Why Are Some Individuals Resistant to the Cardioprotective Effects of Aspirin? Could It Be Thromboxane A₂?

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It has been slightly more than 100 years since the synthesis, development, and commercialization of acetylsalicylic acid, the most widely consumed drug in the world. It is estimated that 35 000 kg are consumed daily in the United States and 6000 kg in the UK. Who would have ever thought that this widely consumed drug would achieve significant uses other than as an analgesic, antipyretic, and anti-inflammatory? The first report of a possible antithrombotic effect of aspirin appeared in 1953 in the Mississippi Valley Medical Journal. Dr Craven noticed that the patients who took Aspergum had a tendency to bleed more easily. He concluded that aspirin must be thinning the blood, and because thrombosis of the coronary arteries led to myocardial infarction, if his patients took aspirin, they might be less prone to experiencing a myocardial infarction.

These prescient observations were succeeded by the discovery by numerous investigators that aspirin could significantly decrease platelet function. In 1971, the mechanism by which aspirin inhibited platelet function began to unfold when Smith and Willis demonstrated that aspirin inhibited platelet prostaglandin synthesis. It was subsequently shown that aspirin irreversibly acetylated serine-529 close to the active site of the fatty acid cyclooxygenase (COX). In the case of the anucleate platelet, the enzyme is rendered inactive for its lifetime.

Samuelsson and co-workers demonstrated that thromboxane A₂ along with prostaglandin H₂ were the arachidonic acid metabolites responsible for activation of the platelet. Thromboxane A₂ is also a very effective vasoconstrictor and mitogenic substance.

After the discovery of thromboxane A₂, numerous studies followed that investigated its potential role in thrombotic cardiovascular diseases. Platelet thromboxane A₂ synthesis is increased in acute myocardial infarction, unstable angina, thrombolysis therapy, percutaneous transluminal coronary artery angioplasty, and pregnancy-induced hypertension, as well as in patients with diabetes mellitus (for review see Davis-Bruno and Halushka). Platelet thromboxane A₂ (TP) receptors also are increased in patients experiencing acute myocardial infarction and pregnancy-induced hypertension. Collectively, it is easy to envision how platelet-synthesized thromboxane A₂ plays a very important pathophysiological role in these syndromes.

Despite the demonstrated benefit of aspirin in secondary prevention and its possible beneficial effects in selected individuals for primary prevention, there remains a large segment of the population at risk that does not benefit from aspirin. The reasons for the failure of aspirin to be of benefit to these subjects remains unknown. In this issue of Circulation, Eikelboom and co-workers have provided some biochemical data to perhaps begin to unravel the causes for so-called aspirin resistance.

A major urinary metabolite of thromboxane A₂, synthesized from extrarenal sources is 11-dehydro thromboxane B₂. A major portion of this metabolite is believed to come from the platelet, but there are also additional cellular sources. Eikelboom and co-workers measured this metabolite in patients enrolled in the Heart Outcomes Prevention Evaluation (HOPE) trial. Subjects in this study took aspirin (75 to 325 mg daily). The authors subdivided the subjects into quartiles based on the levels of 11-dehydro thromboxane B₂ obtained at the baseline entry into the study. All the subjects were taking aspirin at the time of collection. A multivariate regression model was used to control for all the usual variables. Those subjects whose urinary 11-dehydro thromboxane B₂ levels were in the highest quartile had an odds ratio of 2 for having a myocardial infarction and 3.5 for a risk of having a cardiovascular-related death compared with those subjects in the lowest quartile. The overlap of values among subjects is such that the measurement of 11-dehydro thromboxane B₂ in the urine of a single individual could not be used as a guide for therapy or prediction of success or failure of aspirin treatment. Thus, if failure to adequately suppress thromboxane A₂ synthesis by aspirin plays a role in the pathogenesis of aspirin resistance, then we must try to better understand the mechanism and seek surrogate markers for this phenomenon that may have a greater and more accurate predictive value.

One possible mechanism for the failure to suppress thromboxane A₂ synthesis may simply be noncompliance, an issue that can always be raised in this type of study. However, assuming that all these subjects were compliant and took their aspirin as prescribed, one is compelled to ask why
thromboxane A₂ synthesis was not inhibited by over 90% as would be expected based on its irreversible effect on platelet COX-1. Several explanations should be considered. Is the dose of aspirin adequate? This has been a highly debated issue over the last several years. If there were not a dose-dependent relationship between aspirin and gastrointestinal bleeding, one might consider doses higher than 75 to 81 mg. Although these low doses do carry a risk of increased gastrointestinal bleeding, they are sufficient to produce the maximum inhibition of platelet thromboxane A₂ synthesis. Furthermore, there is no evidence that higher doses are more efficacious for secondary prevention. This raises several possibilities. There could be individuals whose COX-1 is less sensitive to aspirin, or perhaps those patients who were in the lowest quartile had the most sensitive COX-1. The authors raise the possibility that single nucleotide polymorphisms (SNPs) of COX-1 exist and that these make some individuals more or less sensitive to the inhibitory effects of aspirin.

The assignment of a genetic basis for variation in response to drugs represents an exciting new area of study. SNPs are believed to be arbiters of phenotypic variation. The actions of SNPs are believed to alter proteins, which leads to functional changes including amino acid substitutions, promoter element binding site changes, and interference with normal intron/exon splicing. Only a small subset of the millions of predicted SNPs are likely to have functional consequences. However, SNPs in multiple genes have already been shown to correlate with cardiovascular disease and function.

Although the authors point to COX-1 as a likely site of variation, any gene in the pathway that converts arachidonic acid to thromboxane A₂ and ultimately to 11-dehydro thromboxane B₂ can, in theory, be responsible for their observed differences within the population. As part of the human genome project, high throughput methods have identified, mapped, and made publicly available over a million SNPs within the human genome (http://www.ncbi.nlm.nih.gov/SNP). A search of this record identified well over 100 SNPs within the loci of genes in the thromboxane A₂ synthesis pathway. An important step in correlating genetic variation with aspirin response will be to determine what impact on function, if any, these SNPs have. The observation of Eikelboom et al should stimulate the need to understand the role of these genetic variations in the response to aspirin. Whether or not any SNPs are associated with aspirin resistance or sensitivity remains to be determined, but clearly raises some interesting possibilities for improved and safer therapeutic approaches.

Although Eikelboom et al controlled for many variables, an important one of predictive value for unstable angina or myocardial infarction, C-reactive protein, was not one of those. This variable is of significant interest because it may provide a potential surrogate marker for the apparent resistance to aspirin and also a possible mechanism. C-reactive protein is a circulating marker of inflammation and is made in the liver in response to certain inflammatory stimuli, including IL-6. It is now widely acknowledged that atherosclerosis is a chronic inflammatory disease and that the macrophage plays a very important role. The macrophage/macrophage is also a rich source of thromboxane A₂, ranking behind the platelet in potential for synthetic capability. It also has a TP receptor. In the platelet, COX-1 is responsible for arachidonic acid metabolism to eicosanoids. Because the platelet is anucleate, once the enzyme is irreversibly inhibited, the effect lasts for the life of the platelet. In the macrophage, the inducible COX-2 is the enzyme responsible for the major portion of the metabolism of arachidonic acid. Unlike the platelet, the macrophage is capable of synthesizing new COX-2 after it has been inhibited by aspirin. This raises the possibility that the daily low dose of aspirin may not be sufficient to maximally inhibit COX-2. Thus, the macrophage resident in the atherosclerotic plaque may contribute significantly to a pool of thromboxane A₂ that is not inhibited by the lower doses of aspirin. The macrophage metabolism of arachidonic acid to prostaglandin H₂ and thromboxane A₂ may contribute to the pathogenesis of acute cardiovascular syndromes. As pointed out by Eikelboom et al, prostaglandin H₂ synthesized by the macrophages may be shunted to the platelet, which can then be metabolized to thromboxane A₂ and activate platelets. The thromboxane A₂ synthesized by the macrophages may also directly activate the platelets. Recently, it has been demonstrated that human peripheral blood monocytes obtained from patients with recurrent unstable angina are more responsive to inflammatory stimuli than those obtained from control subjects. Liuzzo et al found that patients’ monocytes synthesized increased quantities of interleukin (IL)-6. These observations raise the possibility that the activated monocytes may also make increased quantities of other inflammatory mediators such as thromboxane A₂ and prostaglandin E₂.

Evidence is beginning to accumulate that COX-2 and prostaglandin E synthase may be important contributory factors in plaque rupture. In symptomatic carotid plaques, there is an increased expression of these 2 enzymes, and they in turn appear to be responsible for increased levels of matrix metalloproteinases 2 and 9, two enzymes that may play roles in plaque rupture. Thus, macrophage COX-2 may play a very important role in atherosclerosis, which raises the possibility that selective inhibition of COX-2 may slow the atherosclerotic process and some of its sequelae. Furthermore, SNPs of COX-2, if they exist, and their potential involvement in the pathogenesis of atherosclerosis and effect on sensitivity or resistance to aspirin poses a fertile ground for future investigations.

However, selective inhibition of COX-2 in patients who require low-dose aspirin therapy but do not receive it may be associated with an increased risk of an acute cardiac event. Subsequent studies have not borne this out, but the question still remains.

Thromboxane A₂ may also play a very important role in the pathogenesis of atherosclerosis. Cayatte et al have shown in the apolipoprotein E (ApoE) knockout mouse that a TP receptor antagonist significantly reduced aortic root lesions and intercellular adhesion molecule-1 (ICAM-1) expression, whereas aspirin did not. The TP receptor antagonist also blocked the TP receptor agonist–induced expression of ICAM-1 by endothelial cells in vitro. TP receptor antagonists have an advantage over aspirin because several eicosanoids, including the nonenzymatically synthesized isoprostanes,
which have the potential to contribute to the pathogenesis of atherosclerosis, can stimulate the TP receptor. Thus, their actions would be blocked.

The platelet COX-1 has long been the accepted target for aspirin therapy, because of its synthesis of large quantities of thromboxane A2 and its pivotal role in the pathogenesis of acute cardiovascular syndromes. It is now time to consider the macrophage’s COX-2 as an additional target for aspirin and whether it can adequately inhibit it. The other target that needs to be considered to slow the progression of atherosclerosis and its sequelae is the TP receptor. TP receptor antagonists may have significant potential therapeutic benefit in preventing not only the progression of atherosclerosis but also the acute cardiovascular events associated with it.

Perhaps the ideal future therapeutic approach to treating the acute and chronic sequelae of atherosclerosis is the combination of a TP receptor antagonist and a selective COX-2 inhibitor. This would have the theoretical advantage over low-dose aspirin and a selective COX-2 inhibitor because the lack of gastrointestinal bleeding seen with selective COX-2 inhibitors is lost in patients taking low-dose aspirin therapy.

Aspirin has served us well during its first 100 hundred years. Will it still be around for another 100 years? Still another chapter remains to be written about this remarkable drug, both its successes and its failures.

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References

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