Reversal of Abnormal Platelet Aggregability and Change in Exercise Tolerance in Patients with Angina Pectoris Following Oral Propranolol

By William H. Frishman, M.D., Babette Weksler, M.D., James P. Christodoulou, M.D., Charles Smithen, M.D., and Thomas Killip, M.D.

SUMMARY

Nineteen patients with severe but stable angina pectoris who had positive ECG response to exercise on a bicycle ergometer and 11 normal subjects were studied. Patients received placebo for six weeks and were then randomized into placebo (n = 9) and propranolol (n = 10) treatment groups. Threshold for platelet aggregation in response to adenosine diphosphate (ADP) was measured in fresh platelet rich plasma.

Mean concentration of ADP necessary for a biphasic threshold aggregation response was 1.56 μM (geometric mean) in patients and 3.85 μM in normals (P < 0.01). Serial studies with placebo showed no significant change in ADP threshold. With propranolol, 80 mg/day, platelet aggregation in response to ADP was entirely normalized; 3.79 μM ADP produced maximal aggregation compared to 1.32 μM before therapy (P < 0.01). No additional changes were noted with propranolol, 160 mg/day. In the propranolol-treated patients (80 mg/day) who demonstrated reduction in ADP-induced platelet aggregation, total work during exercise increased by 128%, from 765 ± 125 (standard error of mean) kpm during the control period to 1,792 ± 285 kpm (P < 0.01).

Thus patients with angina and abnormal exercise tolerance demonstrate increased platelet aggregability in vitro which is restored toward normal with propranolol in dosage sufficient to improve exercise tolerance.

Additional Indexing Words:

Coronary artery disease Arteriosclerosis

Platelet dysfunction may be a factor in the pathogenesis of coronary artery disease. Platelets initiate thrombosis by aggregating at the site of previous vascular injury and it is speculated that altered or accelerated platelet aggregation may play a significant, albeit not yet fully defined role in the development and progression of arteriosclerotic lesions. There are conflicting reports concerning the status of abnormal platelet function in patients with coronary artery disease. Reliable and reproducible studies of human platelet aggregation are difficult because of the variety of factors which alter platelet responsiveness such as age, sex, diet, blood lipid and glucose levels, and smoking history. Many commonly used drugs significantly influence platelet aggregability in vitro. Venipuncture techniques, rapidity of sample processing, and ambient changes in pH can all contribute to altering platelet responsiveness with any of the standard laboratory methods measuring aggregation threshold. Platelet aggregates have been observed in the coronary microvasculature in an animal model following catecholamine infusion and after acute, severe stress. Infusions of adenosine diphosphate (ADP) induce coronary arterial platelet thrombi associated with myocardial necrosis in swine similar to that noted following catecholamine administration. It has been postulated that therapy directed at altering the platelet response to endogenous release of catecholamine or local release of ADP might alter the likelihood of acute thrombotic events in patients with coronary artery disease.

The present study was designed to test the hypothesis that patients with angina pectoris have altered platelet aggregability when compared to a normal group similar with regard to age and sex.
addition, the effects of treatment with therapeutic
doses of oral propranolol, a beta-adrenergic blocking
agent with known anti-anginal properties but inade-
quately defined effects on platelet function, were also
assessed.

Methods

Nineteen patients with presumed ischemic heart disease
were studied. Criteria for inclusion were: 1) At least three
attacks of angina pectoris per week, responsive to
nitroglycerin, with no evidence of an accelerated course; 2)
absence of valvular heart disease, hypertension, congestive
heart failure, chronic obstructive pulmonary disease,
diabetes, lipoprotein abnormalities, anemia, smoking
history, or myocardial infarction within six months; 3) 
definite electrocardiographic (ECG) evidence of myocardial
ischemia associated with chest pain during submaximal ex-
ercise stress testing.

Pertinent clinical data of these 19 patients are presented
in tables 1 and 2. There were eleven male and eight female
patients with an average age of 54. Four patients had a
history of previous myocardial infarction. Three patients
without previous myocardial infarction had undergone cor-
ony angiography. In each case, narrowing of at least 70% of
one or more of the major coronary arteries was seen.

Eleven normal subjects with a negative stress test were
studied along with the patient group. These subjects had no
evidence of ischemic vascular disease, lipid abnormalities or
diabetes, and each had a negative history for cigarette smok-
ing and drug ingestion of any sort. Pertinent clinical data of
the patients with angina are compared with those of the nor-
mal subjects in table 2.

Protocol

All normal subjects had platelet aggregation studies and
exercise tests as described below.

The patients with angina pectoris were followed bimonthly by the same physician (W.F.). Informed consent
was obtained, and all cardiovascular medications were dis-
continued except for nitroglycerin. An oral placebo was ad-
ministered for six weeks. Every patient received a resting
electrocardiogram, chest roentgenogram, urinalysis, and
blood analysis for cholesterol, triglycerides, glucose, blood
urea nitrogen, creatine phosphokinase (CPK), lactic
der hydrogenase (LDH), serum glutamic oxaloacetic trans-
maminase (SGOT), hematocrit, hemoglobin, white blood cell
count, and platelet count. Platelet aggregation studies were
performed and exercise testing on a bicycle ergometer
carried out as described below.

After the six week placebo period, patients were ran-
domized into placebo and propranolol treatment groups.
The placebo group (n = 9) remained on the same placebo
regimen while the drug treatment group (n = 10) was
started on 80 mg of oral propranolol daily in four divided
doses. Neither patient nor physician knew whether therapy
was placebo or drug. All patients continued taking
nitroglycerin as needed. After two weeks, platelet aggrega-
tion studies were repeated. The propranolol dosage was then
raised to 160 mg/day, again "double blind" as the pill was
identical in appearance to the placebo. The drug was given

* Mannitol, 225 mg. (Ayerst Laboratories).

in four divided doses and two weeks thereafter studies were
repeated in both groups of patients.

Platelet Aggregation Studies

Blood was obtained after a 12-hour fast. After an initial
resting period of 30 min, a 19 gauge needle was inserted,
without use of a tourniquet, into an antecubital vein and a
slow infusion of physiological saline begun. After 15 min of
rest, with the patient relaxed, blood was sampled by free
flow into plastic tubes containing 1/10 volume acid citrate
dextrose (ACD). The blood specimens were then im-
mediately processed for analysis. The specimens were coded
so that those performing the aggregation studies had no
knowledge of the patient’s identity, the diagnosis, or type of
drug therapy.

Platelet aggregation studies were performed using the
turbidometric method of Born, as modified by Mustard et
al. The blood sample collected a few minutes earlier was
plated in sterile polypropylene tubes and platelet rich
plasma separated (PRP) by immediate centrifugation for 15
min at room temperature at 225 g. After removal of the up-
er 2/3 of the PRP, the remaining PRP was recentrifuged
at 4,300 g for 10 min at 4 C to yield platelet poor plasma
(PPP). Platelet counts were performed on a Coulter Model F
counter, the count of the PRP was adjusted to the range
300–400,000/mm3 when necessary with the autologous PPP.

Platelet aggregation studies were performed in duplicate
within 90 min of venipuncture. The sample was kept tightly
covered at room temperature until analyzed. Samples of
0.45 ml PRP were prewarmed for 1 min with stirring of
siliconized cuvettes placed in a Payton dual channel
aggregation module (Payton Associates, Buffalo, N.Y.)
before aggregating agents were added. The light transmit-
tance was recorded on a Riken Densi linear recorder.
The blank for each study was a similarly treated sample of PPP.
The aggregating agent tested was adenosine diphosphate
(ADP) (Sigma, Inc., St. Louis, Mo.), maintained as a 10–M
frozen stock and diluted essentially twofold just before use
over a range of 10–7M to 10–4M (0.1 to 100 M). The ADP
concentrations producing aggregation were 0.5, 1, 2, 5, 10,
and 20 M. The lowest concentration producing a full
biphasic response was recorded as the threshold dose. The
optimal ADP concentration was assessed without extrapolation
between different concentrations. The change in light transmittance to increasing concentrations of ADP and the
characteristic biphasic response at the threshold dose are il-
ustrated in figure 1. In repeated determinations using
coded samples, reproducibility was within 10% of mean.

Exercise Testing

Bicycle ergometry testing was performed without
knowledge of the patients’ drug status during the study. A
modification of the ear-ensiform system of Abarquez et al. was
used with three apical leads recorded in the supine posi-
tion and on the bicycle ergometer prior to exercise. The
placement of electrodes was as follows: right arm in the left
V4 position, left arm on the forehead, left leg over the en-
siform (xiphoid) process and right leg over the right V4 posi-
tion. Simultaneous recordings were taken of aV1, aV1, and aV5, yielding modified orthogonal X, Y, and Z leads. The
precordial leads were placed in the V1 and V4 position and
the third placed either in the V2 or V3 positions so as to
record the transitional QRS over the precordium. The
modified X, Y, and Z leads were monitored continuously on

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Table 1

Clinical Data in Nineteen Patients with Angina Pectoris

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<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
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Normal values: Hematocrit, 38–53%; blood urea nitrogen, <22 mg/dl; cholesterol, <300 mg/dl; triglycerides, 0–160 mg/dl; fasting blood sugar, 62–108 mg/dl; glutamic oxaloacetic transaminase, 4–40 units; lactate dehydrogenase, 90–225 units.

*Myocardial infarction.
tim:

- Blood pressure in the brachial artery was recorded by sphygmomanometer prior to and immediately after exercise. The heart rate-blood pressure product, an approximation of myocardial oxygen consumption, was calculated from measurements obtained immediately after cessation of exercise.

**Propranolol Blood Levels**

The concentration of propranolol in blood was measured from coded samples without knowledge of previous therapy. The fluorometric method of Black and associates, as modified by Shand et al., was used. Four milliliters of plasma, obtained approximately two hours after the most recent dose of propranolol or placebo, were alkalinized with 1 ml of 1 N NaOH and extracted into 12 ml of heptane containing 1.5% isomyl alcohol. After centrifugation, 10 ml of the organic phase was extracted into 1.5 ml of 0.1 N HCl and the fluorescence of the acid phase measured in an

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**Figure 1**

Aggregation response of platelets from a normal subject induced by different concentrations of adenosine diphosphate (ADP). Degree of aggregation is related to the increase in light transmission since clumping reduces light absorption. Arrows indicate the addition of different concentrations of ADP to stirred platelet-rich plasma. 5 μM ADP induces the characteristic biphasic aggregation threshold response of an initial plateau followed by maximal aggregation. 1 μM and 2 μM ADP are inadequate to achieve threshold or maximal aggregation. 10 μM ADP induces maximal aggregation but at this higher concentration the biphasic threshold cannot be detected.
PROPRANOLOL AND PLATELET ACTIVITY

Aminco-Bowman spectrophotometer (Maximum excitation, 295 μ; maximum emission, 360 μ, uncorrected). For each patient duplicate plasma blanks and a standard of 100 ng per ml of known propranolol added to the plasma blank were determined.

Statistical Analysis

For all of the variables except ADP, arithmetic group means with the standard error of the mean are presented. For ADP data, geometric means are presented. Geometric means are preferred since the concentrations of ADP used were essentially geometric dilutions. Tests of significance for results obtained in normals and untreated patients, as well as between the two groups of untreated patients, were performed utilizing the Wilcoxon Rank Sum Test. Comparison of data from the same group with various experimental protocols were performed using the Wilcoxon Signed Rank Test or by the Sign Test.

Results

Platelet Aggregation Studies

Patients with angina pectoris demonstrated increased platelet sensitivity to aggregating concentrations of ADP when compared to the control subjects exposed to the same concentration of ADP. Mean concentration of ADP necessary for a biphasic aggregation response was 1.56* μM in the group with angina pectoris, a value significantly less than the 3.85* μM observed in the normal controls (P < 0.01) (table 3, figure 2).

Serial studies performed on the patients with angina pectoris receiving placebo showed no change in the increased platelet sensitivity to ADP (table 3).

Administration of propranolol in a dose of 80 mg/day had a dramatic effect in reducing platelet sensitivity to ADP in patients with angina pectoris. After propranolol a mean of 3.79* μM ADP was required to produce a biphasic aggregation response compared to 1.32* μM before therapy (P < 0.01). No additional changes in ADP concentration to effect aggregation threshold were noted after the propranolol dosage was increased to 160 mg/day, (fig. 3, table 3). The mean concentration of ADP required to produce the threshold aggregation response after 80 mg/day of propranolol was not significantly different from the value observed in the normal controls, but was significantly different from placebo-treated patients (P < 0.01) (table 3). An example of the effect of propranolol in increasing the dose of ADP required for platelet aggregation in a patient with angina pectoris is shown in figure 4.

Mean serum concentration of propranolol was 21 ± 4.0 ng/ml (range: 11–49 ng/ml) at a dose of 80 mg/day and 49 ± 9 ng/ml (range: 25–110 ng/ml) at a dose of 160 mg/day.

*Geometric mean.

There were no significant changes from control in the mean level of blood glucose, urea nitrogen, SGOT, CPK, LDH, cholesterol, triglycerides, hematocrit, or platelet count (table 4) during the serial sampling periods in either the control, placebo or drug treatment group.

Exercise Tests

During the control period, all 19 patients with angina had a positive electrocardiographic response to

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Table 3

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3.85†

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1.78† 1.73† 1.67†

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1.32† 3.79† 3.46†

*Adenosine diphosphate in concentration needed to reach aggregation threshold.
†Geometric mean.
exercise. Administration of propranolol was followed by a significant increase in exercise tolerance in the patients with angina pectoris. In the ten patients who received propranolol (80 mg/day), total work during exercise increased by 128%, from \(765 \pm 125\) kpm during the control period to \(1,790 \pm 285\) after propranolol \((P < 0.01)\) (fig. 5). This beneficial effect on work performance was associated with a significant drop in the heart rate-blood pressure product from \(16,800 \pm 1,500\) before to \(12,000 \pm 890\) after propranolol \((P < 0.01)\) (fig. 6). In each case typical anginal pain was the end-point. In contrast, there was no significant change in exertional tolerance in patients with angina pectoris who received a placebo. When the dose of propranolol was increased to 160 mg/day, work performed during maximal tolerated exercise decreased slightly with either pain or fatigue the end-point, but the mean for the group was not significantly different from that observed with 80 mg/day.

**Figure 2**
Comparison of ADP-induced platelet aggregability in normal subjects and patients with angina pectoris. Platelets of normal subjects require significantly higher concentrations of ADP (geometric mean) to attain aggregation threshold than do platelets of patients with angina pectoris.

**Figure 3**
Effect of propranolol on platelet aggregability in patients with angina pectoris. After propranolol, 80 mg/day, platelets of treated patients are less sensitive to ADP (geometric mean) compared to before treatment and not significantly different from normal. Platelet responsiveness was not further changed when the dose of propranolol was doubled to 160 mg/day.
Discussion

A number of abnormalities in platelet function have been reported in patients with ischemic heart disease.7-11,32-34 Platelet hyperresponsiveness has been described in myocardial infarction7-9,32 and cerebrovascular disease.35 However, other investigators have failed to detect differences in platelet function in patients with arteriosclerotic heart disease when compared to normal subjects.9,10,12,36 Wide differences in methodology and patient selection may account in part for these divergent findings.22,36

Some factors that influence the reliability and reproducibility of platelet aggregation analyses have been identified. Repeated measurements of human

Figure 4

Aggregation with and without propranolol in a patient with angina pectoris. Left panel, during placebo therapy, 2 μM ADP required for aggregation threshold. Right panel, after 80 mg/day propranolol orally, 2 μM ADP is insufficient to achieve aggregation threshold. Five μM ADP is now required and response is within normal limits.

Figure 5

Changes in work performance after propranolol treatment in patients with angina pectoris. Circles depict individual values before and squares after propranolol treatment. A significant increase in mean total work performance occurs with 80 mg/day of oral propranolol. Work is measured in kilo-pond meters (kpm).

Figure 6

Changes in heart rate-systolic arterial pressure product (HR × BP) after propranolol in patients with angina pectoris. Circles depict values before and the squares after propranolol treatment. A significant decrease in HR × BP at the onset of angina, even though exercise level was higher after treatment, is demonstrated at the end of exercise with 80 mg/day of oral propranolol.
platelet aggregation induced by ADP vary from day to day depending on the subject’s emotional state, smoking history, or with acute changes in blood lipids, glucose, free fatty acids, catecholamines, hematocrit, and platelet count. Differences in age and sex of the subjects studied may also affect the comparability of previously reported studies. The technique of blood sampling and processing and the rapidity with which studies are performed is also important. Since many drugs influence platelet function, it is imperative that this factor be rigidly controlled. In our study an attempt was made to minimize the effects of the variable factors influencing platelet function by carefully selecting and closely following a population of reliable patients with documented ischemic heart disease and comparing them to a group of normal controls, similar in age and sex.

The present study demonstrates a highly significant increase in ADP-induced platelet aggregability in patients with angina pectoris when compared to normal subjects. There are several possible explanations for this observation. Platelets are known to react with components of injured vascular endothelium such as exposed basement membrane and collagen by adhesion and aggregation, an effect mediated by ADP. Platelets passing through narrow and sclerotic vessels may thus be rendered hyperaggregable. Platelet aggregation induced by these or other mechanisms might cause intravascular thrombi further narrowing the lumen. Thrombi produced by platelet aggregation on ruptured arteriosclerotic plaques may induce acute myocardial infarction or ischemia by obstruction or distal embolization of the aggregates. Our results may be reflecting the secondary effect of vascular disease on platelet aggregation. We demonstrated coronary artery disease in three of our patients by coronary arteriography. The other patients had typical angina pectoris with abnormal exercise electrocardiograms and it seems probable that most of them had significant coronary vascular disease as well.

Since catecholamines are known to alter platelet function, an elevation of plasma catecholamine levels in the patient population could have influenced our findings. Experimentally, catecholamine infusion or acute stress induce platelet aggregation in the coronary microcirculation with subsequent myocardial necrosis. Antiaggregation substances protect against this effect. Catecholamine levels were not measured in either the normal or patient group, and it is possible that anticipation of exercise, particularly in those patients prone to pain, might have acutely elevated plasma catecholamines and affected platelet aggregability. However, in the patient group no evidence of excessive catecholamine release could be detected by gross measurements such as heart rate, and all studies were done with the patients relaxed and reassured. Furthermore, platelet aggregability studies were performed in other clinical settings, when establishing reproducibility of assay, and the results were consistent with pre-exercise values.

Free fatty acids can also alter platelet aggregability but increased levels are generally secondary to stress-induced rise in catecholamines. An alpha-globulin that inhibits platelet aggregation has recently been described in normal plasma. The role of this factor in platelet aggregability of patients with angina pectoris is conjectural.

Alternatively, our findings may be a reflection of a primary platelet abnormality in patients with angina pectoris. This was suggested by Hampton and Gorlin who showed abnormalities in platelet function not only in patients with symptomatic coronary vascular disease, but also in their healthy relatives, raising the possibility of a familial defect.

Although the exact mechanism for the increased platelet aggregability in patients with angina pectoris remains unknown, the clinical implications of this observation are of interest. Experimental myocardial infarction has been observed secondary to platelet aggregation although the aggregates persisted for only a few minutes. Haerem reported that platelet aggregates in the epicardial coronary arteries of patients who died suddenly of cardiac causes were more numerous and larger than those found in patients without cardiac Disease. Extension or reinfarction is common in patients dying with cardiogenic shock and may be due to formation of microaggregates mediated by a local release of ADP from ischemic myocardial cells, circulating platelets, and red blood cells. Agents which could alter platelet aggregability may have a role in the treatment of coronary artery disease, possibly in the prevention of acute ischemic complications. Further clinical data will be required, however, to evaluate the significance of platelet dysfunction in the infarction syndrome.

In this study, 10 patients who received propranolol in dosage of 80 mg/day showed a dramatic and significant increase in performance during standard exercise. Propranolol improved the exercise response and restored the increased platelet aggregability induced by ADP toward normal in most patients with angina pectoris. The platelet effect was noted with serum levels of propranolol as low as 11 ng/ml, was not further influenced by increasing the dose, and persisted over a long period of time with chronic administration of the drug. It is also of interest that the improvement in exercise tolerance was also maximal at the lower dose of propranolol and was not enhanced by increasing dosage.

The improved exercise tolerance in patients with
PROPRANOLOL AND PLATELET ACTIVITY

angina pectoris receiving propranolol was associated, in all cases, with a decrease in the heart rate-blood pressure product, an effect which has been demonstrated by several investigators.6, 47 While the improved exercise performance in patients with angina pectoris receiving propranolol can be correlated with reduction of myocardial oxygen consumption,6, 48 the possibility that the decrease in platelet aggregability influenced cardiac performance, perhaps by improving capillary blood flow, merits consideration.

The effect of propranolol itself on platelet function is controversial.18, 50-54 It has been suggested that catecholamine-induced aggregation is an alpha-adrenergic response since phentolamine prevents epinephrine-induced platelet aggregation.59 Others have speculated that with beta-adrenergic blockade, the alpha receptor on the platelet could be unmasked, and aggregation responsiveness heightened.51 Thomas, however, showed that propranolol, in vitro, using a concentration of 10 μM, reduced platelet aggregability.52 Haft and his associates have recently demonstrated that propranolol prevents isoproterenol-induced intravascular platelet in the rat.64

Propranolol has multiple physiologic actions and may be of potential value in patients with ischemic heart disease through several mechanisms. In addition to reducing cardiac work during exercise and thus reducing myocardial oxygen demand,65 the drug shifts the oxyhemoglobin dissociation curve to the right66 and also has an antitremor effect.67 In addition, the present study documents that propranolol abolishes platelet hyperresponsiveness to ADP in patients with angina pectoris.

If hyperaggregable platelets play a role in the pathogenesis of myocardial ischemia, an agent which reverses this abnormality may be an important adjunct in chronic treatment. The mechanism of action of propranolol in reducing platelet hyperaggregability and the long-term effectiveness of oral propranolol in preventing thrombotic events in patients with coronary artery disease remains to be determined.

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Reversal of Abnormal Platelet Aggregability and Change in Exercise Tolerance in Patients with Angina Pectoris Following Oral Propranolol

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