Evaluation of Thromboxane Production and Complement Activation During Myocardial Ischemia in Patients With Angina Pectoris

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Background. The complement system and arachidonic acid metabolites are involved in severe myocardial ischemia such as myocardial infarction. Furthermore, there is experimental evidence for C5a participation in thromboxane production.

Methods and Results. We examined whether C5a and thromboxane are produced during brief and reversible episodes of myocardial ischemia induced in patients with stable angina. Twenty-five patients underwent either atrial pacing or percutaneous transluminal coronary angioplasty associated with arterial and coronary sinus blood sampling. Rapid atrial stimulation of patients with effort angina caused significant ST segment depression (ΔST = −1.7 ± 0.2 mm), decreased fractional lactate extraction (from +12.8 ± 2.5% baseline to −13.7 ± 4.6% at peak ischemia, n = 13, p < 0.001), and increased coronary sinus plasma thromboxane B2 levels (from 345 ± 85 pg/ml baseline to 1,684 ± 64 pg/ml at peak ischemia, p < 0.01). Changes of fractional lactate extraction correlated significantly with changes of coronary sinus plasma levels of thromboxane B2. There was no change of coronary sinus 6-keto-PGFα levels. Similar pacing of control subjects (n = 6) did not cause release of lactate or thrombaxane. Seventeen other patients underwent exercise testing with noninvasive measurements of thromboxane and prostacyclin metabolites in urinary samples collected before and after the test. No detectable increase of urinary 11-dehydrothromboxane B2 was measured in patients with stable angina after exercise-induced myocardial ischemia. However, basal 11-dehydrothromboxane B2 levels were significantly higher in patients with angina (105 ± 25 pg/mmol creatinine, n = 9) than in control patients (45 ± 8 pg/mmol creatinine, n = 8, p < 0.05 between groups). Coronary sinus plasma levels of the anaphylatoxin C5a always remained below 4 ng/ml in patients undergoing pacing. More severe myocardial ischemia after coronary angioplasty (percent lactate extraction decreased from +24.8 ± 2.7% baseline to −41.6 ± 22.4% at peak ischemia, p < 0.05) was not associated with C3a or C5b-9 generation. In all patients, there was never platelet sequestration nor platelet α-granule release (no changes of β-thromboglobulin/platelet factor 4 levels) into the coronary sinus plasma.

Conclusions. Patients with stable angina have chronically increased thromboxane synthesis as assessed by excretion of urinary metabolites. Thromboxane is acutely released into the coronary sinus during pacing-induced ischemia without significant intracoronary platelet aggregation. Complement does not appear to be activated in stable angina during brief and reversible episodes of myocardial ischemia and does not contribute to thromboxane production.

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Thromboxane $A_2$ (TXA$_2$) is a potent coronary vasoconstrictor and platelet proaggregant. Enhanced thromboxane synthesis occurs during unstable angina, recent myocardial infarction, and coronary thrombolysis. The dynamics of cardiac release of TXA$_2$ during myocardial ischemic episodes in patients with stable angina are controversial. Although plasma thromboxane B$_2$ (TXB$_2$) levels are not increased at rest in patients with effort angina, enhanced thromboxane production has been reported during pacing-induced ischemia in these patients.

Complement is activated during myocardial ischemia with deposition of C1q, C3, C5, and C5b-9 complexes in ischemic areas of myocardium. Complement anaphylatoxins may participate directly or indirectly in the induction and spread of ischemia. Indeed, C5a and its derivative C5a des Arg have constrictor activity on vascular strips and are potent leukotactic factors responsible for activation, aggregation, and trapping of granulocytes in myocardium, which lead to decreased coronary blood flow. Even short episodes of ischemia are associated with C5a-induced leukocyte infiltration into the myocardium. In addition, inhibition of the complement system can reduce the extent of myocardial injury after experimental occlusion of a coronary artery. Complement activation occurs in patients with acute myocardial infarction but data are limited concerning angina pectoris, and it is unknown whether complement is activated during episodes of myocardial ischemia in patients with angina pectoris.

There is evidence for a relation between activation of the complement system and thromboxane synthesis. In animals, infusing zymosan-activated complement or activating complement in vivo (e.g., by heparin-prostacycline complexes) causes pulmonary leukosequestration, thromboxane release into plasma, and severe pulmonary vasoconstriction. Partial inhibition of complement activation significantly reduces thromboxane release into plasma and pulmonary vasoconstriction. Moreover, high plasma levels of C5a and TXB$_2$ are associated with the profound pulmonary vasoconstriction caused by heparin-prostacycline complex formation in humans.

Recent experimental studies have demonstrated that intracoronary administration of C5a in pigs induces thromboxane production; moreover, thromboxane receptor blockade blunted the myocardial ischemia and contractile dysfunction caused by C5a administration.

We investigated complement activation during episodes of myocardial ischemia in patients with stable angina to determine whether C5a anaphylatoxin might contribute to thromboxane release. Thromboxane synthesis during transient myocardial ischemia was assessed by exercise testing and analysis of the urinary excretion of 11-dehydroTXB$_2$ (a major metabolite of thromboxane) and by atrial stimulation (ischemia comparable with effort-induced angina) and measurements of TXB$_2$ levels in coronary sinus plasma. In another group of patients with stable angina, we studied more severe ischemia caused by a brief coronary artery occlusion during percutaneous transluminal coronary angioplasty (PTCA).

**Methods**

**Patient Population**

Forty-two patients (37 men and five women aged 56±1 years, mean±SEM) without a previous myocardial infarction were included in this study. The study protocol was accepted by the ethical committee of our hospital and all patients gave their informed consent. In the first group, patients with typical effort angina pectoris underwent atrial stimulation (group 1, n=13). All these patients had a positive exercise stress test, and they developed significant ischemic ST segment depression after mild exercise (greater than 1 mm at 80% or less of the predicted maximal heart rate). Their condition was stable and without chest pain during the 48 hours before catheterization. They received neither antianginal medications for 24 hours nor drugs inhibiting cyclooxygenase for 10 days before catheterization. In the second group of patients (group 2, n=6), we performed PTCA of stenosis (greater than 75% of the lumen diameter) of a major coronary artery. Antianginal medications were not stopped and aspirin (250 mg per day) was prescribed before the procedure. In group 3, six additional patients without angina, taking neither prostaglandin-active nor antianginal drugs, underwent atrial stimulation. These patients developed neither chest pain, ST segment changes, nor lactate production during pacing and served as control subjects. Patients in group 4 had angiographically proven coronary artery disease and underwent exercise test associated with analysis of urinary metabolites of TXA$_2$ and prostacyclin. They took neither antianginal medications for at least 24 hours nor drugs inhibiting cyclooxygenase for 10 days before the exercise test. Urinary samples were collected immediately before and after exercise. Patients who were not able to void twice and those with a negative stress test were excluded from the study. Nine patients who had myocardial ischemia during stress test (defined as chest pain and greater than 1 mm ST segment depression) were included in this group. In group 5 (n=8), healthy volunteers of the same age and sex as patients of group 4 underwent a maximal exercise test that did not provoke signs of myocardial ischemia. Urinary samples were collected before and after the test for eicosanoid measurements.

**Study Protocol**

**Atrial pacing.** After an overnight fast, a 7F pacing and sampling catheter (United States Catheter and Instrument Company [USCI], Billerica, Mass.) was introduced via a peripheral vein into the coronary sinus (CS) and a 7F polyurethane (pigtail) catheter was placed in the ascending aorta for arterial blood sampling. The lines were kept patent by intermittent flushing with saline without heparin. A 12-lead elec-
trocardiogram (ECG) was continuously recorded. After a 10-minute rest, we began CS pacing at the rate of 90 beats/min, which was progressively increased to 150 beats/min over 3 minutes and maintained for 10 minutes. If atrioventricular block was induced, atropine (0.5 mg i.v.) was administered. Our criteria for ceasing atrial stimulation were chest pain, significant ST segment depression (greater than 1 mm), or a 10-minute period of pacing at the maximum rate (150 beats/min). In preliminary studies, we found that peak CS lactate concentration occurred either at the time of chest pain during pacing or within the first minute after the end of pacing, probably corresponding to metabolite washout (data not shown). Therefore, three sets of blood samples were collected: resting before pacing, during the last minute of pacing, and 1 minute after pacing was discontinued. To appreciate the dynamics of metabolite release into the CS, one more sample was drawn 5 minutes after the end of pacing in a subset of patients (n=5). The sampling time with maximal decrease of percent lactate extraction was considered to be the moment of peak ischemia. During each measurement interval, blood samples were taken simultaneously from the aorta and CS and placed on ice.

Percutaneous transluminal coronary angioplasty. We placed a 7F USCI sampling catheter into the CS. A 9F USCI introducer was then inserted into the right femoral artery by using the Seldinger technique. An 8F guiding catheter was placed into the coronary orifice, and baseline samples were drawn from the CS and the side arm of the arterial introducer. The guide wire crossed the stenosis and was placed far beyond the lesion, and the balloon catheter was advanced over the guide wire. The first inflation was short (15 seconds) and at low pressure (3 bars). Guided by clinical and electrical signs of ischemia, we performed a second inflation of the balloon at higher pressure lasting 1–2 minutes; the balloon was then deflated, and samples were simultaneously withdrawn from the artery and the CS. Blood samples were immediately chilled on ice until completion of the procedure.

Exercise testing. All patients provided a basal urinary sample without prior hydration. The exercise was performed on an electrically braked bicycle ergometer (Case 12, Marquette Electronics Inc., Milwaukee, Ill.) according to the Bruce protocol. Blood pressure was measured by a sphygmomanometer, and the 12-lead ECG was continuously monitored. Patients without chest pain or significant ECG changes were asked to continue as long as possible to reach the predicted maximal heart rate. The test was considered positive when both chest pain and 1 mm or more ST segment depression (flat or downward sloping) occurred. After the end of the test, patients were asked to deliver a second urinary sample. After collection, urinary samples were immediately stored at −70°C.

Eicosanoid Measurements

Plasma TXB2 concentrations were measured in patients undergoing atrial stimulation but not in patients who underwent PTCA because they had received aspirin to prevent acute closure. Coronary angioplasty is known to be associated with thromboxane release, and this production is prevented by aspirin regimen. To measure plasma TXB2 concentrations in other patients (groups 1 and 3), 4 ml of blood was collected in test tubes containing ethylene-diaminetetraacetic acid (EDTA) and indomethacin (5 mmol and 10 μmol final concentration, respectively). Samples were immediately transferred to ice and centrifuged at 3,000g for 10 minutes, and plasma was stored at −70°C until further analyses. TXB2 and 6-keto-PGF1α analyses were performed by enzyme immunoassay.28

The urines were analyzed using a thin-layer chromatography purification procedure, and quantitation was done by enzyme immunoassay analysis28 after a previously published procedure that has been validated by gas chromatography/mass spectrophotometry.29

C3a, C5a, and C5b-9 Complex Determinations

Blood samples were drawn on EDTA (54 μl of 15% K3 EDTA) and stored on ice. After centrifugation, plasma was stored in polypropylene tubes at −70°C until analyses were performed. The plasma concentrations of C3a-C3a des Arg and C5a-C5a des Arg were measured using a radioimmunoassay (Amersham, Les Ulis, France).

Plasma C5b-9 complex levels were measured using an enzyme-linked immunosorbent assay (ELISA) for SC5b-9.30 The monoclonal antibody clone 3B1 (against a neoantigen of C5b-9) was absorbed onto polystyrene micro-ELISA plates and used to capture C5b-9; bound antigen was detected using polyclonal rabbit antibodies in combination with biotinated donkey anti-rabbit IgG. Assays were developed using streptavidin-biotinylated horseradish peroxidase complexes. The positive control was inulin-activated serum.

Lactate Determinations

Arterial and CS blood was drawn (2-ml samples) into glass test tubes containing 80 μM fluoride/EDTA reagent. After centrifugation at 3,000g for 10 minutes, lactate levels were measured on supernatants by enzymatic assay (Boehringer Mannheim). We calculated the percent lactate extraction (%L) by subtracting the CS from the arterial concentration and dividing by the arterial concentration.

Hematologic Measurements

Five-milliliter blood samples were collected in EDTA. Arterial and CS blood was drawn simultaneously. Total white blood cell (WBC) and erythrocyte concentrations, hematocrit, hemoglobin, and platelet concentrations were measured by an automated counter (ELT 800, Ortho Diagnostics).
Four-milliliter blood samples were collected in a Diatube-H® containing anticoagulants and inhibitors of platelet aggregation and cooled immediately on ice. Plasma was obtained by centrifugation at 2,500g for 30 minutes. Plasma β-thromboglobulin (βTG) and platelet factor 4 (PF4) levels were measured on the same samples by an enzyme immunoassay (Asserachrom, American Bioproduits Company). We then calculated the ratio of βTG to PF4, which is considered a better marker of in vivo platelet activation than levels of both platelet proteins.31,32

Statistics

All data are expressed as mean±SEM. Changes of variables within groups were evaluated by the Wilcoxon test for paired data. Comparisons between two groups were made by the Mann-Whitney test. Overall comparisons among three groups were made by the Kruskal-Wallis nonparametric analysis of variance followed by the Dunn test for post hoc comparisons.33 Association between variables was determined by linear regression analysis, and a correlation coefficient was calculated between selected biochemical variables. A probability value of less than 0.05 was regarded as significant.

Results

Myocardial Ischemia Induced by Atrial Pacing, PTCA, and Exercise Testing

Atrial stimulation (group 1) caused a significant decrease of fractional lactate extraction (more than 5%) and ST segment depression (greater than 1 mm) in all patients with effort angina, and nine of 13 patients complained of typical chest pain during pacing. Maximum changes of ST segment from baseline were measured in the same lead at peak pacing-induced ischemia. Mean ST segment depression induced by pacing was −1.7±0.2 mm. The percent lactate extraction in these patients with angina decreased from +12.8±2.5% baseline to −13.7±4.6% at peak ischemia (n=13, p<0.001; see Figure 1). In contrast, the fractional lactate extraction did not change in control patients undergoing a 10-minute pace (group 3, n=6; Figure 1). There were no significant ST segment changes in any of these control patients; one patient complained of chest pain during pacing, but this pain was not believed to be of ischemic origin.

In group 2, balloon inflation during PTCA treatment induced reversible chest pain and ST segment elevation in all patients. We performed coronary angioplasty of the left anterior descending artery in four patients and the circumflex artery in two patients. Acute and profound ischemia was measured after occlusion of the artery (%L decreased from 24.8±2.7% baseline to −41.6±22.4% at peak ischemia, p<0.05; see Figure 1). Fractional lactate extraction measured after PTCA- and pacing-induced ischemia (groups 2 and 1, respectively) differed significantly from %L extraction measured in control patients undergoing atrial pacing (p<0.01, see Figure 1).

All patients in group 4 had chest pain during exercise, and mean ST segment depression was −1.8±0.1 mm. In contrast, control patients (group 5, n=8) had no chest pain and no significant ST segment depression during the bicycle exercise test.

Plasma Eicosanoid Levels

Pacing-induced ischemia was associated with the acute release of thromboxane into CS plasma (mean CS plasma TXB2 level increased from 345±85 pg/ml baseline to 1,684±640 pg/ml at peak ischemia; n=13, p<0.01; see Figure 2), causing a positive CS-arterial gradient of TXB2 (from 53±115 pg/ml baseline to 1,201±521 pg/ml at peak, p<0.01). Coronary sinus plasma TXB2 levels did not increase during pacing in control patients (from 338±150 pg/ml to 170±73 pg/ml, n=6, NS). Therefore, after rapid atrial pacing, groups 1 and 3 differed significantly for both CS plasma level and transmyocardial gradient of TXB2 (p<0.05 between groups, see Figure 2). In five patients with angina (group 1), we measured CS plasma TXB2 levels 5 minutes after the end of pacing, demonstrating a rapid decrease of TXB2 levels after pacing was stopped (TXB2 levels were 188±24 pg/ml at baseline, 372±85 pg/ml at peak ischemia, and 230±25 pg/ml 5 minutes after the end of pacing). Concomitant fractional lactate extraction in these five patients was 6±3%, −21±6%, and −9±7%, respectively.

We did not observe significant changes of CS plasma 6-keto-PGF1α levels in patients during atrial stimulation. All plasma 6-keto-PGF1α levels (of both
arterial and CS samples) remained below 300 pg/ml, indicating that TXB₂/PGL₂ ratio was shifted toward thromboxane. There was a 2.6-fold increase in this ratio (p<0.05) after ischemia was induced in group 1, whereas it did not change significantly in group 3.

Two series of plasma samples were successively analyzed for thromboxane levels. Although TXB₂ values (both baseline and peak values) were higher in the first series, the percentage of increase was comparable in both series. The increase of TXB₂ concentrations in CS plasma correlated significantly with the simultaneous decrease of percent lactate extraction in the first series (r=0.68, p<0.05, n=9) as well as in the second series (r=0.91, p<0.001, n=10). The correlation was still significant in the second series when control patients were excluded to consider only patients with angina (r=0.88, p<0.01, n=7).

**Urinary Eicosanoid Levels**

The excretion of 11-dehydroTXB₂ did not change after exercise in both control patients and patients with proven coronary artery disease and exercise-induced myocardial ischemia (see Figure 3). However, urinary 11-dehydroTXB₂ levels were significantly higher in angina patients (105±25 ng/mmol creatinine at rest, n=9, group 4) than in control patients (45±8 ng/mmol creatinine, n=8, group 5, p<0.05 between both groups).

There were no changes of prostacyclin metabolites in group 4 (from 22±3 ng/mmol creatinine at rest to 37±13 ng/mmol creatinine after exercise, NS) and in group 5 (from 36±16 ng/mmol creatinine at rest to 30±5 ng/mmol creatinine after exercise, NS). Subsequently, thromboxane metabolites/prostacyclin metabolites ratio was significantly higher in angina patients than in control patients (4.7±0.9 versus 2.0±0.4), and effort-induced ischemia did not change this ratio in group 4 (from 4.7±0.9 at rest to 3.7±0.7 after exercise, NS).

**C5a, C3a, and C5b-9 Complex Plasma Levels**

All plasma C5α levels in patients undergoing atrial stimulation (groups 1 and 3) remained below 4 ng/ml, and no increases were measured during the episodes of myocardial ischemia.

No significant changes in plasma C3α levels were found during PTCA procedures in group 2. Coronary sinus plasma levels of C3α were 227±35 ng/ml before and 257±41 ng/ml after balloon inflation (p=NS). No significant changes in plasma levels of C5b-9 complex were demonstrated in these patients, as all values remained below 80 ng/ml.

**Leukocyte and Platelet Concentrations**

We measured no significant changes from baseline of WBC and platelet concentrations in both CS and arterial blood after atrial stimulation (see Table 1). Therefore, pacing-induced myocardial ischemia was associated with neither a CS–arterial white cell gradient nor a CS–arterial platelet gradient. Similarly, there were no changes of WBC and platelet concentrations after balloon inflation during PTCA (Table...
Table 1. Hematologic Data

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (pacing)</th>
<th>Group 2 (PTCA)</th>
<th>Group 3 (control)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Peak ischemia</td>
<td>Rest</td>
</tr>
<tr>
<td>WBC (cells/mm³)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>6,896±457</td>
<td>6,786±443</td>
<td>7,666±1,037</td>
</tr>
<tr>
<td>CS</td>
<td>7,020±434</td>
<td>6,980±417</td>
<td>7,900±973</td>
</tr>
<tr>
<td>Platelets (×1,000 cells/mm³)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>260±10</td>
<td>259±9</td>
<td>253±11</td>
</tr>
<tr>
<td>CS</td>
<td>245±9</td>
<td>238±12</td>
<td>244±13</td>
</tr>
</tbody>
</table>

WBC, white blood cell concentrations; CS, coronary sinus; PTCA, percutaneous transluminal coronary angioplasty.

1). No changes of hematocrit and erythrocyte count were measured in any group.

Platelet Factor 4 and β-Thromboglobulin Levels

PF4 and βTG levels did not change significantly after either atrial pacing or PTCA. βTG/PF4 ratios did not increase after atrial pacing in either control patients (from 4.9±0.6 baseline to 5.4±1.4 at peak pacing, \(p=NS\)) or patients with angina (from 5.0±0.7 baseline to 5.2±0.8 at peak ischemia, \(p=NS\)). Similarly, coronary angioplasty induced no change of βTG/PF4 ratio, from 5.4±1.1 baseline to 5.5±1.1 at peak ischemia.

Discussion

We have demonstrated that rapid atrial stimulation of patients with effort angina causes the cardiac release of lactate and thromboxane. The local release of thromboxane is brief, and total amount produced during the period of ischemia is small. Further dilution in circulation may make these acute changes undetectable in peripheral plasma and urines and, in fact, exercise-induced myocardial ischemia is not associated with detectable increased urinary excretion of thromboxane metabolites. However, basal concentrations of these metabolites are significantly higher in patients with angina than in control patients of similar age and sex, suggesting chronic stimulation of thromboxane synthesis in patients with coronary heart disease. Another important finding of this study is that myocardial ischemia and prostanoid synthesis were not associated with C5a release into CS plasma or with leukocyte trapping in the myocardium. Ischemia induced by rapid atrial pacing is mild, the relative efficiency of C5a cleavage is low relative to C3a cleavage, and C5a is rapidly bound to neutrophils, all conditions that could minimize evidence for C5a generation in vivo. However, coronary artery occlusion during PTCA caused more severe myocardial ischemia and induced neither C3a release into CS plasma nor C5b-9 complex formation; both are more accurate markers of complement activation in vivo than C5a anaphylatoxin levels. Thus, in patients with stable angina, the complement system is not activated during brief and transient episodes of myocardial ischemia and does not correlate with thromboxane release. Increased synthesis of thromboxane was also not associated with platelet aggregation because there was neither coronary platelet sequestration nor significant platelet α-granule release into CS plasma. These data may help to define mechanisms of thromboxane release in humans.

Activation of the complement system with generation of C5a and C5a des Arg anaphylatoxins occurs in experimental models of myocardial ischemia. Intracoronary administration of C5a anaphylatoxin to pigs causes myocardial leukocyte sequestration and thromboxane release into the coronary circulation. In humans, after acute myocardial infarction, complement is activated and the accumulation of C5b-9 complexes has been detected in infarcted regions. It was not known whether complement was activated during reversible episodes of myocardial ischemia in patients with angina pectoris. In the present study, we did not demonstrate C5a generation or myocardial sequestration of leukocytes during pacing-induced ischemia. Complement activation generates much larger amounts of C3a than C5a, and detection of SC5b-9 is a more sensitive indicator of terminal pathway activation than the production of C5a. Although the brief coronary artery occlusion during PTCA caused more serious ischemia than pacing, it did not induce C3a or C5b-9 release. Both types of ischemia (during pacing and PTCA) are short and reversible, and may be too mild to produce significant complement and leukocyte activation. Complement may play a role after more prolonged cessation of flow, thereby enhancing severe ischemia and myocardial injury. Experimental studies in dogs corroborate this hypothesis since complement C1q deposition in myocardium was observed after 45 minutes of coronary occlusion and reperfusion but not after 5 minutes of ischemia. Yasuda et al recently reported complement activation in patients with myocardial infarction but there was no complement activation at rest in patients with stable angina. Our results are consistent with their data, further demonstrating that complement is not activated during brief episodes of myocardial ischemia induced by either atrial pacing or PTCA in these patients.

Pacing-induced ischemia was associated with increased CS plasma TXB₂ levels in patients with stable angina, whereas there was neither lactate nor thromboxane release in control patients. Our data demonstrating thromboxane production are similar to those reported by some studies but at variance with other studies. Any differences may be
related to differences in methodology such as inclusion criteria, medications, myocardial viability, pacing rates, sampling periods, and degree of ischemia. We recruited patients with a high probability of pacing- and exercise-induced ischemia (group 1 and 4, respectively) and no treatment that might influence the occurrence of myocardial ischemia and prostanooid synthesis. Myocardial ischemia occurred consistently in these patients during atrial pacing and lactate levels paralleled TXB2 levels in CS plasma. The correlation between the changes of lactate extraction and thromboxane release suggests a role for thromboxane in this type of myocardial ischemia. Measurements of transcardiac gradients have been used extensively to overcome dilution problems when evaluating markers of platelet activity. Potential activation during this invasive sampling technique must be considered, and study protocols should always be conducted over a short period of time with control measurements. In our study, thromboxane release was acute, returning to baseline values after the end of pacing with a transmyocardial gradient of thromboxane during ischemia and no thromboxane release in the control group (group 3), indicating that real myocardial production of thromboxane related to myocardial ischemia.

In an in vivo noninvasive method of evaluating thromboxane production, we could not demonstrate increased urinary excretion of thromboxane metabolites after exercise-induced myocardial ischemia (group 4), confirming recently published data. However, considering the fact that these metabolites represent 6.8% of the generated thromboxane, the small amounts of thromboxane locally released cannot be differentiated from the basal level. The significant difference of thromboxane metabolite concentrations between patients with angina and control patients (group 5) indicates a chronic stimulation of thromboxane synthesis in patients with stable angina pectoris. The ratio calculated from the urinary metabolites of thromboxane and prostacyclin confirms the significant difference between groups 4 and 5, reflecting in patients with angina a chronic imbalance of the ratio that has been incriminated in mechanisms of myocardial ischemia. Repeated episodes of ischemia with no clinical symptoms (silent ischemia) may have occurred and been associated with the release of small amounts of thromboxane similar to those measured in CS during atrial pacing. We cannot exclude that ischemia and thromboxane synthesis may have been exacerbated by ceasing antianginal therapy 24 hours or more before inclusion.

The βTG/PF4 ratio is a marker of in vivo platelet activation, with increases from 5:1 to 10:1 indicating platelet activation. Both pacing- and PTCA-induced ischemia had no effect on βTG/ PF4 release into CS blood. Our results as well as those of Verheugt et al demonstrate that in stable coronary artery disease, there is no significant intracoronal platelet α-granule release either at rest or during pacing. These findings associated with the lack of platelet trapping in the myocardium during the ischemic period confirm that platelet aggregation was only minor in our patients. Although the hypothesis of platelet aggregation in stable angina was initially proposed, several reports strongly suggest that platelets are not activated in stable angina, contrasting with the major platelet activation measured in patients with unstable angina.

We measured elevated CS plasma TXB2 levels during transient myocardial ischemia without concomitant global platelet activation. Similar data have been reported in the same type of patient population. In vitro, stimulation of platelet thromboxane synthesis can occur without platelet release or aggregation and may explain this apparent dissociation. Platelets or the interaction of platelets with endothelium might release thromboxane during myocardial ischemia without further activation or aggregation of platelets. An alternative hypothesis would require a different cellular source of thromboxane. Experimental studies have demonstrated that subtotal platelet depletion does not prevent thromboxane release and thromboxane-dependent vasoconstriction. Leukocytes, monocytes, macrophages, or resident cells can synthesize TXA2 and may participate in the thromboxane release measured into coronary sinus plasma during acute myocardial ischemia.

Conclusions

In patients with stable angina, thromboxane is acutely produced during pacing-induced myocardial ischemia without concomitant platelet α-granule depletion and platelet sequestration. Elevated urinary concentrations of thromboxane metabolites in patients with stable angina may reflect chronic stimulation of prostanoid synthesis. The intracoronal release of thromboxane does not require C5a generation, and the complement system is not activated during pacing and PTCA. The precise cellular origin and pathophysiological role of thromboxane during transient myocardial ischemia in patients with stable angina remain to be elucidated.

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