Specific Platelet Mediators and Unstable Coronary Artery Lesions
Experimental Evidence and Potential Clinical Implications

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Acute coronary heart disease syndromes, including unstable angina, variant angina (Prinzmetal's angina), and acute myocardial infarction are usually caused by a primary decrease in myocardial oxygen delivery.\textsuperscript{1-15} Both unstable angina and Prinzmetal's angina are caused by a primary reduction in myocardial oxygen delivery, but in all probability, the mechanisms responsible for the decrease in myocardial blood flow in these two syndromes are generally different.\textsuperscript{4-15} Unstable angina occurs in some patients with endothelial injury or ulceration at the site of a coronary artery stenosis; some patients also have an intraluminal coronary artery thrombus.\textsuperscript{4-6,9-11} Specifically, Falk\textsuperscript{7} and Davies and Thomas\textsuperscript{8} have suggested that atherosclerotic plaque rupture or fissuring may lead to coronary arterial thrombi and the development of unstable angina, acute myocardial infarction, or sudden death. Patients with Q wave (usually transmural) myocardial infarcts often have ulcerated or fissured atherosclerotic plaques and the subsequent development of occlusive coronary artery thrombi.\textsuperscript{2,3} A similarity exists in the coronary arteriographic appearance in the relevant coronary artery in patients with unstable angina and in those patients who develop acute myocardial infarction.\textsuperscript{15} Patients with non-Q wave infarcts (usually subendocardial infarcts) much less commonly have coronary artery thrombi that are permanently occlusive,\textsuperscript{3} but these patients do have transient reductions in coronary blood flow followed by reperfusion.\textsuperscript{16} The transient reductions in coronary blood flow may be related to intermittent platelet aggregations and dynamic vasoconstriction occurring at sites of coronary artery stenosis and endothelial injury.\textsuperscript{4,5}

We have suggested that unstable angina, non-Q wave myocardial infarction, and Q wave myocardial infarcts represent a continuum, such that transient reductions in coronary blood flow associated with platelet aggregation and dynamic vasoconstriction at sites of coronary artery stenosis and endothelial injury lead to the abrupt development of crescendo or unstable angina.\textsuperscript{4,5} If important reductions in coronary blood flow and oxygen delivery are sustained for 20 minutes to 2 hours, a non-Q wave myocardial infarction may occur, and if the period of important reduction in myocardial oxygen delivery persists for more than 2 hours, a Q wave myocardial infarction usually occurs.\textsuperscript{4,5} In this suggested pathophysiologic scheme, factors responsible for the conversion from chronic to acute coronary heart disease syndromes include endothelial injury at sites of coronary artery stenosis\textsuperscript{4,5}; the endothelial injury could be, among other factors, the result of plaque fissuring or ulceration,\textsuperscript{7,8} hemodynamic factors, systemic arterial hypertension, infection, smoking, catheterization, or balloon angioplasty.

With endothelial injury, platelets attach to the exposed subendothelium and collagen, and the platelet aggregation quickly leads to the release and local accumulation of thromboxane and serotonin that promotes further platelet aggregation, dynamic coronary artery vasoconstriction, and consequent reduction in coronary blood flow\textsuperscript{4,5,10-14,17-28} (Figure 1). Platelet aggregation and dynamic coronary artery vasoconstriction probably result from a combination of the local arterial accumulation of thromboxane and serotonin (and possibly other mediators) and relative or absolute decreases in local arterial concentrations of endothelially derived vasodilators and inhibitors of platelet aggregation, such as endothelially derived relaxing factors (EDRFs) and prostacyclin\textsuperscript{20,29,30} (Figure 1).

Chronic endothelial injury at sites of coronary artery stenosis lasting from several hours to days is
associated with the accumulation of platelets, white and red blood cells, and a fibrin mesh, and potentially, this injury may be associated with the accumulation of other mediators contributing to platelet aggregation and dynamic vasoconstriction, including platelet activating factor, selected leukotrienes (LTC₄ and LTD₄), histamine, prostaglandin D₂, and possibly endothelin.⁴,⁵,³¹

This editorial feature presents a conceptual scheme based on experimental and clinical data that may explain, in at least selected patient subsets, the abrupt progression from endothelial injury (plaque fissuring or ulceration or one of the other potential causes for endothelial injury cited above) at sites of coronary artery stenosis to unstable angina and, as part of a continuum, acute myocardial infarction.

**Unstable Angina Pectoris**

Unstable angina pectoris is defined as angina that increases in frequency with progressively less effort, and often, it occurs at rest. Any factor that results in abrupt or progressive coronary artery luminal diameter narrowing may cause diminished myocardial perfusion with consequent myocardial ischemia and unstable angina. Table 1 lists several possible causes of unstable angina. In some patients, the syndrome may result from the progression of severe, multifocal coronary atherosclerosis. However, in many patients, we believe that other mechanisms are involved because a good correlation often does not exist between acute coronary heart disease and the anatomic extent and severity of coronary atherosclerosis.

**Mechanisms Potentially Responsible for the Development or Sustainment of Unstable Angina**

In 1979 and more recently, our group¹⁰ hypothesized the following: 1) Increases in thromboxane and decreases in prostacyclin concentration at sites of endothelial injury and significant coronary artery stenosis cause platelet aggregation and dynamic coronary vasoconstriction. These, in turn, decrease regional coronary blood flow below a critical level and lead to the development or sustainment of unstable angina pectoris. 2) Mechanisms responsible for endothelial injury at sites of coronary artery stenosis vary but probably include a) progression, fissuring, or rupture of atherosclerotic plaques⁷,⁸; b) hemodynamic factors, including flow turbulence; c) cigarette smoking; d) systemic arterial hypertension; e) infection or immune complex deposition; and, on occasion, f) catheter-induced endothelial injury associated with coronary arteriography and angioplasty.

The hypothesis that unstable angina is associated with increases in transmyocardial thromboxane A₂ concentrations in patients was tested from 1979 to 1980.¹⁰ In 60 patients undergoing cardiac catheterization, blood samples were obtained from the coronary sinus and ascending aorta, and prostaglandins were measured by radioimmunoassay.¹⁰ By history, noninvasive evaluation, and the results of cardiac catheterization, patients were separated into five groups. Group A patients (n=6) had congenital and acquired noncoronary cardiac lesions, but they were without significant coronary stenoses; group B patients (n=14) complained of chest pain, but they

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**Table 1. Potential Causes of Unstable Angina Pectoris**

| Progression of coronary artery stenosis causing myocardial ischemia and unstable angina. |
| Platelet aggregation and white blood cell adhesion and platelet- or white blood cell-derived mediator release at the site of endothelial injury and coronary artery stenosis leading to increased luminal narrowing from anatomic obstruction and dynamic increases in coronary vascular resistance. |
| Any combination of plaque fissure, hemorrhage, or thrombosis with progressive coronary artery narrowing. |
| Coronary artery spasm. |

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**FIGURE 1. Schematic diagram indicating the possible mechanisms by which thromboxane A₂ (Txₐ₄₋₅) and serotonin (5HT) promote platelet aggregation and decrease coronary blood flow in the patient with unstable angina.**

Aggregating platelets (stars) release thromboxane A₂ and serotonin at sites of coronary artery stenosis and endothelial injury, which cause further platelet aggregation at that site and downstream, dynamic coronary vasoconstriction, and partial or total coronary artery thrombosis. Absence or reductions in endothelially derived relaxing factor (EDRF) and prostacyclin (PGI₂) at vascular sites with endothelial injury probably contribute to the development of vasoconstriction and thrombosis. See text for details. Modified and reproduced from Hirsch et al.⁴² by permission.
is not clear why three patients in group D had elevated transcardiac concentrations of thromboxane $B_2$. However, two possible explanations include 1) persistent increases in transcardiac thromboxane concentration after the relief of unstable angina and 2) continuing unstable angina not detected clinically or identified by the patient, that is, “silent myocardial ischemia.” We were unable to show differences in transmyocardial prostacyclin concentrations among these patients.$^{10}$

These data showed a temporal relation between continuing unstable angina and increases in transcardiac thromboxane concentrations, and they were consistent with the hypothesis that thromboxane $A_2$ accumulation and consequent platelet aggregation and coronary vasoconstriction are important factors in the pathogenesis of unstable angina in patients. However, these data did not prove a causal relation.

Subsequently, Fitzgerald et al.$^{11}$ at Vanderbilt and Hamm et al.$^{12}$ have shown that many patients with unstable angina have elevated urinary concentrations of a major thromboxane metabolite, that is, 2,3-dinor-thromboxane $B_2$ and that in some patients, further increases occur in the thromboxane urinary metabolite with new episodes of chest pain.

Evaluation of the Physiologic Importance of Thromboxane Accumulation in Causing Intracoronary Platelet Aggregation, Thrombus Development, and Reductions in Coronary Blood Flow

Experimental Animal Models

In the canine heart, a severe proximal coronary artery stenosis with associated endothelial injury causes cyclical coronary blood flow reductions as originally described by Folts et al (Figure 3).$^{17}$ In this model, endothelial injury is caused by the application of an external constrictor and by gentle stroking of the left anterior descending coronary artery with cloth-covered forceps. The cyclical coronary flow alterations are related to platelet aggregation and leukocyte and red blood cell accumulation at the stenotic site (Figures 3 and 4). Dazoxiben (UK-37-248, Pfizer Pharmaceuticals, New York, New York), a thromboxane synthetase inhibitor,$^{18}$ or SQ29,548 (Squibb Pharmaceuticals, Princeton, New Jersey), a thromboxane receptor antagonist,$^{19}$ abolishes or significantly attenuates the frequency of cyclic flow reductions in approximately 70% of treated animals (Figure 3). Thromboxane $B_2$ concentrations in the distal portion of the narrowed coronary artery and at the site of the coronary stenosis and endothelial injury are increased during cyclical coronary flow reductions and are reduced to control values after administration of dazoxiben.$^{18,20}$ In contrast, 6-keto-prostaglandin $F_1\alpha$ (the inactive metabolite of prostacyclin) generation is reduced at the site of coronary artery constriction compared with generation in the nonconstricted, noninjured
coronary artery. The 6-keto-prostaglandin F1\_x levels distal to the narrowed coronary artery increase significantly during cyclic flow reductions and remain elevated after the administration of dazoxiben. In the canine model, systemically administered dazoxiben (2.5 mg/kg body wt i.v.) suppresses arachidonic acid--induced thromboxane A2 (but not prostaglandin E2) production by platelets, but it does not significantly influence prostacyclin synthesis by coronary artery rings. Furthermore, the topical application or intra-atrial administration of a thromboxane-mimetic (U46619) generally restores cyclic coronary flows in this model after they are abolished by a thromboxane synthesis inhibitor. Folts et al have shown that aspirin often eliminates cyclic coronary artery flow reductions in the same experimental model.

Therefore, thromboxane concentration increases at the site of a coronary artery stenosis and endothelial injury in the canine model, and the nar-
rowed, injured artery makes substantially more thromboxane when arachidonic acid is added in vitro.\textsuperscript{18,20} The administration of a thromboxane synthetase inhibitor or receptor antagonist usually abolishes cyclic flow alterations in this model.\textsuperscript{17-19} More recently, we have shown that dynamic coronary vasoconstriction occurs adjacent to the site of stenosis in this canine model and that thromboxane and serotonin receptor antagonists prevent the dynamic vasoconstriction.\textsuperscript{21} Thromboxane synthesis inhibitors and receptor antagonists are also protective against the development of in situ platelet aggregation\textsuperscript{22} in this experimental model.

### Additional Humoral Mediators Influencing Platelet Aggregation and Dynamic Changes in Coronary Artery Tone

In our initial studies, we found that a thromboxane synthesis inhibitor or receptor antagonist was protective and abolished cyclical alterations in coronary blood flow in approximately 70\% of the treated animals. Thus, another mediator(s) appeared to contribute to the development of cyclic flow alterations in this canine model. Subsequent studies showed that serotonin concentration increases by at least 18-fold at the site of a coronary artery stenosis and endothelial injury when cyclic coronary flow reductions occur.\textsuperscript{23} Ketanserin, a serotonin receptor antagonist, usually abolishes cyclic flow reductions\textsuperscript{23,24} and works in essentially every instance in which a thromboxane synthetase inhibitor or receptor antagonist, or both, have failed (Figure 5).\textsuperscript{25} Other serotonin receptor antagonists without \(\alpha\)-adrenergic antagonist properties at the concentrations at which they were used also abolish cyclic coronary flow alterations in approximately 90\% of animals and are effective when thromboxane synthetase inhibitors or receptor antagonists have failed.\textsuperscript{25} After cyclic flows are abolished by serotonin receptor antagonists, they may be restored by the intra-atrial administration of serotonin.\textsuperscript{23} There is also an amplification of thromboxane and serotonin’s effects on cyclic coronary flow variations, such that interfering with the effect of either is generally protective in eliminating cyclic flow alterations.\textsuperscript{25} Thus, both thromboxane and serotonin are important in initiating or sustaining cyclic flow reductions in this experimental model.\textsuperscript{25} If the contribution from both thromboxane and serotonin receptor stimulation is eliminated, cyclic coronary flow reductions are nearly always abolished in this experimental model\textsuperscript{25} and are very difficult to restore even if systemic epinephrine concentrations are markedly increased.\textsuperscript{26}

Recently, subsets of patients with limiting angina and significant coronary heart disease were shown to have increased transcardiac serotonin concentrations.\textsuperscript{13} Moreover, subsets of patients with limiting angina have a vasoconstrictor substance in their coronary sinus effluents whose vasoconstrictor effect is blocked by a serotonin receptor antagonist.\textsuperscript{14}

![Figure 5. Panel A: Bar graph of number of cyclic coronary artery flow variations (CFVs) per hour in dogs during a control period, after abolition of cyclic coronary flows by ketanserin (a serotonin receptor antagonist), and after reestablishment of cyclic coronary artery flows by the infusion of serotonin (1-3 \(\mu\)g/min) directly into the left atrium. Panel B: Bar graph of severity of CFVs, obtained by averaging the two lowest mean flow velocity values (nadir) as a percentage of controlled unconstituted blood flow velocity throughout a 30-minute interval, is shown during the control period of cyclic flows and after the serotonin receptor antagonist, ketanserin, had abolished cyclic alterations in coronary blood flow. The severity of cyclic coronary flows after their restoration by serotonin administration is shown in the far right. The mean flow velocity nadir in serotonin-restored CFVs was taken as a percentage of mean flow velocity after infusion of serotonin but before the restoration of CFVs. \(*p<0.05, \text{ significantly different from control, unconstituted coronary blood flow velocity; } \text{**p}<0.05, \text{ significantly different from coronary blood flow velocity during infusion of serotonin and before CFVs were restored. Reprinted from Ashton et al}\textsuperscript{23} \text{ by permission.}"

Coronary Artery Stenosis and Endothelial Injury in Closed-Chest, Awake, Unsedated Dogs

Chronically instrumented, awake, unsedated dogs develop cyclic coronary flow variations or a persistently reduced coronary blood flow 2-7 days after their original instrumentation with an external coronary artery constrictor and after endothelial injury.\textsuperscript{27} Cyclic coronary flow variations and the low-flow state are often reversed to normal coronary blood flow patterns by thromboxane and sero-
tonin receptor antagonists in this experimental model, just as they are in the open-chest, anesthetized animals. In the awake, closed-chest dog with coronary artery stenosis and endothelial injury, there is a continuum from cyclic coronary flow alterations to a low-flow state and finally an occlusive coronary thrombus.

Interestingly, the awake, unsedated, closed-chest dog also develops cyclic coronary flow alterations or a severe and sustained reduction in coronary blood flow when excited by the presence of a person (Figure 6), by food, or by low-level exercise. This suggests that excitement and low-level effort may lead to platelet aggregation with consequent mechanical obstruction and dynamic reductions in coronary blood flow when coronary artery stenosis and endothelial injury coexist. Thromboxane and serotonin receptor antagonists may prevent the cyclic coronary flow alterations caused by low-level exercise in this model.

One to three weeks after the application of the coronary artery constrictor and endothelial injury and after frequent cyclic coronary flow alterations, we have noticed that chronically instrumented dogs develop marked intimal proliferation that converts a moderately severe coronary artery stenosis to a more critical one. Similar intimal proliferation has been described previously as occurring in endothelially injured carotid arteries in the rat and in the patient who develops restenosis after coronary artery angioplasty. We have suggested that in the stenosed coronary artery with endothelial injury and chronic cyclic coronary flow alterations in the awake, unsedated dog and in patients with restenosis after coronary artery angioplasty, the marked intimal proliferative responses found may be caused by the accumulation of selected growth factors from aggregating platelets or infiltrating white blood cells and activated T cells, including one or more of the following: platelet-derived growth factors, transforming growth factors, epidermal growth factor, fibroblast growth factor, serotonin, endothelin, or a heparin-associated growth factor. Further studies are needed to test this suggestion and to identify which growth factor(s) and which cell population(s) may contribute to the fibroproliferative reaction.

**Clinical Relevance**

**Clinical Studies With Inhibitors of Platelet Aggregation**

If platelet aggregation and the consequent release of thromboxane and serotonin are important in the initiation or sustaining of selected acute coronary heart disease syndromes, including unstable angina, an intervention that interferes with platelet aggregation and the subsequent release of thromboxane or serotonin should reduce morbidity and mortality in patients with unstable angina. Clinical studies have shown that aspirin, which is an inhibitor of cyclooxygenase, thromboxane synthesis, and platelet aggregation, reduces the risk of subsequent nonfatal myocardial infarction and death in patients with unstable angina. Specifically, a multicenter Veterans Administration Medical Center study established that one tablet of aspirin daily (325 mg/day) reduces mortality and the risk of nonfatal myocardial infarction in the weeks after the onset of unstable angina in men. A Canadian study showed that the equivalent of four aspirin tablets per day reduces mortality and the risk of subsequent nonfatal myocardial infarction in the ensuing 2 years in men and women with unstable angina. These data are consistent with the hypothesis that platelet aggregation and subsequent increases in local thromboxane and serotonin concentrations may affect the abrupt conversion from chronic to acute coronary heart disease syndromes in humans through thromboxane and serotonin accumulation leading to further platelet aggregation, coronary vasoconstriction, and, sometimes, the development of occlusive coronary thrombi (Figures 1 and 4).

**Summary**

We have speculated previously that the abrupt conversion from chronic stable to unstable angina and the continuum to acute myocardial infarction may result from myocardial ischemia caused by progressive platelet aggregation and dynamic vasoconstriction themselves caused by local increases in thromboxane and serotonin at sites of coronary artery stenosis and endothelial injury. Platelet aggregation and dynamic coronary artery vasoconstriction probably result from the local accumulation of thromboxane and serotonin and also relative decreases in the local concentrations of endothelially derived vasodilators and inhibitors of platelet aggregation, such as endothelium-derived relaxing factor (EDRF) and prostacyclin. With severe reductions in coronary blood flow caused by these mechanisms, platelet aggregates may increase, and an occlusive thrombus composed of platelets and white and red blood cells in a fibrin mesh may develop. When coronary arteries are occluded or narrowed...
for a sufficient period of time by these mechanisms, myocardial necrosis, electrical instability, or sudden death may occur. We believe that unstable angina and acute myocardial infarction are a continuum in relation to the process of coronary artery thrombosis and vasoconstriction. When the period of platelet aggregation or dynamic vasoconstriction at sites of endothelial injury and coronary artery stenosis is brief, unstable angina or non-Q wave infarction may occur. However, when the coronary artery obstruction by these mechanisms is prolonged for several hours, Q wave myocardial infarction results. Chronic endothelial injury and coronary artery stenosis are probably associated with the accumulation of platelets, white and red blood cells, and a fibrin mesh at the site of stenosis and endothelial injury. This endothelial injury may lead to the accumulation of other mediators that contribute to the development of dynamic vasoconstriction and platelet aggregation, including histamine, platelet activating factor, selected leukotrienes (including LTC₄ and LTD₄), prostaglandin D₂, and possibly endothelin. Future clinical studies should test these hypotheses, thereby providing further insight into possible additional mediators and mechanisms that may affect the abrupt transition from chronic to acute coronary heart disease syndromes in humans.

References


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