Cyclooxygenase-2 polymorphism: another piece in the cardiovascular puzzle

Francesco Cipollone* and Donato Santovito

Clinica Geriatrica e Centro di Eccellenza Europeo e di Riferimento Regionale per l’Aterosclerosi, l’Ipertensione Arteriosa e le Dislipidemie, Nuovo Policlinico ‘SS. Annunziata’, Università ‘G. d’Annunzio’, Via dei Vestini 66, 66100 Chieti (CH), Italy

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This editorial refers to ‘Association of cyclooxygenase-2 genetic variant with cardiovascular disease’ by S. Ross et al., page 2242.

Prostaglandin–endoperoxidase synthases, commonly referred as cyclooxygenases (COX), are key enzymes in the metabolism of arachidonic acid because they lead to the production of prostaglandins (PGs). These molecules are critically involved in key biological processes, including inflammation, blood clotting, and vascular homeostasis. Two main isoforms of cyclooxygenases have been described, named COX-1 and COX-2, which are encoded by two separated genes. In particular, COX-1 is encoded by a gene mapped to chromosome 1q25.2-q25.3 and its expression is induced by pro-inflammatory stimuli. In contrast, COX-2 is encoded by a gene mapped to chromosome 1q25.2-q25.3 and its expression is induced by pro-inflammatory stimuli.

The role of COX-2 in atherosclerosis and cardiovascular diseases has been thoroughly investigated in both pre-clinical and clinical settings. It has been shown that COX-2 expression is increased in response to several pro-atherosclerotic stimuli (i.e. angiotensin II, settings. It has been shown that COX-2 expression is increased in animal and human atherosclerotic plaques.1–4 Interestingly, COX-2 (as well as COX-1) catalyses the biosynthesis of prostaglandin H2 (PGH2), a highly unstable molecule, which is further metabolized by other enzymes to produce different series of prostaglandins and thromboxane. Although every cell may produce PGH2, only one or two final eicosanoids tend to be prevalent for a specific cell type, owing to the differential expression of downstream enzymes. In particular, in macrophages COX-2 is functionally coupled with type 1 microsomal PG synthase (mPGES-1), leading to the production of PGE2, which interacts with the E-prostanoid receptor-3, promoting vascular smooth muscle cell proliferation and neo-intima formation in mice.6 Conversely, in endothelial cells (ECs) COX-2 is a major contributor to prostacyclin production in vivo, which promotes protective effects against atherosclerosis and thrombosis.7

Thus, the ultimate effects of COX-2 and the consequences of its therapeutic inhibition are mainly a function of the cell source of that enzyme. Indeed, although the selective deletion of COX-2 in macrophages results in beneficial effects on atherothrombosis progression in mice,8 COX-2 deletion in ECs impairs vascular function and predisposes to thrombosis in both microvasculature and large vessels.9 The clinical relevance of these findings is highlighted by the controversial results in terms of cardiovascular events in patients undergoing prolonged treatment with selective COX-2 inhibitors (so-called ‘coxibs saga’).

Interestingly, genome analysis showed that several single-nucleotide polymorphisms (SNPs) might affect the COX-2 gene sequence (over 500 active SNPs for human COX-2 are enlisted in single nucleotide polymorphism database). Most of these are intronic or synonymous changes, thus functional relevance is unlikely. However, some of them have been shown to have a potential biological (and clinical) relevance by effectively influencing gene expression. In particular, the rs20417 polymorphism involves the COX-2 promoter with a guanine-to-cytosine substitution at position −765 (−765G → C) within a putative binding site for the transcriptional factor Sp1 (a positive inducer of COX-2 transcription), and therefore results in a reduced promoter activity.10,11 Notably, patients undergoing elective coronary bypass surgery carrying the −765C allele had significantly lower levels of C-reactive protein. More recently, in a prospective matched case–control study we have shown that the prevalence of the −765C allele, both in homozygosity and in heterozygosity, was significantly higher in patients with acute ischaemic stroke or myocardial infarction when compared with control subjects.11 However, since these first

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reports, other studies have investigated the impact of this functional polymorphism on the cardiovascular risk, often providing fairly controversial results, mainly as consequence of underpowered sample sizes or heterogeneity in the populations studied.

In this issue of the *European Heart Journal*, Ross et al. report the results of a large meta-analysis of 49,232 patients who were participants in six large prospective multicentre randomized clinical trials in the cardiovascular area. In particular, the authors had access to data of patients enrolled in the trials ACTIVE-A (1061 subjects), CURE (4662 subjects), DREAM (14,104 subjects), ONTARGET (3610 subjects), RE-LY (2501 subjects), and Women’s Health Study (23,294 subjects). Overall, they found that subjects carrying the -765C allele showed a reduced risk of cumulative major cardiovascular outcomes [odds ratio (OR), 0.78; 95% confidence interval (CI), 0.70–0.87], vascular death (OR, 0.76; 95% CI, 0.63–0.90), myocardial infarction (OR, 0.78; 95% CI, 0.67–0.92), and stroke (OR, 0.83; 95% CI, 0.70–1.00). Notably, a subgroup analysis performed to explore whether specific cardiovascular risk factors may modify the association between the -765C allele and cardiovascular outcomes showed that none of the major cardiovascular risk factors has a significant impact. The association between COX-2 rs204417 polymorphism and cardiovascular risk factors was also explored by analysing the data from the additional population of the INTERHEART study. Although only a weak association between rs20417 polymorphism and cardiovascular outcomes was found using an additive genetic model (OR, 0.92; 95% CI, 0.85–0.99), overall results confirmed no significant interactions with major cardiovascular risk factors (hypertension, diabetes, obesity, apolipoprotein B, and smoking habits), with the only exception being a marginal interaction with apolipoprotein A1. Finally, the authors also provide a demonstration of the inhibitory effect of the polymorphism on COX activity by showing that -765C allele carriers have lower urinary levels of both 11-dehydrothromboxane B2 and 2,3-dinor-6-keto prostaglandin F1α, two main metabolites of thromboxane A2 and prostacyclin, respectively.

The study by Ross and colleagues is remarkable because of the large population analysed, the accurate statistical approach, which allows exclusion of potential interactions with aspirin treatment, and for having excluded deviation from the Hardy-Weinberg equilibrium in each ethnic group for each study thus reducing the chance of false positives. Nevertheless, some limitations should be taken into account when reading the paper. Firstly, the nature of the study (a meta-analysis) includes clear limitations, such as the heterogeneity between study populations, trial outcomes, and pharmacological treatments. Secondly, the populations enrolled in two trials (RE-LY and ONTARGET) were not genotyped, and a proxy was used instead. This may explain the lower prevalence of the minor allele carriers in these studies and may represent a potential bias. Finally, the reduced levels of urinary prostacyclin metabolites found in this study might suggest a slight reduction of COX-2 activity also in ECs, which are the main source of protective prostacyclin. Thus, other determinants, apart from macrophage-specific inhibition of COX-2 activity, might also contribute to the protective effects of the -765C allele. Future experimental studies will help to clarify these aspects definitively.

In conclusion, for the rs20417 polymorphism as well as for many other polymorphisms discovered, any consequences in daily clinical practice are probably still a long way off. Nevertheless, the findings reported in this article represent an attractive starting point for future research aiming to understand fully the intimate mechanisms...
of COX regulation in vascular cells and their crucial role in atherothrombosis in humans.

Conflict of interest: none declared.

References


