Is cytochrome P450 *2C9* genotype associated with NSAID gastric ulceration?

Jennifer H. Martin,[1] Evan J. Begg,[1] Martin A. Kennedy,[2] Rebecca Roberts[2] & Murray L. Barclay[1]

[1]*Department of Clinical Pharmacology, Christchurch Hospital, Christchurch and* [2]*Christchurch School of Medicine, Christchurch, New Zealand*

Aims The aim of this study was to explore whether genetic variation of cytochrome P450 2C9 (CYP2C9) contributes to NSAID-associated gastric ulceration. The hypothesis tested was that *CYP2C9* poor metabolizer genotype would predict higher risk of gastric ulceration in patients on NSAIDs that are metabolized by CYP2C9, due to higher plasma NSAID concentrations.

Methods Peripheral blood DNA samples from 23 people with a history of gastric ulceration attributed to NSAIDs metabolized by CYP2C9, and from 32 people on NSAIDs without gastropathy, were analysed to determine *CYP2C9* genotype.

Results The following genotypes were found: **1/*1* (wild type) in 70% of cases and 58% of controls, **1/*2* in 17% of cases and 29% of controls, **1/*3* in 13% of cases and 13% of controls. The difference between case and control nonwild-type genotype frequency was 11.5% (95% CI −14,37%), with the direction of the difference being against the hypothesis. No individuals with homozygote poor metaboliser genotype were identified. The differences in genotype frequencies between the two groups were not significant and the frequencies were similar to those in a large published population study. Ninety-five percent binomial confidence interval analysis confirms that there is no apparent clinically significant relationship between *CYP2C9* genotype and risk of gastric ulceration although a small difference in risk in poor metabolizers cannot be excluded.

Conclusions These results do not support the hypothesis that gastric ulceration resulting from NSAID usage is linked to the poor metabolizing genotypes of *CYP2C9*.

Keywords: CYP2C9, gastric ulcer, NSAIDs

Introduction

Gastric ulceration has been associated with the use of non steroidal anti inflammatory drugs (NSAIDs) [1, 2]. Known additional risk factors include advanced age, previous history of ulceration, concomitant use of steroids, higher doses of NSAIDs including the use of more than one NSAID, concomitant administration of anticoagulants, and coexisting serious systemic disorders [2]. Gastrointestinal bleeding is also related to the type of NSAID and the dosage [3].

The cytochrome P450 system is a large group of haemoproteins that catalyses the phase I metabolism of most drugs [4]. Although 12 mammalian cytochrome P450 families are known, members of only four (CYP1, CYP2, CYP3 and CYP4) are thought to be important in drug activation and detoxification [5]. *CYP2C9* is one of the four known members of the human 2C family. Genetic polymorphism has been observed for some of the major drug metabolizing families including *CYP2D6*, *CYP2C19*, and *CYP2C9*.

Several polymorphisms have been observed in *CYP2C9* [6]. Of these, two alleles (**2* and **3*) are thought to lead to reduced function of the enzyme. The **2* allele has a single base substitution resulting in an amino acid change from Arg to Cys at position 144. The **3* allele possesses a single base substitution at position 359, resulting in a change from Ile to Leu, in a substrate recognition site of the enzyme. Point mutations such as in the **3* allele, that alter amino acids in the substrate recognition sites of CYP2C proteins, can appreciably affect metabolic capability of the enzyme [6].

Correspondence: Associate Professor Evan J. Begg, Department of Clinical Pharmacology, Christchurch Hospital, Private Bag 4710, Christchurch, New Zealand. Tel.: 64 03 3641055; Fax: 64 03 3641003; E-mail: evan.begg@chmeds.ac.nz

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Polymorphism in the CYP2C9 gene has been shown to be clinically relevant. An association between warfarin dosage and INR level was recently found to relate to genetic polymorphism of *CYP2C9* [7]. Furthermore, a Chinese study has found that a lower dosage of warfarin is required for anticoagulation in Chinese compared with Caucasians, and that gastric ulcers are more common in this population [8]. Most NSAIDs are metabolized by CYP2C9. The warfarin study results, and the apparent dose dependency of the adverse effect, suggest that *CYP2C9* poor metabolizer genotype may result in increased NSAID plasma concentrations leading to an increased risk of gastric ulceration. This led us to hypothesize that the possession of a poor metabolizing form of the *CYP2C9* gene may predispose to gastric ulcers in patients taking NSAIDs metabolized by CYP2C9.

Methods

Study population

Two populations were studied. The first, the cases, were a subgroup of patients who had undergone upper gastrointestinal tract endoscopy at the Endoscopy Unit in the Department of Gastroenterology at Christchurch Hospital, a tertiary referral hospital. Patients with gastric ulcers and who were on NSAIDs were identified from a computerized endoscopy reporting database (Endoscribe™ version 2.05), analysing the period from September 1997 to November 1999. Using a built-in searching tool within Endoscribe, 425 reports were isolated by searching using the terms 'gastric ulcer' or 'gastric ulcer scar' within the diagnosis and comment fields of the reports. These reports were then reviewed individually and 93 patients were identified as being on aspirin or a NSAID when they developed gastric ulceration. Of these, 39 patients were excluded after hospital case note review because they were on aspirin, either alone or concurrently with a NSAID. Aspirin is not metabolized by CYP2C9. No patient was on an NSAID that was not metabolized by CYP2C9 to some extent.

Other exclusion criteria included inability to give informed consent, concurrent use of warfarin or corticosteroids, use of other medications metabolized by CYP2C9, and known *Helicobacter pylori* infection as determined by gastric biopsy urease testing or antibody serology.

Seventeen patients were excluded because it was not documented clearly in the case notes whether an NSAID was being taken at the time that the gastric ulceration developed. Of other patients excluded, four had known *Helicobacter pylori* infection, three were deceased, one had dementia, and three were unable to travel easily to have a blood test. Therefore, 23 cases were left for genetic analysis. The type and daily dosage of NSAID, concurrent medical problems, and other medication were documented from the case notes for these patients.

The second population, the control group, was identified from the Rheumatology outpatient clinic at Christchurch Hospital. Patients were included if they were taking a NSAID that is metabolized by CYP2C9, and were able to give informed consent for the study. They were excluded if they were on concurrent ulcer preventative medication, if they had ever had a gastric or duodenal ulcer, or if they experienced epigastric discomfort. The aim was to recruit twice as many control subjects as index cases. Approval for the study was obtained from the Canterbury Ethics Committee.

Genotyping

Genomic DNA was extracted from peripheral blood samples using the guanidiumisothiocyanate method of Ciulla *et al.* [9]. Genotyping for *CYP2C*1* (wild type), **2*, and **3* alleles was performed using PCR-RFLP analyses [10, 11, 12]. Exons 3 and 7 were amplified for each DNA sample. Exon 3 PCR products were digested with the restriction endonuclease *Ava*II. Only the *CYP2C9*2* contains an *Ava*II site. For analysis of the *CYP2C9*3* allele, two different forward primers were used to generate exon 7 PCR products. One primer generates a *Nsi*I site in the *CYP2C9*1* and **2* alleles, while the other primer generates a *Kpn*I site in the *CYP2C9*3* allele. All restriction enzymes were supplied by New England Biolabs (Beverly, MA). Digested PCR products were separated by 3% NuSieve GTG (FMC, Rockland, ME, USA) agarose gel electrophoresis, and visualized by ethidium bromide staining.

Statistics

The Chi squared test was used to compare the genotype frequencies in the cases with those of the controls, and to compare the genotype frequencies in the study population with those reported in a large population study. Ninety-five percent binomial confidence intervals for the frequency of the poor metaboliser genotype were also determined for cases and controls and compared.

Results

Fifty-four patients (23 cases, 31 controls) qualified for inclusion in the study. The median age was 68 years (range 28–87) in the case group, and 52 (range 28–72) in the control group. There were 57% females in the case group and 58% in the control group. The group with gastric ulceration had similar demographic features to those in other published studies [2, 3] in that most of the cases

suffered from other comorbidity, mainly cardiovascular, and many were elderly. The controls all had osteoarthritis or rheumatoid arthritis, but were otherwise generally well. There was no significant difference in gender, or NSAID type and dose (Table 1), between the groups. In particular, the average relative risk of gastrointestinal toxicity of the individual NSAIDs was the same between the two groups, using published meta-analysis data on relative risk [3]. There were two non-Caucasian participants, one Cook Islander and one Maori, both in the case group and both of whom had wild type (**1/*1*) genotypes.

No patients were taking drugs that have been reported to inhibit or to induce CYP2C9 metabolism [11]. Three patients in the control group were found at a later date to be taking ulcer preventative medications occasionally for epigastric discomfort (one used omeprazole 20 mg daily, and two used ranitidine 150 mg daily as required). Their genotypes were **1/*1*, **1/*1* and **1/*2*, respectively. Reanalysis after excluding these patients did not affect the results or conclusions. Five patients in the case group were also found to have taken intermittent aspirin after they were recruited into the trial. Their genotypes were **1/*2*, **1/*1*, **1/*2*, **1/*1*, **1/*3*. In relation to possible bias, reanalysis without these patients would have strengthened, not weakened, the study conclusions.

The *CYP2C9* genotypes of the 54 subjects are shown in Table 2. There were no significant differences in the frequencies of any of the genotypes between the two groups of patients studied, or between the control group and subjects in reported population studies [6, 11, 13]. The *P* value for these Chi squared test comparisons was never less than 0.4. The difference between case and control nonwild type genotype frequency was 11.5% (95% CI −14,37%). This difference, albeit nonsignificant, was in the opposite direction to that in the hypothesis.

Table 1 Average daily dose of each NSAID in the cases and controls.

NSAID	*Dose (mg) in cases* (n)	*Dose (mg) in controls* (n)
Sulindac	– (0)	325 (5)
Indomethacin	150 (1)	142 (4)
Piroxicam	20 (1)	20 (1)
Ketoprofen	175 (4)	200 (1)
Diclofenac	131 (12)	133 (9)
Ibuprofen	667 (3)	1600 (2)
Naproxen	1125 (2)	1055 (9)

Table 2 Genotypes of study subjects compared with reported frequencies.

Genotype	*Cases*	*Controls*	*Stubbins* et al. *[6]*
**1/*1* (wild type)	0.70	0.58	0.62
**1/*2*	0.17	0.29	0.19
**2/*2*	0	0	0.03
**1/*3*	0.13	0.13	0.15
**3/*3*	0	0	0.01

Discussion

CYP2C9 has been shown to be solely responsible for the human hepatic 4′-hydroxylation of flurbiprofen, and is the major contributor to the 2-and 3-hydroxylations of ibuprofen. Similarly, the 4-hydroxylation of diclofenac and the 5-hydroxylation of piroxicam, tenoxicam (the major metabolic pathways) are linked to CYP2C9. However, although CYP2C9 is involved in the *O*-demethylation of S-naproxen (as is *CYP1A2* to a minor extent), these are minor pathways for naproxen metabolism, the major one being acyl glucuronidation [14].

The functional effects of the *CYP2C9* mutations studied here are poorly understood, although there is some evidence of reduced activity of the mutant alleles. For example, there is *in vivo* evidence that heterozygotes of the **2* allele require less warfarin (also metabolized predominantly by CYP2C9) for anticoagulation than **1* homozygotes [12]. *In vitro* and *in vivo* evidence suggests that tolbutamide and phenytoin hydroxylation are reduced by the **2* and **3* substitutions [11–13]. The **3* variant is associated with higher K_m and lower V_{max} values than the wild type enzyme [11–13], although a recent report suggests that diclofenac 4-hydroxylation is unaffected by the **3* substitution [12]. The **3* substitution has been found to affect the V_{max} of different CYP2C9 substrates to different degrees. For example, the V_{max} for this substitution is low for tolbutamide, and much lower still for phenytoin [15]. Finally, inheritance of the **3* allele is associated with poor metabolism of warfarin [7].

We hypothesized that reduced function of *CYP2C9* variant alleles might lead to higher plasma concentrations of the drug and greater likelihood of side-effects.

We did not identify any NSAID-ulcer patients with homozygote poor metabolizer status (**2/*2*, **2/*3*, **3/*3*) i.e. the genotypes with the slowest NSAID metabolism rate in theory. If these genotypes are an important risk factor for NSAID ulceration then we should have identified patients with this genotype during the 2-year study enrolment period.

Thirty percent of our cases with NSAID-induced ulceration had the nonwild type *CYP2C9* genotype and the upper end of the 95% binomial confidence interval for this frequency was 46%. Therefore, there is only a 5% chance that the true frequency of nonwild type genotypes in those with NSAID-associated ulcers is more than 46%. The frequency of the nonwild type genotype in the large population study [6] was 38% with a 95% CI of 28–48%.

Our upper 95% CI of 46% falls within this CI. It is therefore possible to say with some confidence that poor metabolizer *CYP2C9* genotype status does not influence the induction of ulcers by conventional NSAIDs in a clinically significant way and, by corollary, that other patient characteristics are more important. However, because of the small size of the study population, it is not possible to exclude a small increase in risk of ulceration in poor metabolisers.

On average, our cases with NSAID-associated ulceration were older than the controls without ulceration. This difference is not unexpected due to the different populations from which the two groups were recruited. We could not postulate a mechanism by which the age difference may cause result bias with regard to genotype frequency, however.

If there are to be other studies in the future that address the association of *CYP2C9* genotype with NSAID ulcers, it would be useful to measure plasma concentrations of NSAIDs. To our knowledge, NSAID plasma concentrations have not been measured in relation to CYP2C9 genotype.

In conclusion, our results do not support the hypothesis that NSAID-induced gastric ulceration is related to *CYP2C9* genotype, although we cannot exclude a small difference in risk in homozygote poor metabolizers as none were detected in our study populations.

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References

1 Bakowsky VS, Hanley JG. Complications of non-steroidal antiinflammatory drug gastropathy and use of gastric cytoprotection: Experience at a tertiary care health center. *J Rheumatol* 1999; **26**: 1557–1563.

2 Wolfe MM, Lichenstein DR, Singh G. Gastrointestinal toxicity of non-steroidal antiinflammatory drugs. *N Engl J Med* 1999; **340**: 1888–1899.

3 Henry D, Lim LL-Y, Rodriguez G *et al*. Variability in risk of gastrointestinal complications with individual non-steroidal anti-inflammatory drugs: results of a collaborative meta-analysis. *Br Med J* 1996; **312**: 1563–1566.

4 Nebert DW, Gonzalez FJ. P 450 genes structure, evolution, and regulation. *Annu Rev Biochem* 1987; **56**: 945–993.

5 Nebert DW. Polymorphisms in drug-metabolizing enzymes: what is their clinical relevance and why do they exist? *Am J Hum Genet* 1997; **60**: 265–271.

6 Stubbins MJ, Harries LW, Smith G, Tarbit MH, Wolf CR. Genetic analysis of the human cytochrome P450 CYP2C9 locus. *Pharmacogenetics* 1996; **6**: 429–439.

7 Aithal GP, Day CP, Kesteven PJL, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and the risk of bleeding complications. *Lancet* 1999; **353**: 717–719.

8 Yu HC, Chan TY, Critchley JA, Woo KS. Facts determining the maintenance dose of warfarin in Chinese patients. *Quart J Med* 1996; **89**: 127–135.

9 Ciulla T, Slar R, Hauser S. A simple method for DNA purification from peripheral blood. *Ann Biochem* 1988; **174**: 485–488.

10 Yasar M, Eliasson E, Dahl ML, Johansson I, Ingleman-Sundberg M, Sjoqvist F. Validation of methods for CYP2C9 genotyping: frequencies of mutant alleles in a Swedish population. *Biochem Biophys Res Comm* 1999; **254**: 628–631.

11 Sullivan-Klose TH, Ghanayem BI, Bell DA, *et al*. The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. *Pharmacogenetics* 1996; **6**: 341–349.

12 Veronese ME, Doecke CJ, Mackenzie PI, *et al*. Site-directed mutation studies of human liver cytochrome P450 isoenzymes in the CYP2C subfamily. *Biochem J* 1993; **289**: 533–538.

13 Bhasker CR, Miners JO, Coulter S, Birkett DJ. Allelic and functional variability of cytochrome P4502C9. *Pharmacogenetics* 1997; **7**: 51–58.

14 Furuya H, Fernandez-Salguero P, Gregory W, *et al*. Genetic polymorphism of CYP2C9 and its effect on warfarin maintenance dose requirement in patients undergoing anticoagulation therapy. *Pharmacogenetics* 1995; **5**: 389–392.

15 Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol* 1998; **45**: 525–538.

