Platelet Glycoprotein IIIa PlA Polymorphism and Effects of Aspirin on Thrombin Generation

To the Editor:

Michelson et al\(^1\) recently reported differences in platelet glycoprotein Ib/IIIa function in relation to the common PlA\(^{A1,A2}\) polymorphism. PlA\(^{A2}\)-positive platelets showed a lower threshold for activation. This was supported by the gene dosage effect: PlA\(^{A2}\) homozygotes had the highest activation of their platelets using a range of ADP concentrations.

We are concerned about the conclusions regarding the antiplatelet effects of aspirin reached by the authors. At a low concentration of epinephrine (0.4 μmol/L), there was no difference in platelet aggregation between the PlA\(^{A1,A1}\) and PlA\(^{A1,A2}\) genotypes, whereas increased aggregation was observed in the PlA\(^{A2,A2}\) group. Unexpectedly, the inhibitory effect of aspirin on epinephrine-induced (2.6 μmol/L) platelet aggregation was found in the PlA\(^{A1,A2}\) group, but the opposite was found in PlA\(^{A2,A2}\) subjects. Two facts could explain this inconsistency. First, in experiments on the platelet aggregation response to aspirin, the number of PlA\(^{A1,A2}\) subjects included in the final analysis was diminished by 35%, because 7 of the 20 subjects did not achieve >60% aggregation at 10.0 μmol/L epinephrine. Because platelet response to aspirin was calculated as a percent of aggregation determined in the absence of the inhibitor, the exclusion of “weak responders” could be the cause of a relevant bias. We wonder whether the results obtained could be attributed to an altered sensitivity to epinephrine, which was used in 3 different concentrations. Second, experiments performed in platelet suspensions do not necessarily reflect the wide array of platelet functional responses observed in vivo, particularly the reaction to vascular injury. The limitations of in vitro studies on platelet glycoprotein activation have been critically reviewed.\(^2\)

Platelets contribute to the explosive generation of thrombin by providing membrane surfaces for the assembly of the prothrombinase complex, which converts prothrombin to thrombin. In our study on the effects of aspirin on the formation of thrombin at the site of microvascular injury,\(^3\) we found that PlA\(^{A2}\) carriers, most of them heterozygotes, had an impaired response to 75 mg of aspirin administered for 7 days. In fact, the odds for a failure of aspirin treatment in our study was 3.5 in PlA\(^{A2}\) carriers. A recent report on the increased risk of restenosis after coronary stent placement in PlA\(^{A2}\) carriers treated with aspirin and ticlopidine corroborates our observations. Moreover, these results are consistent with the finding that in patients treated with aspirin after coronary artery bypass surgery, the PlA\(^{A2}\) allele is a hereditary risk factor for bypass occlusion, myocardial infarction, and death.\(^5\)

Given the high frequency of the PlA\(^{A2}\) allele in the general population and growing evidence for lower clinical efficacy of aspirin treatment in PlA\(^{A2}\) carriers with atherosclerotic vascular disease, the results of the in vitro platelet aggregation experiments reported by Michelson et al\(^1\) should be interpreted with caution.

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Response

Szczeklik et al seem to agree with our conclusions about the prothrombotic phenotype of the platelet PlA\(^{A2}\) polymorphism, and we were pleased to see their own data, which was presented at the 1999 meeting of the International Society of Thrombosis and Haemostasis, showing shorter bleeding times in PlA\(^{A2}\)-positive subjects. They were concerned by the fact that our PlA\(^{A1,A2}\) study group was not intermediate to the other genotypes on aggregation with 0.4 μmol/L epinephrine. This was almost certainly due to the relatively small numbers studied, because when Feng et al\(^1\) assessed platelet hyperreactivity in 1422 subjects, they found a clear PlA\(^{A2}\) allele–dose response. The mechanism responsible for this PlA\(^{A2}\)-induced hyperreactivity seems to involve greater cell spreading, actin cytoskeleton reorganization, and postreceptor occupancy signaling.\(^2\)

Szczeklik et al thought that our finding that PlA\(^{A1,A2}\) platelets were more sensitive to the inhibitory effects of aspirin was inconsistent with their observation that PlA\(^{A1,A2}\) subjects were less sensitive to oral aspirin inhibition of thrombin generation.\(^3\) We used turbidimetric aggregation, the standard assay that has been used in countless patients to refine the efficacy of glycoprotein (GP) Ib/IIIa inhibitors. The assay used by Szczeklik et al measured prothrombin fragment (PF) 1.2 in blood from bleeding time-wounds. This latter assay has no clinical correlate and is not obviously related to GP Ib/IIIa function. Prothrombin competes with fibrinogen for activated GP Ib/IIIa but, unlike fibrinogen, prothrombin can bind to GP Ib/IIIa on resting platelets.\(^4\) Perhaps these PF 1.2 measurements reflect the mirror image of aggregation and our 2 sets of data are not inconsistent: if aspirin therapy causes greater platelet and GP Ib/IIIa inhibition in PlA\(^{A1,A2}\) platelets (our data), there would be less fibrinogen binding, which requires activated GP Ib/IIIa; this would permit greater prothrombin binding to resting GP Ib/IIIa, leading to more PF 1.2 production, which is consistent with the data of Szczeklik et al. In addition, their concerns that excluding “weak responders” to epinephrine might affect the results do not seem logical: if we had a greater proportion of “strong responders” in the PlA\(^{A1,A2}\) group, one would expect less (not more) inhibition by aspirin.

Finally, Szczeklik et al refer to 2 reports that found PlA\(^{A2}\) was a risk factor for poor outcomes after coronary revascularization procedures, despite treatment with aspirin. However, to say that there was an increased risk in PlA\(^{A2}\) patients treated with aspirin seems erroneous because it is a conclusion about data that was not presented in the article by Kastrati et al,\(^5\) who found that female sex was the only characteristic that interacted with PlA\(^{A2}\). Furthermore, because virtually none of the 40+ studies on PlA\(^{A2}\) risk specifically tested for an aspirin interaction, despite its common use in cases and controls, this issue cannot be addressed from the available literature. Unfortunately, a perfect opportunity to address the question of an interaction between aspirin and PlA\(^{A2}\) was missed by the Physician Health Study when they did not analyze their patients who had myocardial infarction separately when patients were randomized to receive aspirin or placebo.\(^6\)

Szczeklik et al conclude by saying our data should be interpreted with caution. We agree and would like to quote our
own discussion: “...our data should be interpreted cautiously until they are confirmed in a larger series.” With respect to aspirin and PlA2, we will reiterate that “future clinical epidemiology studies of platelet genetic variations and cardiovascular disease would be wise to consider possible treatment effects.”

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