Effects of Propranolol Therapy on Platelet Release and Prostaglandin Generation in Patients with Coronary Heart Disease

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SUMMARY Suppression of platelet function is thought to be a mechanism of propranolol's beneficial action in angina pectoris. To study the effects of propranolol on platelets, we measured plasma β thromboglobulin and plasma thromboxane B2 (TXB2, stable metabolite of TXA2) levels by radioimmunoassay as indexes of platelet α-granule and TXA2 release, respectively. Platelet TXA2 generation in vitro in response to arachidonate and thrombin was also quantitated. Twenty-nine patients with coronary disease — 15 not taking propranolol (group A) and 14 taking propranolol (group B) — and 15 normal subjects were studied. Plasma β-thromboglobulin levels were increased in group A and B patients (mean 63 ± 8 and 96 ± 14 ng/ml, respectively) compared with normal subjects (mean 46 ± 6 ng/ml). Plasma TXB2 levels were similar in group A and B patients and in normal subjects (mean 148 ± 41, 149 ± 36 and 216 ± 39 pg/ml). Arachidonate-induced platelet TXA2 generation was significantly higher in group A patients than in normal subjects (725 ± 393 vs 82 ± 25 pg TXB2/108 platelets, p < 0.001). In contrast, platelets from group B patients had very low TXA2 generation (mean 21 ± 18 pg) compared with platelets from group A patients or normal subjects (p < 0.001). Similar results were obtained using thrombin. These data show that propranolol therapy does not affect platelet-released β thromboglobulin or TXA2 at rest, but significantly reduces the capability of platelets to generate TXA2 in vitro. Reduction in platelet TXA2 generation may be an important mechanism of action of propranolol in patients with coronary artery disease.

The purpose of this study was to examine platelet release reaction as an index of platelet activation and platelet TXA2 generation in patients with coronary heart disease and in normal subjects.

Materials and Methods

Study Population

Twenty-nine patients with history of stable angina pectoris and 15 normal subjects with no clinical evidence of coronary heart disease were included in this study. Of the 29 patients with angina pectoris, 23 had coronary artery disease (> 50% luminal narrowing) documented by angiography. Four had one-vessel disease, 10 two-vessel disease, and nine three-vessel disease. Eleven patients had had a myocardial infarction. Six patients who did not undergo coronary angiography were believed to have coronary heart disease because of previous myocardial infarction, angina pectoris and ECG evidence of myocardial ischemia with exercise.

Fifteen of the 29 patients were not taking propranolol (group A). Fourteen patients had been taking propranolol for periods of 1–5 months (group B). The total daily dose of propranolol was 120–1320 mg (mean 231 ± 22 mg). There were no significant differences in the extent of coronary artery disease between group A and group B patients. None of the study subjects had taken aspirin or other platelet-active drugs in the preceding week. All short- and long-acting nitrates were withheld from coronary heart disease patients for 6 hours, as these agents may interfere with platelet studies. Blood samples were collected in all subjects in the morning before eating and after 15 minutes of rest.

Blood Collection

All blood samples were collected from untraumatized peripheral veins. Blood was collected without tourniquet pressure, and only if blood flowed freely.
The first 4 ml of blood were discarded. The subsequent 2.5 ml of blood were collected in a mixture of EDTA and theophylline (2.5:0.5, vol/vol) for β-thromboglobulin assay, and the tube was immediately placed at 0°C after gentle shaking. Another 4 ml of blood was collected in a tube containing 1 mM of aspirin and 4.5 mM of EDTA (4:1, vol/vol) for TXB2 assay. The last blood sample (4.5 ml) was collected in sodium citrate (0.5 ml) for study of in vitro platelet TXA2 generation.

Beta-thromboglobulin determination was done using radioimmunoassay.22–24 The blood sample was centrifuged at 4°C for 30 minutes within 1 hour after collection. After centrifugation, the top 0.5 ml was pipetted with a disposable plastic tip into a separately labeled specimen tube. The sample was stored at −20°C for a maximum of 3 weeks. Samples were thawed and well mixed before use. Reconstituted standards and test samples (each 0.05 ml) were pipetted into assay tubes. Beta-thromboglobulin–iodine-125 (0.02 ml) and anti-β-thromboglobulin serum (0.02 ml) were added to each tube. The tubes were then incubated at room temperature for 1 hour. Ammonium sulfate solution (0.05 ml) was added to each of the assay tubes, which were then centrifuged for 15 minutes at 1000 g. They were then removed and placed in decantation racks and supernatant liquids were discarded. The precipitate remained undisturbed during this procedure. After draining of supernatant material, the precipitates were counted in a gamma counter. The concentration of β-thromboglobulin (ng/ml) was determined from a standard curve established from readings of radioactivity counts/min from a known standard.

Plasma TXB2 determination was also made by radioimmunoassay.25,26 The blood sample was immediately centrifuged at 1500 g for 8 minutes, and the plasma sample was stored at −60°C until assay, which was completed within 1 month. Lyophilized TXB2 standards (New England Nuclear) and the test samples were dissolved in 50-mM phosphate buffer containing 0.1% gelatin and 0.01% thimerosal (final pH 7.3). Tracer solution (0.1 ml of 125I TXB2) and antiserum (0.1 ml) were added to all tubes, incubated for 16 hours at 4°C, placed in an ice bath and reincubated. Charcoal suspension was added to each of the tubes, which were vortex mixed and centrifuged at 1000 g for 10 minutes at 4°C. Supernatant was decanted into scintillation vials containing Atomlight organic stabilizing cocktail. The radioactivity in the supernatant was counted in a β-liquid scintillation counter (Tracor Analytic, Searle Co.). TXB2 levels in the test samples were determined by comparison with the TXB2 standards. By this method, the cross-reactivity with other prostaglandins is as follows: PGE1, 0.2%, PGA1, 0.2%, PGF2α, <0.2% and 6-keto-PGF1α, <0.2%. All measurements were made in duplicate and the results expressed in pg/ml.

**Platelet TXA2 Generation**

Platelet-rich plasma (PRP) was prepared as described previously.19 The technique for in vitro TXA2 generation has been described.26 The platelet count in PRP was adjusted to 300,000—400,000/mm3. To aliquots of PRP, arachidonic acid (33 μg/ml) or thrombin (10 IU/ml) were added. The PRP samples were incubated at 37°C for 5 minutes and then stored in solution containing aspirin and EDTA (4:1, vol/vol). TXB2 was measured in the samples as described above. All samples were assayed in duplicate, and the average expressed in pg/10⁸ platelets was used for calculations.

**Statistical Calculations**

The average of all duplicate values was used to calculate the mean ± SEM. The t test for unpaired data, the rank-sum test and linear regression analysis were used for statistical calculations. A value less than 0.05 was considered significant.

**Results**

**Study Population**

The mean age of coronary heart disease patients was similar in groups A and B (mean 60 ± 2 and 57 ± 3 years, respectively; NS). The normal subjects were somewhat younger (mean age 45 ± 3 years).

Groups A and B were similar in terms of duration of symptoms (11 ± 3 and 12 ± 2 months, respectively) and nitrate therapy. Angina was more frequent in group A patients than in group B patients (mean 4.4 ± 1.1 and 2.5 ± 0.5 episodes of chest pain per week, p < 0.01). However, none of the patients had a myocardial infarction in the preceding 2 months or an episode of chest pain within 12 hours of blood collection; therefore, an acute event did not account for platelet function abnormality.

**Plasma β-thromboglobulin Concentrations**

Duplicate determinations of plasma β-thromboglobulin concentrations showed a mean variation of 5%. Plasma β-thromboglobulin levels in the normal subjects were 21–97 ng/ml (mean 46 ± 6 ng/ml). In patients with coronary heart disease (n = 22), plasma β-thromboglobulin levels were significantly higher (mean 80 ± 8 ng/ml, p < 0.001). The increased concentrations were observed in group A patients (range 30–123 ng/ml, mean 63 ± 8 ng/ml) as well as in group B patients (range 35–161 ng/ml, mean 96 ± 14 ng/ml) (fig. 1). However, there was no significant difference in plasma β-thromboglobulin concentrations in group A and group B patients (63 ± 8 and 96 ± 14 ng/ml, respectively; NS).

**Plasma TXB2 Concentrations**

Duplicate determinations of TXB2 concentrations in plasma samples showed a mean variation of 8%. In the normal subjects, TXB2 levels in the peripheral venous blood ranged from undetectable to 450 pg/ml (mean 216 ± 39 pg/ml). These levels are in the same range as described by others.8–12 The plasma concentrations of TXB2 in patients with coronary heart disease (mean 148 ± 27 pg/ml, n = 29) were not significantly different from those in normal subjects. In group A, plasma TXB2 levels ranged from undetectable to 700 pg/ml (mean 148 ± 41 pg/ml). Plasma TXB2 levels in group B patients ranged from undetectable to 440 pg/ml (mean 149 ± 36 pg/ml) and were similar to those in
group A patients and normal subjects (fig. 2). There was no significant correlation between plasma $\beta$-thromboglobulin and TXB$_2$ concentrations in either the normal subjects ($r = 0.22$) or coronary heart disease patients ($r = 0.35$).

**Platelet TXA$_2$ Generation**

Normal subjects and coronary heart disease patients showed important differences in in vitro TXA$_2$ generation by platelets. The mean arachidonate-induced platelet TXA$_2$ generation in normal subjects was $82 \pm 25$ pg TXB$_2$/10$^8$ platelets (range 0–350 pg/ml). In group A, TXA$_2$ generation was more than 2 standard deviations above the normal mean in eight of the 12 patients studied. The mean value in this group of patients was significantly increased (mean $725 \pm 393$ pg TXB$_2$/10$^8$ platelets, range 49–5000 pg) compared with normal subjects ($p < 0.001$). In contrast, in eight of 10 group B patients (propranolol-treated), platelets did not generate detectable amounts of TXA$_2$; in the remaining two patients, only very small amounts of TXA$_2$ (20 and 186 pg TXB$_2$/10$^8$ platelets) were generated (fig. 3). The mean TXA$_2$ generation in group B patients (mean $21 \pm 18$ pg TXB$_2$/10$^8$ platelets, range 0–186 pg) was significantly reduced compared with that in group A patients and that in normal subjects (both $p < 0.001$) (fig. 3).

Thrombin-induced TXA$_2$ generation was very similar. In the normal subjects, TXB$_2$ recovery was $476 \pm 157$ pg TXB$_2$/10$^8$ platelets (range 10–1789 pg). In group A, TXA$_2$ generation was more than 2 standard deviations above the normal mean in six of the 12 patients studied. The mean value in this group of patients was significantly increased (mean $2430 \pm 683$ pg TXB$_2$/10$^8$ platelets, range 15–5000 pg) compared with normal subjects ($p < 0.01$). In group B patients (propranolol-treated), no platelet TXA$_2$ generation was identified in six of the 10 patients studied, and only small amounts were detected in four (fig. 3). Overall platelet TXA$_2$ generation in response to thrombin (mean $58 \pm 37$ pg TXB$_2$/10$^8$ platelets) was reduced in group B patients compared with either group A patients or the normal subjects (both $p < 0.001$).

Data were examined in relation to platelet TXA$_2$ generation and plasma concentrations of $\beta$ thromboglobulin and TXB$_2$, but no relationships were detected. There were no identifiable clinical features that differentiated patients with high in vitro platelet TXA$_2$ generation from those with low TXA$_2$ generation. Four patients in group A with increased platelet TXA$_2$ generation (above the normal mean $\pm 2$ SD) in response to arachidonate also had high TXA$_2$ generation in response to thrombin. However, two patients (group A) had increased thrombin-induced TXA$_2$ generation without a concurrent increase in arachidonate-induced TXA$_2$ generation. Likewise, two other patients with increased arachidonate-induced TXA$_2$ generation did not have a concurrent increase in thrombin-induced TXA$_2$ generation.

**Discussion**

The results of the present study show that propranolol therapy in patients with stable chronic coronary
heart disease does not affect platelet release of β thromboglobulin and thromboxane A₂ in plasma at rest. However, platelet TXA₂ generation upon stimulation with arachidonate and thrombin in vitro is markedly reduced.

Increased platelet activation has been demonstrated in most patients with coronary heart disease. Beta thromboglobulin is secreted from α granules upon platelet activation. In several studies, plasma β-thromboglobulin levels have been shown to correlate with the state of platelet activity and platelet survival in vivo. Patients with angina pectoris and myocardial infarction have a marked increase in β thromboglobulin as well as in platelet factor 4, another protein released from α granules. A close correlation between these two platelet-release products has been observed. Measurement of these release products is a better index of platelet activity than in vitro tests such as platelet aggregation. The present study again confirms elevations in plasma β-thromboglobulin concentrations in some patients with coronary heart disease.

In vivo platelet activation would also be expected to cause release of TXA₂, a very potent vasoconstrictor and platelet aggregant. However, the very short half-life of TXA₂ precludes its direct measurement. Its stable metabolite, TXB₂, can be measured by several techniques. In most patients with stable coronary heart disease, resting plasma TXB₂ levels have been reported to be normal. Nevertheless, spontaneous or acute exacerbation of coronary heart disease may result in marked elevations in plasma concentration of TXB₂ in some patients. No direct correlation between resting plasma β thromboglobulin and TXB₂ concentrations exists, as seen in this and other reports. This lack of correlation probably relates to different half-lives of the two platelet-derived products.

Propranolol has been reported to inhibit platelet aggregation and adhesion and serotonin uptake and release in vitro. In most patients taking propranolol, a marked reduction in platelet aggregation compared with patients not taking propranolol has been observed. These effects of propranolol are thought to be related to its generalized local-anesthetic-like, membrane-stabilizing activity on the platelets. Since addition of ionophore A23187 in the medium counters reduction in platelet aggregation by propranolol, its platelet inhibitory effects are probably due to interference with calcium mobilization in the platelets. Although the in vitro effects of propranolol have been adequately documented, we found that in the usual oral dosages, propranolol exerted no significant effects on plasma β-thromboglobulin and TXB₂ concentrations. The precise mechanisms are not known, but several possibilities exist. Patients with coronary heart disease have reduced platelet survival and increased platelet turnover and, therefore, a more reactive platelet population. Thus, although propranolol may inhibit a given platelet population in vitro, younger platelets in circulation may overcome the effects of propranolol. Propranolol may affect platelet aggregability in some patients, primarily through interference with intracellular calcium mobilization. Since release of β thromboglobulin in the initial stages of platelet activation process appears to be regulated by α receptors, blockade by propranolol may not influence these components of platelet activity.

The observations of increased TXA₂ generation by platelets when stimulated in vitro in patients with coronary heart disease not taking propranolol may be of significance. Neri Serneri et al. demonstrated increased TXA₂ generation in patients with coronary heart disease in different stages; stable angina, unstable angina, and after myocardial infarction. Szczeklik et al. also demonstrated enhanced conversion of arachidonate to TXA₂ by platelets from survivors of myocardial infarction. Mehta et al. found increased malondialdehyde synthesis by platelets from patients with unstable angina pectoris. Increased ability to generate TXA₂ may become important during states of stress in these patients. Indeed, enhanced TXA₂ release during spontaneous or pacing-induced myocardial ischemia...
and during exercise has been well documented in patients with coronary disease. 11, 12, 35 TXA2 released during stress may modulate vascular tone in atherosclerotic vessels. 12, 33 or hyperactive coronary arteries may be instrumental in gene expression or propagation of myocardial ischemia. Frishman et al. 17 suggested that the absence of an expected increase in platelet aggregation may be part of a mechanism of beneficial actions of propranolol in stress-induced myocardial ischemia. In a recent study by Goldstein et al. 36 propranolol therapy normalized platelet survival. The authors speculated that precipitation of myocardial ischemia after abrupt withdrawal of propranolol is related to exacerbation of platelet hyperactivity. Demonstration of marked reduction in platelet TXB2 generation in most patients taking propranolol in our study is consistent with the observations of Frishman et al. 17 and Goldstein et al. 36 Whether a rebound increase in TXA2 generation occurs after abrupt propranolol withdrawal is not known, but it is a plausible hypothesis.

In atherosclerotic coronary vessels, generation of vasodilator and platelet disaggregant prostacyclin is decreased. 35, 37-40 In addition, platelets from patients with coronary atherosclerosis have enhanced sensitivity to TXA2 and decreased sensitivity to prostacyclin. 33, 34 These mechanisms may be involved not only in the genesis of clinical manifestations of coronary heart disease, but also in the evolution and propagation of atherosclerosis. 35-37 Inhibition of platelet TXA2 generation by propranolol may be important in modifying the abnormal platelet–vessel wall interaction in patients with coronary heart disease.

In conclusion, routine propranolol therapy markedly reduces the capability of platelets to generate TXA2. This finding could be related to modification of hemodynamic effects of stress by propranolol in patients with coronary heart disease. However, variables of platelet activation such as plasma β thromboglobulin at rest are not modified by routine propranolol therapy.

Acknowledgment
The authors thank Pamlette Kinsey for secretarial assistance.

References
The Interaction of Sodium Nitroprusside with Human Endothelial Cells and Platelets: Nitroprusside and Prostacyclin Synergistically Inhibit Platelet Function

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SUMMARY Sodium nitroprusside (NP) is a potent vasodilator that also inhibits platelet aggregation. To test the hypothesis that NP causes both of these effects by altering the balance between prostacyclin (PGI2) produced by endothelial cells and thromboxane A2 (TXA2) produced by platelets, we incubated each of these cell types with NP for 5 minutes and assayed the PGI2 and TXA2 produced. NP at pharmacologically achieved doses (0.01-30 μg/ml) inhibited platelet aggregation and resultant TXA2 synthesis in a dose- and time-dependent manner (p < 0.001). The inhibition was not dependent on cAMP production, external calcium concentration, or suppression of TXA2 synthesis. NP did not alter the production of PGI2, by cultured human endothelial cells as measured by radioimmunoassay for 6-Keto-PGF1α, the stable hydrolysis product of PGI2. However, supernates of NP-treated endothelial cells containing low, noninhibitory concentrations of NP unexpectedly inhibited platelet aggregation. This inhibition of platelet aggregation was due to synergy between PGI2 (0.1-3 nM) and NP (p interaction < 0.003). The synergistic inhibition by NP and PGI2 of platelet aggregation and TXA2 synthesis in vivo may explain some of the beneficial actions of NP in the treatment of hypertension and congestive heart failure.

SODIUM NITROPRUSSIDE (NP) is a vasodilator widely used to treat hypertensive crises and congestive heart failure complicating myocardial ischemia. It is one of a group of vasodilators that includes nitroglycerin, dipyridamole, verapamil and hydralazine, which also inhibit platelet aggregation in vitro.1 NP also inhibits the formation of platelet aggregates in vivo.2,8 and part of its beneficial effect may result from this inhibition.

We recently demonstrated that nitroglycerin induces human endothelial cells in tissue culture to synthesize prostacyclin (PGI2),9 a potent, naturally occurring vasodilator and platelet inhibitor.9,10 The PGI2 produced by these endothelial cells inhibits platelet aggregation in vitro. To explore the hypothesis that NP modulates vascular tone and relieves ischemia by shifting the ratio of PGI2 to thromboxane A2 (TXA2) in the circulation toward PGI2 excess, we studied the effect of NP on endothelial cells and platelets.

Methods

Platelet Aggregation and Thromboxane B2 Production

Platelet-rich plasma (PRP) was prepared from venous blood and platelet counts were determined on a model ZBI Coulter counter by methods described.11 Aggregation was induced with the following aggregating agents (expressed as final concentration in the cuvette): sodium arachidonate (Nu Chek), 0.1-3 mM; fibrillar bovine collagen (Hormon-Chemie), 0.6-1.6 μg/ml; and epinephrine (Parke Davis), 2-4.3 μM. Platelet aggregation was performed in a dual-channel
Effects of propranolol therapy on platelet release and prostaglandin generation in patients with coronary heart disease.

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Circulation. 1982;66:1294-1299
doi: 10.1161/01.CIR.66.6.1294

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/66/6/1294