Marked Interindividual Variability in the Response to Selective Inhibitors of Cyclooxygenase-2

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Background & Aims: Variability in response to drugs may influence both efficacy and safety. Cyclooxygenase (COX)-2 inhibitors pose a cardiovascular risk by potentially increasing the likelihood of thrombosis, hypertension, and atherogenesis. Differences between individuals in response to COX-2 inhibitors would be expected to influence their susceptibility to cardiovascular complications. We examined the variability in degree and selectivity of COX-2 inhibition in humans in response to celecoxib and rofecoxib. Methods: Fifty healthy volunteers received placebo, rofecoxib (25 mg), and celecoxib (200 mg), randomized by order. COX-1 and COX-2 inhibition was determined using ex vivo and in vivo indices of enzymatic activity. A subset of 5 individuals underwent 5 replicate studies to estimate variability in drug response both within and between subjects. Results: Despite the higher COX-2 selectivity of rofecoxib in vitro, the average selectivity attained by 25 mg rofecoxib and 200 mg celecoxib in vivo were not different. However, there was considerable variability at an individual level in the degree of COX-2 inhibition and selectivity attained by both drugs. Approximately one third of the variability was attributable to differences between individuals, suggesting the contribution of genetic sources of variance, such as candidate polymorphisms detected in COX-1 and CYP2C9. Conclusions: The actual degree of selectivity for inhibition of COX-2 achieved by the coxibs relates both to chemical properties of the drug and to factors in an individual that modulate drug response. These sources of variability might be exploited to identify patients uniquely susceptible to benefit or at developing risk of cardiovascular complications.

The coxibs, selective inhibitors of cyclooxygenase (COX)-2, were designed to inhibit the major enzymatic source of the prostaglandins (PGs), which mediate pain, inflammation, and, perhaps, tumorigenesis, while sparing COX-1-derived PGs, which contribute dominantly to gastric cytoprotection.† Two coxibs, rofecoxib2 and lumiracoxib,3 have been shown in controlled trials to reduce the incidence of serious gastrointestinal (GI) adverse effects when compared with traditional nonsteroidal anti-inflammatory drugs (tNSAIDs). Celecoxib reduced the number of colorectal polyps compared with placebo in patients with familial adenomatous polyposis in a controlled clinical trial.4 However, 3 structurally distinct coxibs, rofecoxib, valdecoxib, and celecoxib, increased the incidence of myocardial infarction and stroke in randomized controlled trials,5–8 suggesting that selectivity for inhibition of COX-2 may confer a cardiovascular hazard. Rofecoxib and valdecoxib have recently been withdrawn from the market. Both compounds are more selective for COX-2 than celecoxib in vitro.9 Despite such differences, all of the coxibs depress substantially prostacyclin (PGL1), leaving platelet COX-1-derived thromboxane (Tx) A2 unaffected, in contrast to aspirin or tNSAIDs, which inhibit both COX-1 and COX-2.10,11 PGI2 acts as a constraint on all agonists, which elevate blood pressure, activate platelets, and stimulate atherogenesis.12–14 Thus, drug selectivity for inhibition of COX-2 is thought to contribute to the likelihood of hypertension, myocardial infarction, and stroke resulting from treatment with coxibs.15,16

The detection of cardiovascular complications attributable to coxibs relates in patients to their underlying risk of cardiovascular disease.17 Thus, it seems rational that patients with identifiable cardiovascular risk factors will be excluded from treatment with selective COX-2 inhibitors. However, cardiovascular adverse events attributable to the coxibs also occurred in patients who were apparently at low initial risk of cardiovascular disease,6,7 suggesting that both rofecoxib and celecoxib cause a risk transformation during extended treatment of these individuals. The emerging hazard would be

Abbreviations used in this paper: COX, cyclooxygenase; NSAIDs, nonsteroidal anti-inflammatory drugs; PGs, prostaglandins; PGL1, prostacyclin; PGI-M, 2,3-dinor-6 keto prostaglandin F1α; TxB2, thromboxane; Tx-M, 11-dehydrothromboxane B2.
expected to relate to multiple factors, including the degree of selectivity for inhibition of COX-2 actually attained within an individual, plasma concentrations, and half-life and duration of drug action. Such pharmacokinetic and pharmacodynamic characteristics have been increasingly associated with both drug safety and efficacy and may be successfully exploited to individualize therapy when heritable mechanisms or predictive host factors such as gender, race, age, or weight can be identified. However, environmental sources of variability such as nutritional status and physical activity can mask clinically relevant associations. The relative importance of stable interindividual sources of variability in drug response—such as genetic variants—compared with less predictable, environmental sources of variability in the response to coxibs is unknown.

We examined the variability, both within and between subjects, in response to celecoxib and rofecoxib, in a placebo-controlled crossover study. Our studies suggest that even healthy individuals respond quite differently to coxibs. This variance might confound the detection of cardiovascular events attributable to drug exposure. On the other hand, interindividual variability in drug response might be exploited to identify patients in whom the drug exhibits unique efficacy or in whom the evolution of cardiovascular risk is accelerated. This may permit definition of the parameters within which these drugs might be administered chronically to patients initially at low risk of cardiovascular disease.

### Materials and Methods

#### Study Design

This randomized, double-blind, placebo-controlled protocol was approved by the Institutional Review Board of the University of Pennsylvania Health System and by the Advisory Council of the General Clinical Research Center (University of Pennsylvania, Philadelphia). Screening, enrollment, and follow-up of healthy study volunteers were performed at the General Clinical Research Center from January 2002 to January 2004. Written informed consent was obtained from all volunteers. All had an unremarkable medical history, physical examination, and routine hematologic and biochemical screen and were within 30% of ideal body weight. Subjects were nonsmokers and abstained from the use of aspirin and tNSAIDs, as assessed by history and platelet aggregometry, for at least 2 weeks before enrollment. Routine hematology, biochemistry, and urinalysis were assessed at time of screening and at 24 hours after administration of the drugs on completion of the study.

#### Treatments and Assessment

Fifty healthy volunteers (Table 1), aged between 21 and 43 years, received, in random order, a single dose of placebo, celecoxib (200 mg), and rofecoxib (25 mg) under double-blind conditions, separated by washout periods of at least 2 weeks. The randomization sequence was determined by the General Clinical Research Center pharmacist by allocation of the drugs to numbered containers. Inhibition of platelet COX-1 was assessed by measurement of serum TxB2. Inhibition was assessed ex vivo by measurement of lipopolysaccharide-stimulated PGE2 in plasma. The coefficients of variation for repeatability and reproducibility were 4.3% and 4.1%, respectively, for COX-1 inhibition and 1.9% and 4.3%, respectively, for COX-2 inhibition. Measurements were performed immediately before the administration of drug (0 hrs) and 4 hours thereafter. Urinary 2,3-dinor-6keto PGF2α (PGF-M), an index of PGJ2 biosynthesis, and 11-dehydro TxB2 (Tx-M), reflective of TxA2 formation in vivo, were assessed at 0 and 4 hours in spot urine samples that were collected 30 minutes after voiding. The coefficients of variation for both assays were <10%. A subset of the volunteers (n = 5) progressed through the entire protocol on 5 occasions, each separated by 2 weeks, to assess intraindividual variability in drug response in 5 replicates.

#### Plasma Drug Concentrations

Plasma samples were analyzed by liquid chromatography tandem mass spectrometry (Quattro Ultima, Micromass, Beverly, MA) using L755100 (Merck, Frost, Point Claire, Canada) and SC58125 (Cayman, Ann Arbor, MI) as internal standards for celecoxib and rofecoxib, respectively. Samples were chromatographed on a Luna 3µ column (Phenomenex, Torrance, CA) and quantitated using negative-atmospheric-pressure ionization and selected reaction monitoring of m/z 380.2→316.1 (celecoxib), 384.2→305.1 (SC58125), 314.2→215.1 (rofecoxib), and 328.2→313.1 (L755100). The coefficients of variation for these assays were 5.4% for celecoxib and 8.6% for rofecoxib measurements.

### Table 1. Characteristics of the Subjects

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Table 2. Single Nucleotide Polymorphisms

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\(^a\)Accession numbers refer to the NCBI (rs . . . ) or the EXPASY (VAR . . . ) SNP database.
\(^b\)CYP2C9+2.
\(^c\)CYP2C9+9.
\(^d\)CYP2C9+3.
\(^e\)CYP2C9+4.
\(^f\)CYP2C9+5.

Single Nucleotide Polymorphism Genotyping

Twenty-six single nucleotide polymorphism (SNP) assays (Table 2) were performed, using the Sequenom MassARRAY system (Sequenom, San Diego, CA).\(^25\) These assays robustly detected 1083 out of 1482 genotypes. The additional 399 genotypes were determined by resequencing (ABI 3730xl; Applied Biosystems, Foster City, CA).

Statistical Analysis

The primary response variables were ex vivo COX-2 inhibition, expressed as the ratio of postdrug to predrug plasma PGE\(_2\); ex vivo COX-1 inhibition, expressed as the ratio of postdrug to predrug serum TxB\(_2\); urinary PGI-M inhibition, expressed as the ratio of concentration postdrug to postplacebo administration; urinary Tx-M inhibition, expressed as the ratio of concentration postdrug to postplacebo administration; and plasma drug concentrations 4 hours postdrug administration. Derived variables were ratios of primary measures as estimates of COX-2 selectivity. All variables were log transformed to approximate normality. Four data points (3 measurements of Tx-M and 1 plasma concentration of celecoxib) were excluded because of technical errors in the assays.

Each subject in the study had either 1 or 5 replicate sets of measurements. Hence, the study followed an unbalanced hierarchical design with the repeated factor DRUG nested within measurement REPLICATE nested within study SUBJECT. However, the effects of REPLICATE grouped within SUBJECT were minimal, and a regression model was adopted in which the multivariate outcomes were grouped only by SUBJECT. These semiparametric regression models were estimated by generalized estimating equations using a robust “sandwich” variance estimator and identity link functions for the mean and scale models (geepack v0.2-10 in R 2.0-1; http://www.cran.R-project.org\(^{26}\)). The multiple measures grouped by SUBJECT were assumed to have an exchangeable correlation structure: This is equivalent to a hierarchical model with random intercepts grouped within SUBJECT. Variance components estimates were calculated using Bayesian hierarchical linear models of this type, with Markov Chain Monte Carlo estimation in winBUGS 1.4.1.\(^27\) Comparisons of the primary response variables between the treatment groups had
of the power in excess of 90% to detect a moderate effect size of 0.50 for 2-sided Student t test of paired observations with type I error rate of 0.05 in a study of 50 subjects. Results are graphed on logarithmic scales where error estimations were performed on log transformed data.

Exploratory tests of genetic association on multiple outcome measures were conducted using multivariate analysis of variance (MANOVA), with the dependent variables averaged over replicate measurements for each subject. Analyses were conducted in 2 ways: with data points unweighted and with data points weighted by the number of replicate sets of measurements.

Results

Fifty volunteers received, in random order, placebo and single therapeutic doses of rofecoxib and celecoxib to compare directly the responses to the drugs within the same subjects. Baseline levels of the drug response parameters (medians) were as follows: Serum TxB₂ 408 ng/mL (25%: 280 ng/mL, 75%: 557 ng/mL); LPS induced PGE₂ 30 ng/mL (25%: 16 ng/mL, 75%: 47 ng/mL); urine Tx-M 293 pg/mg Creatinine (25%: 185 pg/mg Creatinine, 75%: 439 pg/mg Creatinine); urine PGI-M 81 pg/mg creatinine (25%: 55 pg/mg creatinine, 75%: 117 pg/mg creatinine). Both rofecoxib and celecoxib inhibited COX-2-dependent PGE₂ formation ex vivo significantly when compared with placebo (Figure 1A). Small placebo effects may be attributable to diurnal variation because predrug and postdrug measurements were performed consistently at 0800 and 1200 hours.

There was no difference in the degree of COX-2 inhibition attained by the 2 drugs (rofecoxib 44.8% vs celecoxib 47.5%). Celecoxib also depressed COX-1-derived serum TxB₂, but the effect was small in comparison with the inhibition of PGE₂ (Figure 1B). Both drugs depressed the formation of PGI₂ in vivo as assessed by urinary PGI-M excretion but not the formation of TXA₂ as assessed by excretion of urinary Tx-M (Figure 1C and 1D). There was no difference between the drugs in their effects on these measures of COX-2 and COX-1 activity in vivo (Figures 1C and 1D).

The degree of selectivity for COX-2 in vitro—a chemical property of a COX inhibitor—is commonly expressed as the ratio of the drug concentrations required to inhibit the enzymatic activities of COX-2 and COX-1 by 50% (Figure 2A). Based on these criteria, rofecoxib is more selective than celecoxib. However, pharmacokinetic and pharmacodynamic variations between individuals would be expected to affect the degree of COX-2 selectivity actually attained in humans, which can be described by the ratio of COX-2 inhibition vs COX-1 inhibition at any given plasma concentration (Figure 2B). When the degree of COX-2 selectivity attained in subjects was estimated using this measure, both drugs differed significantly from placebo but not from each other at this time point after dosing (Figure 2C).

There was considerable variability in both ex vivo and in vivo indices of inhibition of both COX-2 and COX-1 (Figures 1E–H). However, drug response correlated poorly with plasma drug concentrations of rofecoxib (median, 215 ng/mL; range, 115–658 ng/mL) and celecoxib (median, 575 ng/mL; range, 179–1372 ng/mL) at 4 hours after dosing (data not shown). Plasma drug levels after dosing showed a trend toward lower concentrations with increasing body mass index (BMI). However, this attained significance (P < .05) only for rofecoxib: a unit increase in BMI was associated with a drop in plasma concentration of approximately 2%.

We assessed intra- and interindividual variability in drug response to determine the likely contribution of random and environmental fluctuations rather than interindividual factors to variability. Thus, 5 replicate sets of measurements were performed in 5 volunteers (Figure 1I–L). The simplified model underlying this experimental design assumed that the total variability as observed in the whole population (Figure 1E–H) was composed of interindividual variability, intraindividual variability, and some portion of random variability (eg, technical variability). The replicate measurements served to determine the combination of intraindividual variability and random variability (Figure 1I–L). Thus, assessment of the total variability (Figure 1E–H) and its 2 components intraindividual and random variability (Figure 1I–L) allowed estimation of the third component, interindividual variability, using a variance component analysis. The average responses for both drugs in the replicate measurements closely approximate the observations made within the whole population. Variability within these individuals (intraindividual and random variability) was less than the variability in the population as a whole (total variability). This can be visualized by expressing for each individual the variances of the replicate measurements and comparing the average variance of measurements within the same subject with the total variance (Figure 3A–D). The replicate measurements in 3 out of 4 response variables exhibited a lower variation within individuals than in the whole population, indicating that interindividual variability, beyond that attributable to random and environmental influences, is detectable within the population. Indeed, variance component analyses suggested that up to approximately 30% of the total variability in the measures of COX-1 and COX-2 inhibition may be attributed to interindividual variability (data not shown).
Genetic variability is a likely contributor to such differences in drug response between individuals. Thus, we genotyped the study population for polymorphisms in COX-1, COX-2, and cytochrome P450 (CYP) 2C9, the principal metabolizing enzyme of celecoxib, to examine their potential role in drug response (Table 2). We targeted variants potentially affecting either baseline expression or activity of the enzymes or their interaction with the coxibs (Table 2). Fourteen volunteers (28%) were heterozygous for a variant of COX-1 located in the signal peptide or its catalytic site; 3 (6%) were heterozygous for a variant in the catalytic site of COX-2; and 14 (28%) were heterozygous for a variant of CYP2C9. Thus, the population was genetically quite heterogeneous regarding the enzymes most likely to affect drug re-
response; a total of 24 out of 50 individuals had 1 or more genetic variant. No homozygous variants occurred, and they did not associate with gender. This study was not designed to address comprehensively the impact of SNPs on drug response, so we confined our analyses to those SNPs that occurred in 4 or more volunteers. Thus, CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu) were tested for their effect on plasma concentrations of celecoxib, and the Trp8Arg and Pro17Leu variants of COX-1 were tested for their effects on COX-1 response.

CYP2C9*2 occurred in 8 individuals (5 white, 3 Asian) and was associated with higher plasma concentrations of celecoxib, after adjusting for the linear effects of age (Figure 3A). BMI and age were not significant covariates in this model. Effects on the response to celecoxib were not statistically significant. CYP2C9*3 had no effect on plasma concentrations. Only the Pro17Leu variant of COX-1 was associated with a statistically significant (MANOVA, P = .009, unweighted; P = .011, weighted) pharmacodynamic effect—a reduction in COX-1 inhibition both ex vivo and in vivo. Post hoc analysis revealed an association with an absence of the COX-1 inhibition by celecoxib ex vivo (Figure 4B) and an absence of COX-1 inhibition in vivo by rofecoxib (Figure 4C). No effects of BMI or age were found in these analyses; males showed greater Tx-M suppression with both rofecoxib and celecoxib than females (P < .05).
Variability between individuals in their response to drugs is well recognized. Nevertheless, the typical paradigms of drug development and approval infer a common response to a limited number of doses within a therapeutic category. Inhibitors of COX-2 are no exception. Indeed, a considerable variability in the plasma concentration/enzyme inhibition response relationships was noted as the first member of the class, celecoxib, entered the US market. Typically, assays in whole human blood have been utilized to compare the selectivity for inhibition of COX-2 among members of the class. Although the multiple factors that can distort the relationship between the degree of selectivity attained in vitro and in vivo have been noted, the implications of this observation have received little attention. Evidence exists in animal models that suggests that the degree of selectivity attained in vivo may relate to cardiovascular outcomes, including hypertension, myocardial infarction, and stroke. However, this hypothesis has never been addressed in clinical studies.

Rofecoxib has been withdrawn from the market because of an excess of myocardial infarction and stroke in patients receiving this drug (25 mg/day) in the Adenomatous Polyp Prevention on VIOXX (APPROVe) trial. This hazard evolved slowly over time, becoming first evident after 18 months of treatment, in only 1%–2% of patients. A similar pattern, again consistent with a time-dependent transformation of cardiovascular risk was observed in the Adenoma Prevention with Celecoxib (APC) study of celecoxib. Thus, a small minority of the patients in both studies, apparently initially at low risk of cardiovascular disease, proceeded to increase that risk to a point that culminated in clinical events. The exploitation of variability in drug response to identify patients at emerging risk of cardiovascular disease requires first a determination of whether variability in response between individuals exceeds that attributable to environmental variability within individuals.

COX-2 inhibitors depress biosynthesis of PGI₂, while not affecting platelet COX-1-derived TxA₂, unlike aspirin or tNSAIDs. PGI₂ acts as a constraint in vivo on the cardiovascular effects of TxA₂, which include platelet aggregation, elevation of blood pressure, and acceleration of atherogenesis. This last effect may be particularly pertinent to the “latent period” before the emergence of cardiovascular risk in the APPROVe and APC studies. Deletion of the PGI₂ receptor—the IP—accelerates the initiation and early development of atherosclerosis in 2 mouse models. Epidemiologic approaches and, indeed, clinical trials have relatively poor precision for detection of uncommon, but serious, adverse effects, such as clinical events prevalent in the relevant populations. Thus, biomarkers of these mechanistic effects might be combined with measures of drug response (eg, blood pressure) and selectivity to identify individuals at accelerated risk of cardiovascular disease, prior to the occurrence of clinical events. Research is necessary to determine the paradigms within which COX-2 inhibitors can be prescribed for extended periods safely in individuals initially at low cardiovascular risk, including their potential use for cancer prevention.

We sought to explore the variability in response to coxibs to examine the practicality of such an approach based on biomarkers of drug response. We assessed therapeutic doses of celecoxib and rofecoxib, using a placebo-controlled, crossover design to investigate variability in plasma drug concentrations and pharmacodynamic responses, as assessed by both ex vivo and in vivo indices of COX-1 and COX-2 activity. Doses of celecoxib (200 mg) and rofecoxib (25 mg) were selected that have shown comparable effectiveness in clinical models of acute pain. Thus, perceptible pain relief is achieved within 1 hour of administration of these doses, and their maximum analgesic effect (2–3 hours postdosing) is approximately two thirds that of high doses of tNSAID comparators. Despite the higher COX-2 selectivity of rofecoxib in vitro, the average selectivity attained by
these doses of rofecoxib and celecoxib in vivo was not different, suggesting that both efficacy and cardiovascular and gastrointestinal safety profiles of the drugs after acute dosing might be similar. This might differ under chronic dosing conditions when the more prolonged half-life (on average) of rofecoxib would be expected to influence both relative efficacy and the adverse effect profile. However, again, interindividual differences in pharmacokinetics might modulate that expectation at the individual level. We determined both interindividual and intraindividual variability in drug response, the latter to assess the stability of the biochemical phenotype and to get an impression of whether variation attributable to environmental and random effects would be sufficient to obscure a genetic contribution. Significant intraindividual variability was evident in all parameters as 5 individuals progressed through the entire study on 5 occasions. Some of the variation, evident on placebo, may have derived from circadian variability because samples were obtained at discrete time points before (0800 hours) and after (1200 hours) dosing. Diurnal variation in the indices of COX activity has not been described, but diurnal variation in the pharmacokinetics of NSAIDs, including celecoxib, attributable in part to circadian variation in drug metabolizing enzymes, is well recognized. The intraindividual variability in response to both drugs is striking. This may reflect in part variability in assays (although all coefficients of variation were <10%), sources of variability that may be attenuated under steady-state conditions, and random environmental effects that, despite standardization of certain conditions of dosing (time, posture, environment, fasting/feeding), remain unrecognized. Little comparable information is available with other drugs in which intra- vs interindividual variability in response has been compared so comprehensively.

Despite substantial intraindividual variability in response, this was exceeded by the variation in the subject population as a whole. Thus, the variance of both plasma drug concentrations (data not shown) and 3 out of 4 pharmacodynamic parameters were greater in the entire population than for replicate measurements within individuals. We would anticipate that this divergence would be even more pronounced with comparison between and within individuals when dosing had attained steady state.

Thus, additional to the potential value of measurements of pharmacokinetic and pharmacodynamic response, genetic sources of variance might contribute detectably to differences in drug response over and above the background “noise” attributable to analytic variability and fluctuating features of the environment. Our analysis showed that almost half of the study population had variants in the target enzymes, some with allelic frequencies of 5% or more. Information is only beginning to emerge on genetic variation in the COX enzymes, and our study was not designed or sized to afford a comprehensive analysis of genetic variants, which may modulate the response to coxibs. Notwithstanding these restrictions, 2 of the SNPs tested showed evidence of allelic association with elements of drug response. The CYP2C9*2 is a variant associated with reduced activity of a major metabolizing enzyme of celecoxib and was associated with elevated plasma concentrations of the drug 4 hours after administration. However, although this and other genetic variants of CYP2C9 are thought to affect the metabolic clearance of perhaps 20% of all prescribed drugs, acquired or environmental factors may also contribute to variability in drug clearance.

The Pro17Leu variant of the COX-1 enzyme appeared to be associated with a failure of inhibition of thromboxane formation with both drugs. All COX-2 inhibitors are relatively, as opposed to absolutely, selective for COX-2 and, at sufficient concentration, become inhibitors of both COXs in vitro (Figure 2A), just like tNSAIDs. The interindividual variability of inhibition of COX-1 evident 4 hours after dosing with either coxib was substantial and may be attributable in part to this variant. The greater the inhibition of COX-1-derived TxA2 in an individual, the more the gastrointestinal and cardiovascular profile of a coxib would be expected to resemble that of a tNSAID. Thus, determination of the factors that influence the actual selectivity for inhibition of COX-2 attained within an individual may identify biomarkers of relevance to clinical outcome.

In summary, we report substantial variability both within and between individuals in their response to acute dosing with both celecoxib and rofecoxib. SNPs in both COX-1 and CYP2C9 were apparently associated with elements of drug response, although population stratification may confound these findings. It is likely that a more comprehensive approach will shed further light on genetic sources of variation. Validated genetic markers might be usefully combined with biomarkers of atherogenesis, functional (eg blood pressure), kinetic, and dynamic parameters of drug action to identify individuals who accelerate their development of cardiovascular risk during treatment with coxibs. Such a strategy might conserve the value of extended dosing with selective inhibitors of COX-2 for patients at low risk of cardiovascular diseases who have previously exhibited gastrointestinal intolerance of tNSAIDs.
References


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