pmol/mg, and CYP2C9 is responsible for phase I metabolism of approximately 15% of clinically used drugs, some of which are referenced in Table 1 [1-3]. Given this broad range of substrate specificity, altered metabolism by CYP2C9 is responsible for a wide range of individual variation in drug response and drug-drug interactions, which may lead to adverse drug reactions [1-4].

Clinical trials remain costly in terms of time and money, and delay availability to patients in need of therapy. In addition, the risk of a drug recall arises in cases where the long-term use of the drug can result in a narrow therapeutic index, which has clinical implications for patients who rely on these drugs for therapy. The possible link between inter-individual metabolic variability and adverse drug reactions has prompted studies of genetic polymorphisms, especially involving metabolic enzymes with broad substrate specificity, such as CYP2C9 [1,3-8].

The CYP2C subfamily of enzymes is responsible for the metabolism of a variety of drugs on the market, along with an assortment of non-steroidal anti-inflammatory drugs (NSAIDs; Table 1) [1,6-9].

NSAIDs are commonly used for the treatment of rheumatoid arthritis, osteoarthritis, and other pain and inflammation-related conditions, and are among the most popularly prescribed drugs worldwide [10]. The therapeutic effects of NSAIDs are attributed mainly to the inhibition of the cyclooxygenase enzymes COX-1 and COX-2 [5,11]; however, a more recent generation of NSAIDs known as coxibs, including celecoxib, has been engineered to be more effective by increasing selectivity for COX-2 inhibition [10]. Compared with the constitutively expressed COX-1, which is associated with gastrointestinal cytoprotection and thromboxane synthesis, COX-2 is induced mainly by inflammatory stimuli, and is also responsible for prostacyclin synthesis when coupled with prostacyclin synthase [5,12,13]. Therefore, the trade-off for COX-2 selective inhibition is the loss of prostacyclin synthesis, while thromboxane synthesis is maintained. The imbalance between prostacyclin and thromboxane is the mechanism most typically implicated in adverse cardiovascular events (such as myocardial infarctions, hypertension and stroke) in cases of chronic dosing with coxibs [5,13]; however, the manifestation of such injury in patients at low risk of cardiovascular disease has suggested other mechanisms, such as inter-individual variability in drug metabolism, as factors contributing to drug toxicity [5,6,9,11].

Table 1. Agents in clinical use that are metabolized by CYP2C9.

Agent type	Agent name
Angiotensin II receptor antagonist	Losartan
	Irbesartan
	Candesartan
Anti-asthmatics	Zafirlukast
	Zileuton
Anticancer agents	Cyclophosphamide
	Tamoxifen
Anticonvulsants	Phenytoin
	Phenobarbital
	Trimethadione
Anti-inflammatory NSAIDs	Flurbiprofen
	Diclofenac
	Naproxen
	Piroxicam
	Suprofen
	Ibuprofen
	Mefenamic acid
	Celecoxib
	Lornoxicam
	Meloxicam
Diuretics and uricosurics	Torsemide
	Ticrynafen
	Sulfinpyrazone sulfide
Endogenous compounds	Arachidonic acid
	5-Hydroxytryptamine
	Linoleic acid
Oral anticoagulants	Warfarin
	Acenocoumarol
	Phenprocoumon
Oral hypoglycemics	Tolbutamide
	Glyburide
	Glipizide
	Glimepiride
	Nateglinide
	Rosiglitazone
Miscellaneous	Amitriptyline
	Fluoxetine
	Fluvastatin

This table shows the broad variety of substrates that are metabolized by CYP2C9. See references [1,23,30] for further information.

CYP cytochrome P450, **NSAID** non-steroidal anti-inflammatory drug

Because most NSAIDs are metabolized by enzymes in the CYP2C family, principally CYP2C9 [5,6,8,9,11,14], extensive research has been focused on understanding polymorphisms associated with the gene for the CYP2C9 protein [6]. The phenotypes associated with such polymorphisms can help alert healthcare professionals to drug toxicities on a patient-by-patient basis [5,6,12].

Using individual genetic variability associated with drug metabolism may prove instrumental as a prospective predictor of drug toxicities for an individual [5,6,12], or in designing clinical trials that identify susceptible populations that may require dose adjustments for therapy.

This review discussed an important class of pain relievers, the NSAIDs, and the balance between efficacy (potency screening) and toxicity, as represented by variations in drug metabolism. To illustrate these points, selected data for quantitative structure-activity relationship (QSAR) analyses and metabolic prediction are presented [15]. NSAIDs in research can be predicted to be substrates of CYP2C9; when used with information on identified enzyme variants, this can potentially alert healthcare professionals to altered drug responses in certain patient populations.

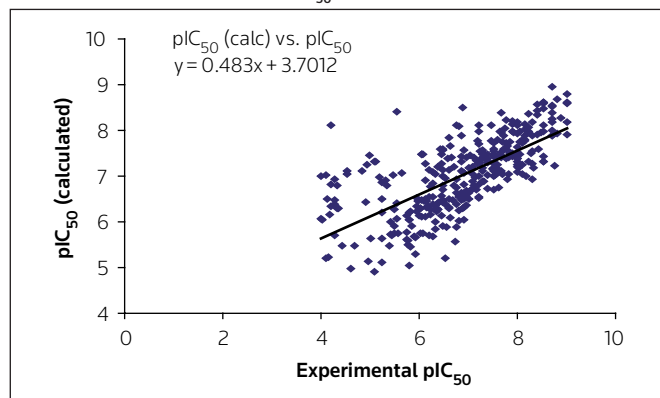
Case study of the polymorphisms of CYP2C9

It is hypothesized that abnormalities in the clearance and elimination rates of experimental substrates of CYP2C9, caused by polymorphisms of the CYP2C9 enzyme, pose a threat of adverse outcomes to certain ethnicities, especially in the case of chronic dosing. This hypothesis was tested by using a dataset of ~ 310 compounds [15] with established potency (pIC_{50} values for COX-2 inhibition) and CYP2C9 substrate specificity. MDL QSAR software was used to perform a multiple regression analysis with genetic algorithm and more than 300 molecular descriptors were examined to determine which of these was most highly associated with potency. The QSAR model was created with the use of a validation set, with 15% of compounds chosen randomly from the original dataset. The chemical structures associated with COX-2 inhibition and substrate specificity for CYP2C9 showed similarities, suggesting that compounds in the dataset [15] would be directed as CYP2C9 substrates. The dataset was therefore analyzed with CYP2C9 models using MetaDrug software [16]. These models included: two substrate models, CYP2C9 K_m for metabolite affinity and CYP2C9 V_{max} for the rate of metabolic reaction; a logarithm intrinsic clearance model, CYP2C9 CL_{int} ; and a model to study inhibition by the substrate, CYP2C9 K_i . The metabolite affinity (K_m) and metabolic reaction (V_{max}) models were used as a means to predict which of the compounds from the dataset were CYP2C9 substrates, the clearance model (CL_{int}) provided data on the rate of metabolic clearance from the system, and, finally, the substrate inhibition model (K_i) was intended to check which experimental compounds were inhibitors (or non-substrates) of the CYP2C9 enzyme. Emphasis was placed on the two substrate models for comparative analysis. Finally, an extensive literature search was conducted to analyze polymorphisms of the CYP2C9 gene. This analysis was divided into the types and frequencies of protein variants within different ethnic groups in order to predict metabolic outcomes and adverse drug reactions.

Results

Analysis of COX-2 binding affinity is presented in Figure 1, in which the QSAR model gave: a multiple R^2 value of

Figure 1. COX-2 inhibition modeling: Experimental pIC_{50} values compared to QSAR-derived pIC_{50} predictions.



The clustering about the regression line implies that most of these structural derivatives share a similar affinity for COX-2 binding. The outliers could signify differences in the parent compounds ($R^2 = 0.6609$).

QSAR Quantitative structure-activity relationship

0.6609, a standard error of 0.6168, an F-statistic of 50.86, a multiple Q^2 of 0.6267, and a cross-validation RSS of 109.3. The results of MDL QSAR's 'One-Touch Regression' routine suggest that the training set was well described by the regression equation, which is statistically significant ($p < 0.0001$). The validity of the selected descriptor set was determined by conducting 100 randomizations of the dependent variable values among the compounds; the most highly influential descriptors of the model included xc3, SssssC, SsNH₂ and SsBr. According to MDL QSAR's Appendix 1, the xc3 descriptor is the chi-index taken from the molecular skeleton of the molecule referring to a collection of fragments of different sizes and complexity. The SssssC, SsNH₂ and SsBr descriptors are associated with the saturated number of carbon hydride, NH₂, and bromine groups, respectively. SssssC, SsNH₂ and SsBr are atom-type e-state descriptors, where the sum of e-state values for all atoms is taken to be groups of a given type that are present in the molecule being described. The conclusions drawn about the molecular properties of the dataset correlate with the biological activity of the CYP2C9 enzyme. More specifically, CYP2C9 is catalytically selective toward acidic or neutral molecules, especially for substrates where the site of oxidation is a discrete distance from the hydrogen bond donor (or anionic heteroatom) [1,17]. Smith *et al* also noted that CYP2C9 substrates tend to have hydrogen bond donating groups that are separated from lipophilic regions [1,17].

Pharmacodynamic analysis using GeneGo Inc's MetaDrug software suggested that CYP2C9 is the primary metabolic enzyme for the experimental compounds analyzed [16]. Binding affinity for COX-2, along with other metabolic criteria, was catalogued for a subset of the dataset, which is listed in Table 2; binding affinity was mostly comparable for the structural derivatives, especially within parent groups. Given the similarities in binding affinities, it is

important to consider the differences in the rates of metabolism and metabolite clearance (CYP2C9 (V_{max}) and CYP2C9 (CL_{int}), respectively, in Table 2). Dissimilarities in V_{max} and CL_{int} values provided an incentive for analyzing the possibility of CYP2C9 polymorphisms. Previous studies provided a background for the different allelic polymorphisms of the CYP2C9 gene [6], in addition to the frequency of these polymorphisms across different ethnic populations [6].

Discussion

With its broad substrate specificity (Table 1), CYP2C9 has been implicated in various instances of adverse drug reactions, especially for drugs which have a narrow therapeutic index [1-4,18-21]. This review has identified associations between certain structural features contributing to the potency of COX-2 inhibition and substrate specificity of CYP2C9. These associations were based on the structure-activity analysis of experimental NSAIDs, including an evaluation of the metabolic tendencies for compounds in the dataset studied [15]. In agreement with QSAR data, the CYP2C9 substrate models demonstrate higher clearance for compounds saturated with carbon hydride and NH₂ groups (Table 2) than for other compounds. Incidentally, binding affinity for COX-2 is reported to be a consequence of CYP2C9 activity [5-7,11], where CYP2C9 is a specific example demonstrating the role that genetic polymorphisms play in drug metabolism.

It has been widely shown that variant forms of the CYP2C9 gene exist, and that these allelic variants are associated with inter-individual variability in drug response [1-3,18-22]. The human CYP2C9 gene is located on chromosome 10, spanning approximately 55 kb across nine exons, which encode a protein of 490 amino acid residues [1,22]. CYP2C9 is highly polymorphic [1,3,22], with as many as 30 identified, although not fully characterized, allelic variants [3]. Allelic variants are identified based on single-nucleotide polymorphisms that result in amino acid changes, essentially altering the wild-type protein. Of the 30 allelic variants, the amino acid changes for five are discussed below. The CYP2C9*3, CYP2C9*4 and CYP2C9*5 allelic variants are due to mutations in exon 7, whereas the mutation for CYP2C9*2 is located in exon 3, and a mutation in exon 5 is responsible for the CYP2C9*6 variant [22]. The CYP2C9*2 variant is due to an Arg¹⁴⁴Cys substitution [1,22,23]. A missense mutation, leading to the substitution Ile³⁵⁹Leu, is responsible for the CYP2C9*3 variant allele [1,22,23]. Similarly, an Ile³⁵⁹Thr substitution gives rise to the CYP2C9*4 variant [1,22]. The CYP2C9*5 variant is reported to arise from an Asp³⁶⁰Glu substitution, and the CYP2C9*6 variant is due to a nucleotide deletion at the 3'-end of exon 5, resulting in a null allele [22].

Allelic variants affect CYP2C9 enzyme activity to different degrees, creating a range of phenotypes that have specific implications in drug metabolism [22,23]. The overall effect of the allelic variants is a decline in the rate of metabolism, such that the overall substrate clearance

Table 2. COX-2 inhibition modeling: Experimentally derived values for metabolite affinity (K_m), rate of metabolite reaction (V_{max}), clearance interval (CL_{int}), and substrate inhibition (K_i).

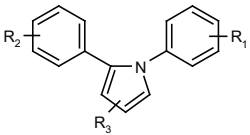
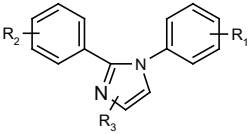
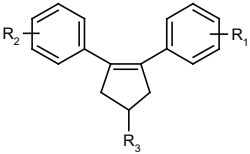
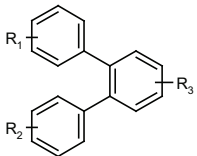
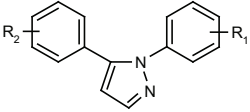
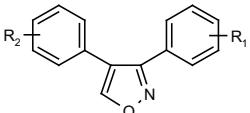
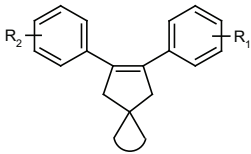
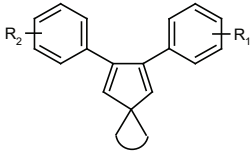
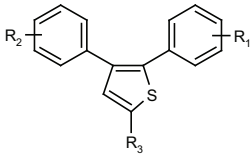
Parent group	Parent structure	Compound number ¹	Substituents	CYP2C9 (K_m)	CYP2C9 (V_{max})	CYP2C9 (CL_{int})	CYP2C9 (K_i)
Pyrroles	 <p>22 compounds</p>	2	$R_1 = 4-SO_2Me$ $R_2 = 4-F$ $R_3 = H$ $R_4 = H$	-0.95	1.43	2.38	-1.03
		10	$R_1 = 4-F$ $R_2 = 4-SO_2Me$ $R_3 = Me$ $R_4 = CH_2N(CH_3)_2$	-0.97	0.64	1.61	-1.15
		20	$R_1 = 4-COCH_3$ $R_2 = 4-SO_2Me$ $R_3 = Me$ $R_4 = H$	-0.87	0.99	1.86	-0.99
Imidazoles	 <p>127 compounds</p>	25	$R_1 = 4-SO_2Me$ $R_2 = H$ $R_3 = CF_3$	-1.46	0.06	1.52	-1.17
		27	$R_1 = 4-SO_2Me$ $R_2 = OMe$ $R_3 = CF_3$	-1.41	0.04	1.42	-1.19
		43	$R_1 = 4-SO_2Me$ $R_2 = 3-CH_2OMe$ $R_3 = CF_3$	-1.48	0.66	2.14	-1.24
Cyclopentenes	 <p>40 compounds</p>	162	$R_1 = SO_2NH_2$ $R_2 = 3,4,5-triF$ $R_3 = 4-OMe$	-1.93	0.31	2.24	-1.09
		165	$R_1 = SO_2NH_2$ $R_2 = 4-OMe$ $R_3 = H$	-1.93	0.31	2.24	-1.08
		174	$R_1 = SO_2Me$ $R_2 = 4-OMe$ $R_3 = H$	-1.93	0.31	2.24	-0.84
Benzenes	 <p>44 compounds</p>	194	$R_1 = 4-SO_2NH_2$ $R_2 = 4-F$ $R_3 = H$	-1.93	0.77	2.7	-1.08
		196	$R_1 = 4-SO_2NH_2$ $R_2 = 3-Cl$ $R_3 = H$	-1.93	0.38	2.3	-1.08
		204	$R_1 = SO_2Me$ $R_2 = 4-OMe-3-OMe$	-1.93	0.27	2.2	-1.08
Pyrazoles	 <p>86 compounds</p>	246	$R_1 = SO_2NH_2$ $R_2 = 2-F$ $R_3 = CF_3$ $R_4 = H$	-1.02	0.33	1.34	-0.72
		255	$R_1 = 4-SO_2NH_2$ $R_2 = 4-NO_2$ $R_3 = CF_3$ $R_4 = H$	-1.07	-0.1	0.97	-0.73
		260	$R_1 = 4-SO_2NH_2$ $R_2 = 4-NH_2$ $R_3 = CF_3$ $R_4 = H$	-1.07	0.23	1.3	-0.71
Isoxazoles	 <p>2 compounds</p>	320	$R_1 = 4-SO_2NH_2$ $R_2 = CH_2OH$	-1.83	0.49	2.31	-0.88
		321	$R_1 = 4-SO_2NH_2$ $R_2 = Me$	-1.93	0.49	2.31	-0.88

Table 2. COX-2 inhibition modeling: Experimentally derived values for metabolite affinity (K_m), rate of metabolite reaction (V_{max}), clearance interval (CL_{int}), and substrate inhibition (K_i). (Continued)

Parent group	Parent structure	Compound number ¹	Substituents	CYP2C9 (K_m)	CYP2C9 (V_{max})	CYP2C9 (CL_{int})	CYP2C9 (K_i)
Spiroheptenes	 30 compounds	339	R ₁ = 4-SO ₂ Me R ₂ = 4-OCF ₃	-1.93	-0.04	1.88	-0.81
		341	R ₁ = 4-SO ₂ Me R ₂ = 4-OMe	-1.93	0.29	2.21	-0.84
		348	R ₁ = 4-SO ₂ NH ₂ R ₂ = 4-OMe	-1.93	0.29	2.21	-0.84
Spiroheptadines	 2 compounds	353	R ₁ = 4-SO ₂ Me R ₂ = 3-Cl-4-OMe	-1.93	0.77	2.7	-1.08
Thiophene	 1 compound	354	R ₁ = 4-SO ₂ Me R ₂ = Br	-1.9	0.77	2.67	-1.14

A selection of the experimental derivatives was used to discern trends in metabolism, with particular attention given to metabolite affinity and rate of reaction. While K_m values are within a narrow range for the different parent groups, the reaction rates (V_{max}) are wide ranging and influence the intrinsic substrate clearance. The substrate models are from reference [16]. ¹Compound number is according to those used in reference [15].

CL_{int} clearance interval, CYP cytochrome P450

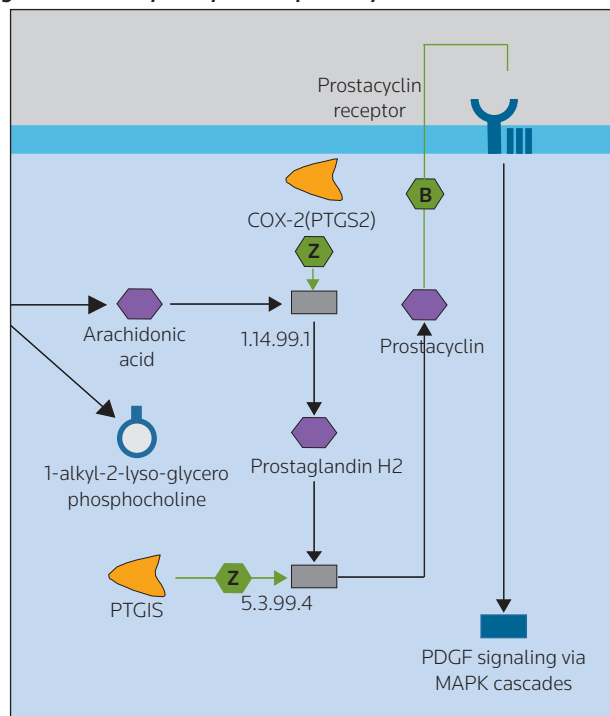
is decreased [1,22]. Of the five variants discussed above, CYP2C9*2 and CYP2C9*3 are the most common polymorphisms of the CYP2C9 gene [1-3,14,22-24], and have been implicated in more severe instances of adverse drug reactions due to decreased intrinsic clearance of clinical substrates [1,3,22,23]. Compared with the corresponding wild-types, CYP2C9*3 significantly reduces drug clearance, whereas the effects of CYP2C9*2 on drug metabolism are comparatively less severe [1,3,22,23]. Furthermore, the extent of reduction in enzyme activity is a direct consequence of the specific genotype of the variant [22-24]. For example, the homozygous genotype CYP2C9*3/*3 decreases drug clearance by as much as 90%, compared with the wild-type (CYP2C9*1/*1); however, this effect is more subdued for the heterozygous variant (CYP2C9*1/*3 or CYP2C9*2/*3), with drug clearance reduced by 66% [22]. More frequent and severe instances of drug toxicity have been reported for CYP2C9 substrates, such as warfarin, phenytoin and celecoxib, especially for carriers of the CYP2C9*2 and CYP2C9*3 allelic variants [1,18-25].

Owing to concerns of increased risk of substrate accumulation, dose adjustments have been recommended for populations with high frequencies of variant alleles [18-23]. CYP2C9*4 is extremely rare and, similarly,

CYP2C9*6 is absent in Caucasian, Hispanic and Asian populations, with allele frequencies of only 0.6 to 1.5% in African-Americans (residing in the region of Middle Tennessee) [22,26]. CYP2C9*5 has been identified in African-American and Hispanic-American populations (with frequencies of 0.2 to 1.7% and 0 to 0.5%, respectively), but is absent in Caucasian, Chinese and Japanese populations [22,26]. Both CYP2C9*2 and CYP2C9*3 are more widespread across different ethnic populations. CYP2C9*2 is not present in Taiwanese, Chinese, Japanese and Korean populations [22,27-29]. This allele is more prevalent in African-American (3.2%), Hispanic (12%) and Caucasian (owing to the heterogeneity of allele frequency, the range is between 8 and 19%) populations [22]. CYP2C9*3 is relatively rare in Korean (1.1%) and African-American (1.3%) populations, but is more prevalent in Japanese (2.2%), Chinese (3.3%), Hispanic (3.4% in Hispanics of Mexican descent), and Caucasian (3.3 to 16.2% because of heterogeneity of allele frequency) populations [22]. Overall, high allelic frequency has been reported for CYP2C9*2 and CYP2C9*3 across Caucasian populations.

The possibility of reduced catalytic activity for CYP2C9 variants has direct implications for the analysis of the experimental NSAIDs mentioned above. The binding

Figure 2. Prostacyclin synthesis pathway.



COX-2 plays a key role in prostacyclin synthesis. The process occurs with the coupling of COX-2 and prostacyclin synthase, and will discontinue with the inhibition of COX-2. Map by Metacore™ from GeneGo Inc [16].

tendencies of the compounds analyzed have raised concerns regarding consequences on the selective inhibition of COX-2, especially cardiovascular outcomes in genetically susceptible patients. In these special circumstances, standard dosages of COX-2 inhibitor drugs could cause greater toxicity, as has been the case for drugs such as warfarin [18-21]. Figure 2 outlines the basic components involved in prostacyclin synthesis. Because COX-2 is located upstream of prostacyclin, a higher binding affinity signifies stronger inhibition of COX-2, with a cascading inhibition of prostacyclin synthesis [13]. This is a critical factor in cardiovascular outcomes because prostacyclin is a potent inhibitor of platelet aggregation, and inhibition of prostacyclin accelerates atherogenesis [5,13]. In addition, platelet activation is facilitated by thromboxane (synthesized by COX-1), which remains unaffected by selective COX-2 inhibitors [5,12,13]. The inhibitory effect on platelet activation and the resulting reduction in damage to the cardiovascular system is lost when COX-2 inhibition leads to reduced levels of prostacyclins, increasing the incidence of cardiovascular outcomes such as myocardial infarction and stroke [5,12,13].

Conclusion

This review identified genetic variability in the CYP2C9 enzyme that relates to differences in metabolic activity of NSAIDs. Caucasians have a greater frequency of the CYP2C9*2 and CYP2C9*3 alleles, especially due to the heterogeneity of alleles, and therefore may be at greater

risk for toxicity from exposure to NSAIDs. This population has an inherited susceptibility to cardiovascular problems which may arise from chronic use of selective COX-2 inhibitors. From a drug design perspective, accounting for genetic variability can be a useful technique to screen for drug toxicities; it can be used as a complement to the traditional drug screening process in order to maximize drug efficacy while limiting drug toxicity.

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