The American Journal of Medicine (2006) 119, 707.e11-707.e16

ELSEVIER

THE AMERICAN JOURNAL of MEDICINE®

CLINICAL RESEARCH STUDY

Paradoxical Rebound Platelet Activation After Painkillers Cessation: Missing Risk for Vascular Events?

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ABSTRACT:

BACKGROUND: Several reliable reports strongly indicate that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase-2 (COX-2) inhibitors is associated with an increased risk of cardiovascular events. Considering the key role of platelets in coronary atherosclerosis and the fact that antiplatelet therapy with aspirin (and more recently, clopidogrel) has been associated with reduced vascular mortality, we sought to determine the effect of therapy and withdrawal of NSAIDs and COX-2 inhibitors on platelet activity.

METHODS: Platelet characteristics from 34 aspirin-naive volunteers who were receiving NSAIDs and COX-2 inhibitors were compared with 138 drug-free controls. Platelets were assessed twice at baseline (at least 1 month of NSAIDs and COX-2 inhibitors) and after a 14-day washout. We used adenosine diphosphate-induced conventional aggregometry, the point-of-care Ultegra analyzer (Ultegra Accumetrics, San Diego, Calif), and whole blood flow cytometry.

RESULTS: Platelet activity during therapy with NSAIDs and COX-2 inhibitors was similar and unremarkable between groups. However, there was a highly significant increase of platelet activity as assessed by conventional aggregometry ($P = .0003$), Ultegra analyzer readings ($P = .03$), and expression of GPIIb/IIIa ($P = .02$), P-selectin ($P = .03$), and platelet endothelial cell adhesion molecule-1 ($P = .001$) after withdrawal from NSAIDs and COX-2 inhibitors.

CONCLUSIONS: These data suggest that drug cessation, rather than continuous therapy with NSAIDs and COX-2 inhibitors, may be associated with rebound platelet activation, which may predispose one to a higher risk of vascular events. This hypothesis requires intensive testing in crossover randomized studies and may justify more aggressive antiplatelet regimens in patients after discontinuation of therapy with NSAIDs and COX-2 inhibitors.

KEYWORDS: NSAIDs; COX-2 inhibitors; Platelets; Cardiovascular risk

Nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase-2 (COX-2) inhibitors are common medications for managing chronic pain and diminishing inflammation in patients with arthritis. Considering the systemic nature of atherosclerosis, and some evidence that inflammatory features are indeed attributed to thrombotic vessel wall damage, NSAIDs and COX-2 inhibitors may potentially exhibit antiatherogenic properties.[1-3] However, by decreasing vasodilatation and reducing prostacyclin production, these agents may lead to increased platelet activity. Therefore, the lowest dose for the shortest duration necessary is now recommended.

Despite broad use and some evidence of increased vascular risk, the effects of NSAIDs and COX-2 inhibitors on biomarkers of platelet activity are not well defined. There are numerous somewhat contradictory reports suggesting that these agents decrease,[4,5] increase,[6,7] or have minimal effect[8,9] on platelet activity when used with or without concomitant aspirin. These discrepancies are mostly related to the sensitivity differences of the tests used to assess platelet function, frequency of monitoring, and treatment duration. Also, most of the reports describe certain platelet characteristics only during the NSAID and COX-2 inhibitor therapy, whereas follow-up studies and testing after drug

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0002-9343/$ -see front matter
doi:10.1016/j.amjmed.2005.11.007

discontinuation are lacking. There is only one report suggesting that platelet function normalizes within 24 hours after withdrawal from ibuprofen in healthy volunteers.[10] In the present study, we sought to retrospectively assess platelet activity during at least 1 month of NSAID and COX-2 inhibitor therapy and at 14 days after withdrawal of NSAIDs and COX-2 inhibitors in aspirin-naive healthy volunteers with the multiple risk factors for vascular disease.

CLINICAL SIGNIFICANCE

- Cessation of NSAIDs and COX-2 inhibitors activates platelets.
- Withdrawal from these agents may lead to worsened cardiovascular outcomes.
- Mild antiplatelet regimens may benefit such patients.

METHODS

Subjects

Subjects were eligible if they met all of the following inclusion criteria: males and females 21 years and older; able to provide informed consent; documented history of vascular disease, or more than 2 of 8 risk factors for vascular disease (family history, sedentary lifestyle, diabetes mellitus, hypertension, morbid obesity, hypercholesterolemia, postmenopausal or surgically sterile females, current or recent smokers); and available and willing to return for follow-up tests. Subjects were ineligible for the study if they received aspirin therapy or any medications containing aspirin within the past 4 weeks or had a known history of blood dyscrasia, gastrointestinal ulcer, or bronchial asthma. The data from 172 subjects with evaluable serial platelet activity characteristics were analyzed. Each platelet biomarker had been validated by quality control and assurance to be included in the present analysis. Thirty-four of the eligible participants were initially receiving NSAIDs and COX-2 inhibitors (the brand names and doses of NSAIDs or COX-2 inhibitors were not specified in the CRF questionnaire). All volunteers were aspirin-free and underwent platelet function assessment twice. Thienopyridines or intravenous platelet glycoprotein IIb/IIIa inhibitors within 3 months from the baseline sample were prohibited as well. Those treated with NSAIDs and COX-2 inhibitors were retested after a washout period of at least 14 days. All participants represent a pool of paid volunteers used as an internal laboratory reference for medical device and pharmaceutical studies conducted from 2001 to the present by the HeartDrug Research Laboratories (Towson, MD). The primary studies were approved by the institutional review board, and informed written consent was obtained from each subject.

Samples

Blood samples were obtained with a 19-gauge needle by direct venipuncture and drawn into 7-mL Vacutainer tubes containing 3.8% trisodium citrate at room temperature. The Vacutainer tube was filled to capacity and gently inverted 3 to 5 times to ensure complete mixing of the anticoagulant. The first 4 to 5 mL of blood were used for lipid profile analysis or discarded. All samples were labeled with a coded number and analyzed by blinded technicians. Research coordinators were not aware of the platelet data, and laboratory personnel did not know the treatment allocation. Platelet studies were performed at baseline and at a prespecified follow-up time point but at least 14 days after NSAID and COX-2 inhibitor withdrawal. The platelet tests were performed in duplicate at least 30 minutes after the sample collection but less than 2 hours after the draw.

Platelet Aggregation

The citrate mixture was centrifuged at 1500 rpm for 10 minutes to obtain platelet-rich plasma, which was always kept at room temperature. Platelet counts were determined for each platelet-rich plasma sample with a Coulter Counter (Coulter Co, Hialeah, FL). Platelet counts were adjusted to 3.5×10^8/mL with autologous platelet-poor plasma. Platelet aggregation was induced by 5 μM of adenosine diphosphate (ADP) (Chrono-Log Corporation, Havertown, PA). Platelet aggregation was determined by using a Chrono-Log optical aggregometer and expressed as the maximum percentage of light transmittance change from the baseline at the end of the recording time. Platelet-poor plasma was used as a reference. Platelet aggregability curves were recorded for 8 minutes and analyzed according to internationally established standards.[11]

Rapid Platelet Function Analyzer

The device (Ultegra Accumetrics, San Diego, Calif) is a turbidimetric-based optical detection system that measures platelet-induced aggregation as an increase in light transmittance with ADP as an agonist. The whole blood citrate mixture is added to the cartridge, and agglutination between platelets and coated beads is recorded.[12] When the activated platelets are exposed to the fibrinogen-coated microparticles, agglutination occurs in proportion to the number of available platelet receptors. The assay results are reported as platelet activation units. The data mirror platelet aggregation and reflect the degree of platelet ADP blockade. An electronic quality control test was performed on each instrument every day before performing any volunteer samples.

Whole Blood Flow Cytometry

The surface expression of platelet receptors was determined by flow cytometry using the following monoclonal antibodies: PAC-1 (GPIIb/IIIa activity), CD 62p (P-selectin) (DAKO Corporation, Carpenteria, Calif), and CD 31 (platelet endothelial cell adhesion molecule-1) (PharMingen, San Diego, Calif). The blood-citrate mixture (50 μL) was diluted with 450 μL tris-buffered saline (10 mmol/L tris, 0.15

mol/L sodium chloride) and mixed by inverting an Eppendorf tube gently two times. The appropriate primary antibody was then added (5 μL) and incubated at 37°C for 30 minutes, and a secondary antibody was applied if needed. After incubation, 400 μL of 2% buffered paraformaldehyde was added for fixation. The samples were analyzed on a Becton Dickinson FACScan flow cytometer (Becton-Dickinson, San Diego, CA) set up to measure fluorescent light scatter as previously described.[13] All parameters were collected using four-decade logarithmic amplification. The data were collected in list mode files and analyzed. P-selectin was expressed as the percentage of positive cells, and platelet endothelial cell adhesion molecule-1 and GP IIb/IIIa were expressed as *log* mean fluorescence intensity.

Statistical Analysis

For clinical characteristics, categoric data are displayed as frequencies and percentages. The chi-square test was used for dichotomous analyses of categoric data. Continuous data are presented as mean ± standard deviation and were compared using one-way analysis of variance. Comparisons between platelet biomarkers dependent on NSAID and COX-2 inhibitor use were made at respective time points using the Student *t* test. Differences between individual flow cytometric histograms were assessed using the Smirnov-Kolmogorov test incorporated in the CellQuest (Becton-Dickinson) software. Statistical analyses were performed with SPSS/11.5 (SPSS Inc, Chicago, Ill).

RESULTS

A total of 180 volunteers were initially considered, but 8 participants had to be excluded from analysis because of clots in the samples (1 participant), baseline cell counts out of institutional limits (2 participants), use of aspirin (4 participants), and noncompliance (1 participant). The remaining 172 aspirin-naive subjects were considered evaluable and grouped into the dataset with complete aggregometry, analyzer, and receptor measures dependent on NSAID and COX-2 inhibitor use. The locked database consists of serial assessments of the platelet biomarkers from 34 participants who were treated with NSAIDs and COX-2 inhibitors for at least 1 month, following at least a 14-day withdrawal. These data were compared with those of 138 participants free of NSAID and COX-2 inhibitor use. Demographic characteristics and risk factors for vascular disease are presented in Table 1.

Age, gender, and race were distributed fairly evenly, and risk factor distribution was similar between groups. However, there were more Asians, fewer obese volunteers, and fewer volunteers with sedentary lifestyle among the subjects treated with NSAIDs and COX-2 inhibitors.

The overall results of the platelet characteristics based on conventional aggregometry, Ultegra readings, and matched surface receptor expression are presented in Table 2.

The data from Table 2 suggest that use of NSAIDs and COX-2 inhibitors is associated with slight inhibition of platelet activity; however, antiplatelet properties of these agents were minimal and reached statistical significance only for PAC-1 antibody expression, suggesting reduced GP IIb/IIIa activity. Moreover, "gold standard" conventional ADP-induced aggregation was almost identical between groups. On the other hand, when assessed by multiple platelet tests, withdrawal from NSAIDs and COX-2 inhibitors was associated with a dramatic increase of platelet activity because all platelet biomarkers indicate significant activation.

Table 1 Demographics and Risk Factors of 172 Analyzed Subjects

Parameter	NSAIDs/COX-2 Inhibitors (−) (n = 138)	NSAIDs/COX-2 Inhibitors (+) (n = 34)
Demographics		
Age	34.4 ± 5.5	35.2 ± 6.1
Male	80 (58%)	18 (52%)
White	106 (77%)	22 (64%)
African-American	28 (20%)	10 (29%)
Asian	4 (3)	2 (6%)*
Risk factors		
Obesity	60 (43%)	11 (32%)*
Family history	50 (36%)	17 (50%)
Hyperlipidemia	44 (32%)	16 (47%)
Smoking	60 (43%)	14 (41%)
Sedentary lifestyle	97 (70%)	17 (50%)*

*Significant by chi-square test.

Individual plots of the ADP-induced platelet aggregation during and after at least 14 days of withdrawal from NSAIDs and COX-2 inhibitors are presented in Figure 1.

Individual plots of the platelet aggregation exhibited in Figure 1 indicate that the discontinuation of NSAID and COX-2 inhibitor therapy is associated with a marked response variability, with the majority of subjects experiencing platelet activation, some exhibiting no effect, and few subjects exhibiting platelet inhibition.

DISCUSSION

The data from the present retrospective analyses are the first to suggest that it is not immediate therapy with NSAIDs and COX-2 inhibitors, but rather withdrawal from these agents that is associated with a paradoxical increase of platelet activity. Despite the obvious limitations of this study, these findings may provide some insights into the existing and growing concern that NSAIDs and COX-2 inhibitors may be associated with worse clinical outcomes. Most important, if such vascular events are in fact occurring predominantly after cessation of NSAID and COX-2 inhibitor therapy, then short-term antiplatelet strategies with aspirin or/and clopidogrel applied after NSAID and COX-2 inhibitor withdrawal may potentially diminish rebound platelet hyperreactivity and result in risk reduction, or even survival benefit.

Numerous reports link the increased risk of acute cardiovascular events with NSAID and COX-2 inhibitor

Table 2 Platelet Activity Characteristics of Volunteers Dependent on Nonsteroidal Anti-inflammatory Drugs and Cyclooxygenase-2 Inhibitors

Parameter	Baseline		After Withdrawal		*P* Values	
	NSAIDs/COX-2 Inhibitors (−)	NSAIDs/COX-2 Inhibitors (+)	NSAIDs/COX-2 Inhibitors (−)	NSAIDs/COX-2 Inhibitors (+)	NSAIDs (+) Before vs After	(+) vs (−) At Baseline
ADP-induced aggregation (%)	64.3 ± 6.3	66.6 ± 6.4	62.3 ± 6.4	73.0 ± 11.6	0.003	NS
Ultegra (PAU) (Ultegra Accumetrics, San Diego, CA)	126.5 ± 34.2	123.8 ± 32.2	128.1 ± 37.4	144.3 ± 33.6	0.03	NS
PAC-1 (MFI)	8.2 ± 2.3	7.7 ± 2.4	8.4 ± 1.9	10.6 ± 2.7	0.02	.04
P-selectin (No. of + cells)	9.1 ± 3.7	9.3 ± 3.8	9.7 ± 3.1	10.9 ± 3.9	0.03	NS
PECAM-1 (MFI)	54.6 ± 14.9	51.6 ± 15.8	53.3 ± 13.8	59.2 ± 16.7	0.001	NS

NSAID = nonsteroidal anti-inflammatory drug; COX-2 = cyclooxygenase-2; NS = nonsignificant; ADP = adenosine diphosphate; PAU = platelet activation unit; MFI = mean fluorescence intensity; PECAM = platelet endothelial cell adhesion molecule.

use.[1,2,10] Although COX-2 inhibitors in general, and rofecoxib in particular, were primary "suspects," recent population-based, case-controlled studies suggest reconsideration of the cardiovascular safety of all NSAIDs as well.[14,15] On the other hand, the precise mechanisms by which NSAIDs and COX-2 inhibitors may increase cardiovascular risk are not clear. Targeting vascular endothelium and reducing prostacyclin production,[16,17] suppressing nitric oxide synthesis,[18,19] diminishing neovascularization,[20,21] abolishing adrenomedullin activity,[22] and enhancing free-radical production[23,24] have been implicated.

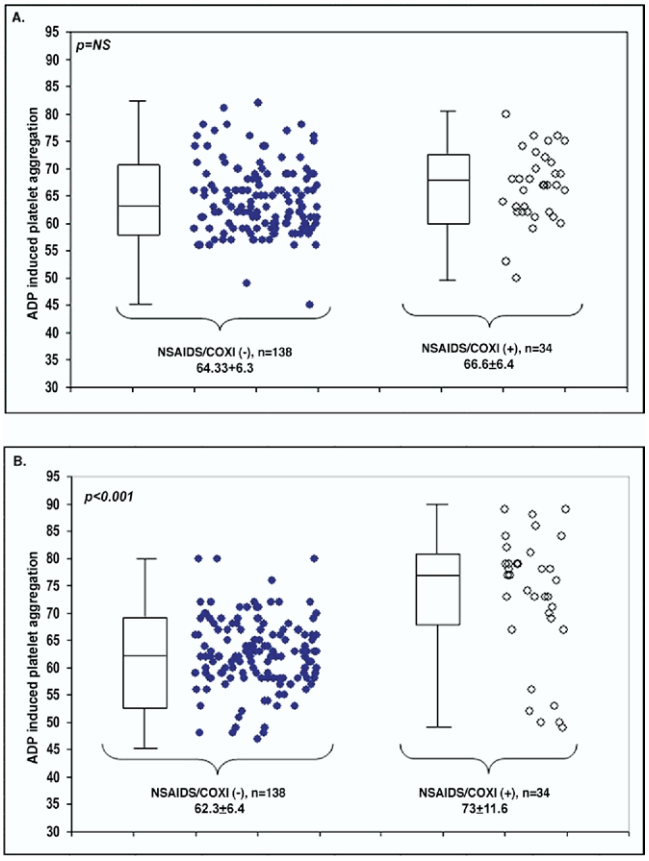

Figure 1 Individual plots of 5 μM adenosine diphosphate (ADP)-induced platelet aggregation on top (A) and after withdrawal (B) of nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase-2 (COX-2) inhibitors. Mean, median, and range (*bars*).

All of the above mentioned mechanisms also are affecting platelet activity. Platelets play a pivotal role in the development of cardiovascular events[25] and stroke,[26] whereas antiplatelet therapy with aspirin[27] and clopidogrel[28] provide a mortality benefit in such high-risk patients. However, there is no consensus on the effects of NSAIDs and COX-2 inhibitors on platelet activation.[4-9] Among the main reasons for such controversy are different experimental or clinical settings, including the specific designs for the earlier studies and underuse of comprehensive and/or conventional methods for the platelet function assessments. It is surprising that there are no published population-based randomized or even prospective platelet substudies assessing the time course of the platelet-related effects for NSAIDs and COX-2 inhibitors. In short, most of our current knowledge is based on the in vitro data[29,30] or observational evidence[31,32] challenging the clinical relevance of the findings. Moreover, no data are available when platelets are serially assessed before and after NSAID and COX-2 inhibitor use simultaneously by conventional "gold standard" aggregometry, rapid cartridge-based analyzers, and flow cytometric detection of activation-dependent surface receptors. Even less is known with regard to the platelet activity after withdrawal from NSAIDs and COX-2 inhibitors. In fact, although hazards of aspirin withdrawal are relatively well defined,[33] there is only one report suggesting that platelet function normalizes after 24 hours after cessation of ibuprofen.[10] This elegant observation, however, represents another major limitation because platelet activity after NSAID was measured solely by the cartridge-based analyzer (PFA-100; Dade-Behring, Miami, Fla). The problem with this particular device is that it is not reliable to

determine enhanced platelet activity and was designed to screen patients for the bleeding disorders in general, and von Willebrand disease in particular. Reported individual analyzer readings[10] show a strong trend toward the shortened closure time in the majority of subjects, suggesting rebound activation rather than "normalization" of platelet function after discontinuation of ibuprofen. Another important report provides strong evidence that recent withdrawers from oral antiplatelet agents had higher 30-day rates of death or myocardial infarction than continuous users or nonusers.[34] This study supports our present data that treatment discontinuation with NSAIDs and COX-2 inhibitors may outweigh any therapeutic antiplatelet benefit.

Relatively large sample size, comprehensive analysis of the platelet function, and serial assessments in the same subjects are among the strengths of our study. Retrospective design; unknown brands, doses, and duration of therapy; unequal group size; and significant differences in the demographics are major limitations, so chance represents a plausible alternative explanation, precluding us from making any definite conclusions. Although the expression of activation-dependent receptors was studied, their individual roles in patients treated with NSAIDs and COX-2 inhibitors are unknown. Similarly, the relation of platelet receptor expression to established platelet function tests is currently under investigation. It will be important to determine whether vascular events occur immediately during use of NSAIDs and COX-2 inhibitors, or after therapy cessation. Prospective studies are needed to confirm whether withdrawal of NSAIDs and COX-2 inhibitors is associated with enhanced platelet activity. If so, randomized evidence will be needed to prove that short-term antiplatelet regimens after NSAID and COX-2 inhibitor cessation will reduce the incidence of vascular events.

We conclude that drug withdrawal, rather than continuous therapy with NSAIDs and COX-2 inhibitors, may be associated with rebound platelet activation, which may result in a higher risk of developing vascular events. This hypothesis requires intensive testing in crossover studies, and in the future may justify more aggressive antiplatelet regimens in patients after cessation of NSAIDs and COX-2 inhibitors.

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