Pathogenesis of Acute Myocardial Infarction

Novel Regulatory Systems of Bioactive Substances in the Vessel Wall

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Changing Concepts of the Role of Coronary Thrombus in Acute Myocardial Infarction

Acute myocardial infarction had generally been considered to be the result of thrombotic occlusion of a major extramural coronary artery until postmortem observations of the infrequency of coronary thrombi began to accumulate in patients who died of extensive myocardial infarction. Roberts and Buja1 found coronary artery thrombi in 40 (54%) of 74 patients with transmural necrosis, in none of 9 with only subendocardial necrosis, and in 2 (8%) of 24 who died suddenly. On the basis of their own data and of the literature, which reported the frequency of coronary thrombosis in fatal acute myocardial infarction as ranging from 21% to 91%, they claimed that coronary thrombi are consequences rather than causes of acute myocardial infarction. Similar conclusions were reached by other investigators.2-5 In a workshop on the role of coronary thrombosis in the pathogenesis of acute myocardial infarction, no unanimous conclusion was obtained as to whether the thrombus precedes infarction as a primary lesion or follows infarction as a secondary effect. The report of the workshop stated that although most evidence continues to affirm myocardial infarction to be caused by thrombotic occlusion of a coronary artery, the idea of coronary thrombosis as a secondary event after infarction is also provocative and deserves serious consideration.6

Most of our knowledge of the lesions associated with transmural infarction before 1980 was derived from autopsy studies. These studies were performed in patients who died days to weeks after the infarction or in patients who died suddenly (less than 6 hours after the onset of symptoms). It is generally accepted that postmortem studies on victims of sudden death often reveal multivessel coronary atherosclerosis but only rarely evidence of an acute coronary event or myocardial necrosis.7,8 However, an autopsy study in which the time interval between the onset of symptoms and death was less than 24 hours and was accurately ascertained revealed a high incidence (88.9%) of coronary thrombi.9 The frequency of coronary thrombi decreased with the time after the onset of myocardial infarction.

During the past decade, the increased use of coronary arteriography has enabled us to explore the morphology of coronary arteries within 4 to 6 hours after the onset of infarction, which was rarely possible in previous (postmortem) studies. DeWood et al10 reported that total coronary occlusion was observed in 110 of 126 patients (87%) evaluated within 4 hours after the onset of symptoms: this proportion decreased significantly to 65% in patients examined 12 to 24 hours after the onset of symptoms. With the development of thrombolytic therapy, the general consensus today is that thrombotic occlusion of a coronary artery is demonstrable in 80% to 95% of patients if coronary arteriography is performed during the early hours after the onset of myocardial infarction.9,11,12 Thus, the controversy, particularly among pathologists, as to whether coronary artery thrombi are the cause or the result of acute myocardial infarction has been resolved by the demonstration by coronary arteriography of thrombotic coronary occlusion in the majority of patients within 4 to 6 hours after myocardial infarction.

Rupture of Coronary Atheromatous Plaque and Acute Myocardial Infarction

On the basis of the postmortem angiographic and histopathologic correlations described by Levin and Fallon,13 Ambrose et al14 stated that an asymmetric narrowing of a coronary artery in the form of a convex intraluminal obstruction with a narrow neck or irregular borders (or both) might represent a ruptured atherosclerotic plaque with or without a superimposed, partially occlusive intraluminal thrombus. These angiographic findings were common in the majority of patients with unstable angina at high risk for a subsequent coronary event.14 Irregular narrowing and a filling defect on coronary arteriography were associated with rupture and hemorrhage of the atheromatous plaque in patients who died a few days after selective intracoronary thrombolytic therapy.15

It can now be generally appreciated that there are three pathological hallmarks in the infarct-related coronary artery at the site of acute myocardial infarction: ruptured lipid-rich atheromatous plaque, intraplaque hemorrhage, and intraluminal thrombus (Fig 1). The theory of Davies and Thomas12 is most attractive as an explanation of the dynamic process from the rupture of an atheromatous plaque to intraluminal thrombotic

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occlusion. Rupture of the fibrous cap in the surface of an atheromatous plaque leads to free communication between the lipid content of the plaque and the blood flowing in the arterial lumen. Macrophages, which are numerous in atheromatous plaques, contain much tissue factor with procoagulant activity.16-18 Blood from the lumen flows into the plaque, causing a large intraintimal thrombus. Over the site of the rupture, a thrombus develops in the lumen and grows to become totally occlusive and to induce myocardial infarction. On the other hand, the intraluminal thrombus may be completely lysed in some cases, and the plaque fissure resales, stabilizing the atheromatous plaque, now considerably larger than before (Fig 2). The frequent presence of plaque contents dispersed deeply in the thrombus clearly indicates that plaque rupture has preceded and is not the result of thrombus formation.

Besides plaque rupture, a variety of hematologic disorders including leukocyte count, plasma fibrinogen, plasminogen activator inhibitor-1 (PAI-1),20 lipoprotein(a) [Lp(a)],21 hemotocrit, and blood viscosity may also contribute to coronary thrombosis formation. A recent study21 provides the first in vivo evidence that elevated concentration of Lp(a) may inhibit fibrinolysis, but further studies are needed to obtain conclusive results.

Why and How Do Plaques Rupture?

Plaque rupture occurs most frequently at the plaque cap with a lipid pool near its lateral margin. The intimal layer of the plaque cap may have been weakened by macrophage-derived foam cells, which release oxygen radicals as well as neutral proteases leading to enzymatic degradation of the connective tissue matrix within the plaque.22,23

Triggering events for plaque rupture may be important by promoting platelet aggregation through increases in catecholamines associated with stress, exercise, or sudden fright at sites of endothelial dysfunction. Even minor plaque fissure might contribute to the development of thrombosis with the accumulation of mediators such as thromboxane $A_2$ (TXA$_2$), serotonin, and adenosine diphosphate (ADP).

On the other hand, recent investigation25 stresses the fact that soft, lipid-rich plaques with progressive weakening of the fibrous cap are prone to rupture. As a result, plaque composition is more important than plaque size. Since most ruptures seem to occur during normal daily activities without any obvious precipitating cause, the composition of plaques probably plays a more important role in rupture than does a triggering event.

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Fig 1. Cross-sectional autopsied specimen of an infarct-related coronary artery at the site of occlusion. Rupture or fissuring (R) of lipid-rich atheromatous plaque (A) in the intima, intraplaque hemorrhage (H), and intraluminal thrombus (T) in the original arterial lumen are shown. Reprinted with the permission of Life Science Ltd, Co, from Kawai C, Fujitaka H, in Japanese in Therapeutic Research 1991;12:85-105.

Fig 2. Schematics of evolutionary changes of a plaque rupture either to reseal and stabilize the plaque or to progress to occlusive intraluminal thrombus. From Davies and Thomas,12 reprinted with the permission of British Heart Journal.
Regulatory System of Bioactive Substances in the Vessel Wall in Evolving Myocardial Infarction

It was thought that plaque rupture was always associated with occlusive thrombus formation if the preexisting luminal stenosis was greater than 95%, whereas occlusive thrombosis rarely occurred with preexisting stenoses of less than 75%. Two retrospective studies, however, suggested that myocardial infarction developed frequently from previously nonsevere coronary lesions. The patients underwent coronary arteriography before and after a myocardial infarction. In 48% of 23 patients in one study and in 66% of 29 patients in the other study, the coronary artery that subsequently occluded had less than a 50% stenosis on the first arteriogram (before infarction). In 78% of the 23 patients and in 97% of the 29 patients, preexisting stenosis of the infract-related coronary artery was less than 70%. It was concluded that myocardial infarction appeared to develop frequently from a coronary lesion of mild to moderate stenosis. A recent prospective study demonstrated similar results. Sudden thrombotic occlusions of mild to moderate stenotic lesions probably produce myocardial infarction because of a lack of collateral vessels.

The mechanism of thrombotic occlusion of mild to moderate coronary atherosclerotic lesions is unclear at this time. Elucidation of the interactions between platelet activities and bioactive substances in the vessel walls with endothelial dysfunction is needed to solve the problem.

Unstable Angina, Acute Myocardial Infarction, and TXA2

Hirsh et al proposed the following hypothesis of the pathophysiology of unstable angina pectoris and acute myocardial infarction. Circulating platelets become activated and adhere to exposed collagen at the site of rupture of an atherosclerotic plaque. Adhering platelets undergo a release reaction: TXA2 is produced and released, and platelet aggregation is initiated. Platelet aggregates may plug a distal coronary artery at the site of stenosis or TXA2 may cause vasoconstriction, allowing platelet plugs to obstruct even a nearly normal coronary artery. Thus, an increase in TXA2 estimated by elevated transcadiac levels of thromboxane B2 (TXB2) may initiate or sustain the syndrome of unstable angina pectoris. It has been noted that measurements of plasma TXB2 and 6-keto-prostaglandin F1α (6-keto PGF1α), a stable hydrolysis metabolite of prostacyclin (prostaglandin I2 [PGI2]) in plasma, are frequently subject to analytic errors. By measuring urinary TXB2 and 6-keto PGF1α to minimize analytic artifacts, Fitzgerald et al demonstrated that the biosynthesis of thromboxane and prostacyclin was increased in patients with unstable angina, suggesting that episodic platelet activation occurs in unstable angina. The increment in PGI2 biosynthesis was thought to be a compensatory response of vascular endothelium that limits the degree of the effect of platelet activation during such ischemic episodes. The first demonstration by Hirsh et al of increases in thromboxane across the coronary bed in patients with unstable angina was thus confirmed. Other investigators have also reported increased TXA2 production and augmented platelet aggregability during acute myocardial ischemic events.

Intracoronary thrombosis treatment since the 1980s has also provided us with a unique opportunity to study the role and genesis of intracoronary thrombus formation in acute myocardial infarction. Moreover, among intracoronary thrombolytic patients, it is not rare to encounter a case in which the coronary artery, once recanalized by the intracoronary infusion of thrombolytic agents, is immediately reoccluded at the same site. The reoccluded coronary artery is again recanalized by a second intracoronary infusion of a thrombolytic agent.

In a study of eight patients, the plasma TXB2 level in the coronary sinus blood was elevated after recanalization of the occluded left anterior descending coronary artery by an intracoronary infusion of urokinase due to the wash-out effect of locally accumulated TXB2, and a further marked increase in the plasma TXB2 level in the coronary sinus blood was confirmed immediately after reocclusion of the once recanalized coronary artery (Fig 3). These differences in the plasma TXB2 level in the three different situations were statistically significant (P<.05).

Therapeutic Effect of TXA2 Synthesis Inhibitor and Combined Inhibition of Several Mediators

The results obtained from our laboratory and from others revealed an enhanced generation of TXA2 in patients with unstable angina, acute myocardial infarction, and vasospastic angina. Since TXA2 is a potent vasoconstrictor and causes platelets to aggregate, it may play a crucial role in the genesis of intracoronary thrombus formation and thus in the development of myocardial infarction. If this is the case, pharmacological agents to inhibit TXA2 synthase might prevent some myocardial infarctions.

OKY-1581 is a pyridine derivative [sodium (E)-3-(4-[3-pyridylmethyl][phenyl]-2-methyl-2-propenoate], one of the selective TXA2 synthase inhibitors developed in Japan. Intravenous injection of OKY-1581 in normal volunteers resulted in (1) a decrease in peripheral plasma TXB2, (2) a reduction of TXB2 generation in serum, (3) an inhibition of rabbit platelet TXA2 synthase, (4) a reduction of arachidonate-induced platelet aggregation, all in a dose-dependent manner, and (5)
concomitant (in contrast to aspirin) increased production of 6-keto PGF₁α, a stable metabolite of PGI₂, in the serum.37

Our experiences have revealed that selective TXA₂ synthase inhibitors appear to be effective in the treatment of some types of angina pectoris, which we call thromboxane-dependent angina pectoris and which are usually refractory to the conventional antiangiinal treatments. Unfortunately, however, it is our impression that cases of thromboxane-dependent angina are the exception rather than the rule among patients with unstable angina or vasospastic angina.38-41 This is due to the fact that TXA₂ is only one of the mediators that is increased at sites of endothelial injury and coronary artery stenosis.24,30,42-46 Subsequently, Bush et al47 and Ashton et al48 have shown in experimental animal models that TXA₂ synthesis inhibitor and TXA₂/prostaglandin H₂ (PGH₂) receptor antagonists and combined TXA₂ synthase inhibitor and receptor antagonists prevent platelet aggregation and thrombus development at sites of endothelial injury and coronary artery stenosis, thereby abolishing cyclic flow variations. Further work has demonstrated that there are additional mediators that cause platelet aggregation, thrombus development, and vasoconstriction in experimental animals and humans. They include serotonin, thrombin, ADP, histamine, platelet activating factor, selected leukotrienes (LTE₄), and possibly endothelin.24,42-46.49-52 Thus, it is reasonable to conclude that inhibition of only one mediator (such as TXA₂) will not completely protect patients with unstable angina and that it is necessary to simultaneously inhibit several mediators to provide more protection than a TXA₂ synthesis inhibitor, a receptor antagonist, or aspirin alone provides.45,49,50 Combined antagonism of TXA₂ and serotonin or combined inhibition of TXA₂ synthase and a receptor antagonist or combined inhibition of ADP-induced platelet aggregation with antagonism of TXA₂ receptors54 are far more powerful in preventing cyclic flows than by using any one antagonist alone. Recently, Eichhorn et al53 have demonstrated the presence of cyclic coronary artery flow variations before and after coronary angioplasty in patients with severe angina. These variations may be related to platelet aggregation or coronary vasoconstriction (or both) at sites of endothelial injury occurring during coronary angioplasty. It has also been reported that an intravenous administration of monoclonal antibody to the platelet glycoprotein IIb/IIIa receptors eliminates cyclic flow variations and prevents abrupt closure during and after coronary angioplasty in humans.54

Factors Regulating Prostacyclin Synthesis and Stabilization in Ischemic Heart Disease

Prostacyclin (PGI₂) is a natural substance produced in the vessel wall (endothelium and smooth muscle), and it has both antiaggregatory and vasodilating properties.55 PGI₂ and TXA₂, having entirely opposite physiological behaviors, constitute an important homeostatic mechanism for the regulation of platelet aggregation and coronary arterial tone.

For the past 10 years or so, the existence of two factors in the plasma or serum to regulate PGI₂ metabolism has been reported from some institutions.56-61 One is the PGI₂ synthesis stimulating factor, which stimulates the synthesis of PGI₂ in the vessel wall. The other is the PGI₂ stabilizing factor, which stabilizes unstable PGI₂ in the circulating blood and prolongs the biological half-life of PGI₂. The prostacyclin synthesis stimulating factor is reported to be decreased in hemolytic uremic syndrome66 and sickle cell anemia67; the PGI₂ stabilizing factor is decreased in thrombotic thrombocytopenic purpura68 and in ischemic stroke.69 In our laboratory, PGI₂ synthesis stimulating factor was measured in blood samples obtained from the pulmonary artery or the peripheral vein in 7 patients aged 47 to 55 years (mean age, 51 years) with acute anterior myocardial infarction. Blood samples were also obtained from 12 patients with stable angina pectoris (mean age, 53 years) and 7 healthy volunteers (mean age, 33 years).60 The serum generation of PGI₂ measured by 6-keto PGF₁α production was significantly lower in the acute phase (2.5±0.8 hours after onset) than in the subacute phase (80±17 hours after onset) of myocardial infarction (15±15 ng/mg per hour versus 68±20 ng/mg per hour, P<.01). Values at 80±17 hours after onset showed no significant difference from those of patients with stable angina pectoris (61±31 ng/mg per hour), but both levels were significantly lower than those of normal volunteers (117±48 ng/mg per hour, P<.01) (Fig 4).

Activities of the PGI₂ stabilizing factor in patients with stable angina pectoris (n=11, 31.3±6.2%), with unstable angina pectoris (n=10, 24.2±9.1%), and in the acute phase of myocardial infarction (n=12, 24.9±3.5%) at 3.4±1.5 hours after onset) were significantly lower than those of the age-matched healthy normal control subjects (n=10, 38.2±4.1%; P<.05 versus stable angina pectoris; P<.01 versus unstable angina pectoris and acute phase of myocardial infarction).40 However, the activity during the subacute phase of myocardial infarction (n=12, 33.2±4.3% at 77.0±14.3 hours after onset) was not different from that in the control subjects. The activities of PGI₂ stabilizing factor in patients with
unstable angina pectoris or during the acute phase of myocardial infarction were significantly lower than those in patients with stable angina pectoris (P<.05) or during the subacute phase of myocardial infarction (P<.01). It was confirmed that the activities of PGI₃ stabilizing factor in patients with unstable angina pectoris and during the acute phase of myocardial infarction were the lowest among these five groups (Fig 5).

**Purification of Serum PGI₃ Stabilizing Factor and Apolipoprotein A-I**

The PGI₃ stabilizing factor in serum was purified from human serum through column chromatography to a single protein with a molecular weight of 28 000 by SDS-PAGE in our laboratory. The NH₂-terminal amino acid sequence (32 residues) was H₂N-Asp-Glu-Pro-Pro-Gln-Ser-Pro-Trp-Asp-Arg-Val-Lys-Asp-Leu-Ala-Thr-Val-Tyr-Val-Asp-Val-Leu-Lys-Asp-Ser-Gly-Arg-Asp-Tyr-Val-Ser-Gln, and the COOH-terminal (three residues) was HOOC-Gln-Thr-Asn. These sequences were identical to those of human apolipoprotein (Apo) A-I. Comparison of the amino acid composition of PGI₃ stabilizing factor with that of human Apo A-I also revealed strong similarities (Table 1). Thus, we concluded that PGI₃ stabilizing factor is identical to Apo A-I.

Albumin has long been claimed to bind to PGI₃ and to prolong its half-life. We also confirmed the activity of PGI₃ stabilizing factor in commercially available human albumin (Cohn fraction V albumin, Sigma). A contaminated band of Apo A-I was detected by SDS-PAGE with silver staining in the Cohn fraction V albumin. This contaminated band also was identified as Apo A-I by Western blotting. Purified albumin lost its PGI₃ binding activity.

The activity of PGI₃ stabilizing factor varies in parallel with the concentration of Apo A-I. Apo A-I is a major apolipoprotein of high-density lipoprotein (HDL). The activity of PGI₃ stabilizing factor was lost when HDL was delipidated by ethanol-diethyl ether. Nascent HDL reconstituted from Apo A-I and the phospholipid revealed PGI₃ stabilizing activity. Delipidated Apo A-I and delipidated nascent HDL showed no binding activity with (³H)-PGI₃. Thus, an a-helix structure of Apo A-I appears to be necessary for the binding with PGI₃. No binding activity of PGI₃ was observed with other apolipoproteins of HDL such as Apo A-II, C-I, C-II, C-III, D, and E, or low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL). The addition of HDL prolonged the half-life of PGI₃ significantly, from 4.8 minutes (at 24°C, pH 7.4) to 22.3 minutes.

**Apo A-I and Apo A-II in Ischemic Heart Disease**

Epidemiological and clinical studies have demonstrated that Apo A-I appears to be an even more useful indicator than HDL cholesterol of resistance to coronary

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**Fig 5.** Plot of prostacyclin (PGI₃) stabilizing factor measured by PGI₃ binding activity in patients with stable angina pectoris (AP), unstable angina pectoris (UAP), during acute myocardial infarction (MI-a, 3.4±1.5 hours after onset), and during subacute myocardial infarction (MI-s, 77.8±14.3 hours after onset). Cont indicates age-matched control subjects. *P<.05, **P<.01.
artery disease. Some reports have disclosed that Apo A-I deficiency is closely related to premature coronary artery disease and severe coronary atherosclerosis.

Our group found that total serum Apo A-I levels were significantly lower in patients with stable angina pectoris, with unstable angina pectoris, and during the acute (3.6±1.7 hours after onset) and subacute (75±15 hours after onset) phases of myocardial infarction than in age-matched control subjects (Table 2). However, there were no significant differences among the four groups of patients with coronary artery disease. Previously, Yui et al[62] demonstrated clearly that PG12 stabilizing activity was manifested only by Apo A-I with an α-helix structure occupying the surface of the HDL particle. Free (delipidated) Apo A-I could not stabilize PG12. The amount of HDL-associated Apo A-I has been reported to be affected by the amount of Apo A-II, which has a greater affinity than Apo A-I for the HDL particle, and 2 mole of Apo A-II is able to displace 1 mole of Apo A-I from HDL particles quantitatively.[74,75] Until now, no consistent results were obtained on the levels of Apo A-II in patients with ischemic heart disease.[67,68,76-78] Our study showed that Apo A-II levels were significantly lower in patients with stable angina pectoris, with unstable angina pectoris, and during the acute and subacute phases of myocardial infarction than in the age-matched control subjects (Table 2). However, values of Apo A-II in patients with unstable angina pectoris and during the acute phase of myocardial infarction were higher than those in patients with angina pectoris or the subacute phase of myocardial infarction. Aoyama et al[73] demonstrated that after incubation of human serum HDL from normal fasting men with increasing amounts of Apo A-II, HDL-associated Apo A-I was displaced by Apo A-II, and free Apo A-I was increased in parallel. PGI2 binding activity decreased with increasing concentrations of Apo A-II (Fig 6).

The regulatory factors involved in the synthesis and secretion of Apo A-II are not fully understood yet. The pentapeptide of proapolipoprotein A-II is cleaved by the proapolipoprotein A-II converting enzyme and then converted into mature apolipoprotein A-II.[79] This specific converting enzyme may play a key role in the synthesis of Apo A-II.

The calculated HDL-associated Apo A-I levels[80] were lower in the four groups of coronary artery disease than in the age-matched control subjects (Table 2). Moreover, the HDL-associated Apo A-I levels in patients with unstable angina pectoris and during the acute phase of myocardial infarction were significantly lower than those in patients with stable angina pectoris and during the subacute phase of myocardial infarction and lowest among these groups, thus coinciding with the level of PG12 stabilizing factor (Figs 5 and 7). In conclusion, HDL-associated Apo A-I, which is regulated by Apo A-II, is able to stabilize PGI2 and appears to play an important role in the prevention of intracoronary thrombus formation in part through the stabilization of PGI2.

Endothelium-Derived Relaxing Factor and Purification of Nitric Oxide Synthase

Since the report of Furchgott and Zawadski[81] in 1980, endothelium-derived relaxing factor (EDRF) has been

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**TABLE 1. Amino Acid Composition of Prostaglandin I2 Stabilizing Factor and Apolipoprotein A-I**

<table>
<thead>
<tr>
<th>Prostaglandin I2</th>
<th>Apolipoprotein A-I</th>
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<tbody>
<tr>
<td>Stabilizing Factor</td>
<td></td>
</tr>
<tr>
<td>Asp+Asn</td>
<td>21.0</td>
</tr>
<tr>
<td>Thr</td>
<td>9.8</td>
</tr>
<tr>
<td>Ser</td>
<td>12.3</td>
</tr>
<tr>
<td>Glu+Gln</td>
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<tr>
<td>Gly</td>
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<tr>
<td>Ala</td>
<td>17.1</td>
</tr>
<tr>
<td>Val</td>
<td>12.1</td>
</tr>
<tr>
<td>Cys-Cys</td>
<td>0</td>
</tr>
<tr>
<td>Met</td>
<td>1.7</td>
</tr>
<tr>
<td>Ile</td>
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</tr>
<tr>
<td>Leu</td>
<td>33.5</td>
</tr>
<tr>
<td>Tyr</td>
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</tr>
<tr>
<td>Phe</td>
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</tr>
<tr>
<td>Lys</td>
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</tr>
<tr>
<td>His</td>
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<td>Arg</td>
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<td>Pro</td>
<td>10.3</td>
</tr>
<tr>
<td>Trp</td>
<td>ND</td>
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</tbody>
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**TABLE 2. Prostaglandin I2 Binding Activities and Apolipoprotein A-I and A-II Levels**

<table>
<thead>
<tr>
<th>Group</th>
<th>PG12 Binding Activities, %</th>
<th>Total Serum Apo A-I, mg/dL</th>
<th>Free Apo A-I/Total Apo A-I, %</th>
<th>HDL-Associated Apo A-I, mg/dL</th>
<th>Total Serum Apo A-II, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.5±3.5</td>
<td>137.3±18.6</td>
<td>10.3±1.7</td>
<td>124.4±8.1</td>
<td>38.0±5.1</td>
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<tr>
<td>AP</td>
<td>31.8±5.6†</td>
<td>105.7±13.0†</td>
<td>8.3±3.3</td>
<td>98.9±13.9†</td>
<td>30.2±4.4†</td>
</tr>
<tr>
<td>UAP</td>
<td>26.2±4.7†</td>
<td>101.4±11.9†</td>
<td>22.1±3.7†</td>
<td>76.9±8.7†</td>
<td>33.0±5.3*</td>
</tr>
<tr>
<td>MI-a</td>
<td>24.8±3.2†</td>
<td>102.2±11.5†</td>
<td>20.8±3.2†</td>
<td>77.8±8.8†</td>
<td>34.5±5.0*</td>
</tr>
<tr>
<td>MI-s</td>
<td>33.3±3.5†</td>
<td>109.3±10.8†</td>
<td>11.5±2.5</td>
<td>95.2±8.3†</td>
<td>26.0±3.5†</td>
</tr>
</tbody>
</table>

PG12 indicates prostaglandin I2; Apo, apolipoprotein; HDL, high-density lipoprotein; free Apo A-I/total serum Apo A-I, ratio of free Apo A-I to total serum Apo A-I; total Apo A-I/total Apo A-II, molar ratio of total serum Apo A-I to total serum Apo A-II; AP, angina pectoris; UAP, unstable angina pectoris; MI-a, acute phase of myocardial infarction (3.6±1.7 hours from onset); and MI-s, subacute phase of myocardial infarction (75±15 hours from onset). Values are mean±SD.

*P<.05, †P<.01 vs control group.

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considered to possess the pharmacological properties of smooth muscle relaxation in a number of different vascular preparations, including inhibition of platelet aggregation.82,83 Through numerous efforts to identify this substance, evidence has been growing that EDRF may be identical to nitric oxide84,85 or a closely related donor of nitric oxide.86,87 It has now become clear that nitric oxide is formed through the biosynthetic pathway involving the conversion of L-arginine into L-citrulline by enzymes termed nitric oxide synthase(s).88,89 In addition to endothelial cells, nitric oxide has been found to be generated in macrophages,90 cerebellum,91 neutrophils,92 and human myocardium.93

Recently, Bredt and Snyder94 have reported that they purified nitric oxide synthase with a molecular weight of 150 000 from rat cerebella and that this enzyme requires calmodulin. Yui et al.95 from our laboratory have succeeded in purifying nitric oxide synthase from rat polymorphonuclear neutrophils (PMN). This enzyme is a monomeric protein with a molecular weight of 150 000 on SDS-PAGE (Fig 8), which produces nitric oxide and L-citrulline from L-arginine as a substrate. Nitric oxide immediately decomposes to nitrite (NO₂⁻) and nitrate (NO₃⁻). The stoichiometry of arginine loss to formation of citrulline and nitrite and nitrate is 1/1/1. The enzyme is dependent on NADPH, calcium ion (Ca²⁺), and (6R)-tetrahydrobiopterin (BH₄), with no calmodulin requirement.

Nitric oxide synthase from rat macrophages also has been purified by our group.96 The macrophage nitric oxide synthase requires the presence of NADPH and (6R)-BH₄ but maintains its activity in the absence of calmodulin and Ca²⁺. The purified enzymes were very unstable, with a half-life of 3 hours at 4°C for the PMN...
nitric oxide synthase and 6 hours at 4°C for the macrophage nitric oxide synthase, but the cytosol preparation for either enzyme was stable after 24 hours because of the presence of a stabilizing factor.

Nitric oxide synthase appears to be regulated by at least two mechanisms. One is a calmodulin and Ca²⁺-dependent type demonstrated in tissues such as endothelium and cerebellum. The other is a calmodulin and Ca²⁺-independent type shown in macrophages. Now we have found a third regulatory mechanism in PMN, which is dependent on Ca²⁺ but not on calmodulin. Nitric oxide synthase in the endothelial cell and the cerebellum is a constitutive enzyme that may respond quickly only when signals come. In contrast, nitric oxide synthase in PMN and macrophage is an inducible enzyme that releases large quantities of nitric oxide slowly for many hours after exposure to cytokines and microbial products. Recent molecular cloning of the two enzymes, constitutive and inducible, has revealed that they share only 51% of their amino acid sequences.

On the other hand, the formation of nitric oxide or endothelium-dependent relaxations are known to be inhibited by N⁶-monomethyl-L-arginine, hemoglobin, methylene blue, and lipoproteins, not only LDL but VLDL and HDL.

Clinical Application of EDRF and Its Precursor, L-Arginine

In stenosed and endothelium-injured coronary and femoral arteries of mongrel dogs, Yao et al. have demonstrated that platelet-derived nitric oxide may protect against platelet aggregation and thrombus formation in these arteries. The endothelium proximal to the site of arterial injury also may contribute nitric oxide to the elimination of cyclic flow variations. Previous studies have shown that an endothelium-dependent vasodilator, acetylcholine, may exert divergent effects on vascular tone. When the endothelium is intact, acetylcholine dilates coronary vessels. In contrast, acetylcholine may actually cause constriction in an endothelium-injured artery.

Based on the animal experiments showing the effect of nitric oxide (EDRF) on the protection against platelet aggregation and thrombus formation in endothelium-injured coronary arteries, the effect of L-arginine, the substrate for nitric oxide, on endothelium-dependent vasodilatation of the coronary artery and on platelet aggregation has been studied recently in humans.

Intracoronary infusion of L-arginine did not affect acetylcholine-induced epicardial artery vasomotion but enhanced endothelium-dependent dilation of the coronary microvasculature in patients with hypercholesterolemia but not in normcholesterolemic patients with coronary artery disease and/or hypertension. Similarly, patients with depressed dilator response to acetylcholine, that is, those with endothelium dysfunction, had a greater response to L-arginine. In addition, infusion of L-arginine inhibited in vivo platelet function but it did not enhance the blunted inhibition of platelet aggregation with EDRF in atherosclerotic patients.

One can expect that therapeutic applications of nitric oxide synthase and L-arginine may prevent thrombosis and vasoconstriction mediated by TXA₂, serotonin, ADP, platelet-derived growth factor, and tissue factor at the sites of endothelial injury through promoting EDRF release from neighboring endothelial sites.

Summary and Conclusions

Rupture of the lipid-rich atheromatous plaque, intraplaque hemorrhage, and intraluminal thrombus are three pathological hallmarks most commonly recognized in the infarct-related coronary artery at the site of acute myocardial infarction. Rupture of the atheromatous plaque is closely related to but does not fully explain the genesis of occlusive intracoronary thrombus formation and thus the development of acute myocardial infarction. Besides a variety of hematologic disorders, one should emphasize the role of the platelet-derived mediators that promote an environment where thrombosis and vasoconstriction occur, including TXA₂, serotonin, ADP, platelet-derived growth factor, tissue factor, and the diminished availability of those natural endogenous substances that inhibit platelet aggregation, such as EDRF, tissue plasminogen activator, and PGI₂.

PGI₂ released from vascular endothelial cells is extremely unstable. Our group provided the first evidence that HDL stabilizes PGI₂ through the newly discovered function of Apo A-I, which is associated with the surface of HDL particles and identified as PGI₂ stabilizing factor. Decrease in HDL-associated Apo A-I in patients with unstable angina and during the acute phase of myocardial infarction indicates that HDL plays an important role in preventing coronary atherosclerosis and intracoronary thrombus formation by stabilizing PGI₂ in addition to the generally accepted biochemical property of HDL to prevent the accumulation of cholesterol by mobilizing free cholesterol from tissues or macrophages. There is also a PGI₂ synthesis-stimulating factor in serum that has not yet been identified chemically.

EDRF or nitric oxide provides another important regulating system in the vessel wall. Lipoproteins are inhibitors of endothelium-dependent relaxation of rabbit aorta. The role of plasma Lp(a) in inhibiting intrinsic fibrinolysis is still to be elucidated.
Damage to the vascular endothelium at the time of atheromatous plaque rupture could provoke the breakdown of the exquisite homeostasis regulating platelet aggregation, prostaglandins, EDRF, and as yet unknown bioactive substances in the vessel wall, resulting in occlusive intracoronary thrombus formation and acute myocardial infarction. The present review clearly demonstrates the importance of the new discoveries to the regulatory systems in the vessel wall for intracoronary thrombus formation.

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