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# Role of Adenosine as Adjunctive Therapy in Acute Myocardial Infarction

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## ABSTRACT

Although early reperfusion and maintained patency is the mainstay therapy for ST elevation myocardial infarction, experimental studies demonstrate that reperfusion per se induces deleterious effects on viable ischemic cells. Thus "myocardial reperfusion injury" may compromise the full potential of reperfusion therapy and may account for unfavorable outcomes in high-risk patients. Although the mechanisms of reperfusion injury are complex and multifactorial, neutrophil-mediated microvascular injury resulting in a progressive decrease in blood flow ("no-reflow" phenomenon) likely plays an important role. Adenosine is an endogenous nucleoside found in large quantities in myocardial and endothelial cells. It activates four well-characterized receptors producing various physiological effects that attenuate many of the proposed mechanisms of reperfusion injury. The cardioprotective effects of adenosine are supported by its role as a mediator of pre- and postconditioning. In experimental models, administration of adenosine in the peri-reperfusion period results in a marked reduction in infarct size and improvement in ventricular function. The cardioprotective effects in the canine model have a narrow time window with the drug losing its effect following three hours of ischemia. Several small clinical studies have demonstrated that administration of adenosine with reperfusion therapy reduces infarct

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size and improves ventricular function. In the larger AMISTAD and AMISTAD II trials a 3-h infusion of adenosine as an adjunct to reperfusion resulted in a striking reduction in infarct size (55–65%). *Post hoc* analysis of AMISTAD II showed that this was associated with significantly improved early and late mortality in patients treated within 3.17 h of symptoms. An intravenous infusion of adenosine for 3 h should be considered as adjunctive therapy in high risk-patients undergoing reperfusion therapy.

## INTRODUCTION

The rationale for myocardial reperfusion therapy originated from experimental studies showing that timely reperfusion improves myocardial salvage by aborting the progressive "wavefront" of myocardial cell necrosis (126,127). While reperfusion is a prerequisite to prevent further myocardial necrosis, Hearse conceptualized that the rapid introduction of oxygen, cellular elements and electrolytes into the previous ischemic bed after reperfusion has deleterious effects that compromise the potential benefits of reperfusion therapy (78). As a result, reperfusion may convert reversibly-injured myocardial and endothelial cells to irreversibly-injured cells, which has become known as myocardial reperfusion injury (18,55,58). This concept implies that cells potentially viable just before reperfusion undergo lethal injury during the peri-reperfusion period. This phenomenon should be distinguished from the entity of acceleration of necrosis of cells that were already irreversibly injured at the time of reperfusion.

Proof that reperfusion injury importantly contributes to ultimate infarct size requires the demonstration that significant populations of cells that are viable just before reperfusion are irreversibly injured by processes triggered by reperfusion. Five lines of evidence are consistent with the hypothesis of myocardial reperfusion injury: 1) Infarct size extension occurs following reperfusion. For example, magnetic resonance imaging (MRI) contrast studies show a progressive increase in infarct size in the canine model undergoing 48 h of reperfusion, and histological studies demonstrate infarct-size extension in the rabbit model following three hours of reperfusion (46,129); 2) Myocyte apoptosis occurs after reperfusion in regional ischemia but not in animals with persistent ischemia (68, 167); 3) Vascular endothelial damage is accelerated by reperfusion compared with persistent ischemia (6,55,79,90); 4) Administration of various therapeutic agents at the time of or soon after reperfusion significantly enhances myocardial salvage (52,114); and 5) Multiple potentially injurious pathways activated by reperfusion have been identified, as discussed below.

Studies demonstrate that reperfusion engages several potentially injurious processes (53). In this regard, the rapid re-introduction of formed elements and molecular components of blood to a myocardial vascular bed primed by ischemic damage leads to: 1) adherence of activated blood-borne neutrophils to sites of ischemic vascular injury, with release of cytotoxic chemicals and pro-inflammatory cytokines; 2) generation of damaging free radicals by a myocardial/vascular substrate primed by ischemia for free radical formation; 3) adherence/aggregation of activated blood-borne platelets to sites of ischemic vascular injury; 4) wash-out of the purine-nucleotide pool by the restored blood flow thus compromising energy generation and storage; 5) wash-in of pro-inflammatory and

vasoconstrictive factors that contribute to further inflammation and regional ischemia; and 6) wash-in of normal electrolytes to injured myocytes that can no longer maintain electrolyte homeostastis. All of these processes then contribute to "downstream" phenomena such as abnormalities of potassium, sodium and calcium homeostasis in injured myocytes, inability to restore myocyte energy balance, and microvascular injury leading to the "no-reflow" phenomenon.

These observations suggest the need to develop pharmacologic agents that would attenuate reperfusion injury, thereby limiting infarct size and improving clinical outcomes. Adenosine is an endogenous nucleoside produced in part from the degradation of adenosine triphosphate (ATP) (15). Adenosine, which activates four well characterized receptors, produces favorable physiological effects on various cell types thought to be involved in the pathogenesis of myocardial reperfusion injury (48,162). Thus adenosine may be a particularly efficacious agent with regard to enhancing the benefits of reperfusion therapy. The purpose of this review is to explain the rationale for the use of adenosine as a cardioprotective agent following reperfusion of the ischemic myocardium, to describe preclinical evidence supporting the use of adenosine as a cardioprotective agent and to review clinical studies verifying the efficacy of adenosine as an adjunctive agent in acute myocardial infarction (MI). Because the microvascular bed most likely is a proximal common pathway for reperfusion injury and is an important site of adenosine action, we first review the role of the microvasculature in the pathogenesis of reperfusion injury.

## ROLE OF MICROVASCULAR INJURY IN THE PATHOGENESIS OF REPERFUSION INJURY

### Physiology of Normal Myocardial Endothelium

The coronary microcirculation consists of small arterioles, precapillary vessels, capillaries and post capillary venules, and is critical for transfer of nutrients to and removal of waste products from the myocardial interstitial compartment. Capillaries are the smallest and most prevalent blood vessels in the heart with a density of 2000–3885 vessels per $mm^2$ of myocardium (79). Endothelial cells account for an exceptionally high surface area in the heart (1,000 $cm^2/g$), and form a continuous lining of the vasculature (63,140). Endothelial cells are attached to blood vessels by subendothelium, which consists of an organized matrix of molecules including collagen, elastin, fibronectin, and von Willibrand's factor secreted by endothelial cells (35). Endothelial cells also secrete $\beta_2$ integrins (VLA-2, VLA-3) that bind collagen to sites on the endothelial cells (35). The endothelial cell provides a selective barrier separating intravascular and interstitial spaces and is therefore in a position to regulate the transfer of nutrients to myocytes, the diapedesis of leukocytes and the interaction between circulating vasoactive substances and vascular smooth muscle.

The endothelial cell is metabolically active and releases factors that regulate platelet activity and the coagulation pathway. Endothelial cells produce numerous antiplatelet factors [prostacyclin, adenosine, nitric oxide (NO)], proaggregatory factors (thromboxane $A_2$), anticoagulation factors [tissue-type plasminogen activator (t-PA), thrombomodulin,

protein S, antithrombin III, heparin sulfate] and procoagulation factors (plasminogen activator inhibitor, factor V, factor VIII, and tissue factor) (2,19,35,85,94,97,99,111). The delicate balance between these factors may be altered by reperfusion-induced damage to the endothelium, resulting in inappropriate intravascular thrombus formation.

Numerous vasoactive compounds that regulate vascular tone are also produced by endothelial cells. These include endothelium-derived relaxing factor (EDRF), endothelins, prostacyclin, adenosine, ATP, endothelium-derived hyperpolarizing factor (EDHF), and angiotensins (19,35,85). Reflecting the integral involvement of the endothelium in regulating vascular smooth muscle tone, endothelial cells have surface receptors for many vasoactive compounds including acetylcholine, histamine, serotonin, catecholamines, bradykinin, and adenine nucleotides (15,19,63).

The endothelium also plays a role in controlling vascular smooth muscle proliferation by producing compounds which both promote and inhibit smooth muscle cell growth. Pro-mitogenic factors include platelet-derived growth factor, basic fibroblast growth factors, interleukin-1 and endothelin (60,64,119). Anti-mitogenic factors include heparin-like oligosaccharides and transforming growth factor-β (TGFβ), NO and adenosine (43, 64).

The endothelium also modulates local inflammatory and immune responses. Neutrophil adherence, activation, and migration involve an interplay between expression of adhesion molecules by endothelial cells, neutrophil activation, and local cytokine activity. Exposure of endothelial cells to thrombin, interleukins (ILs), and tumor necrosis factor-α ($TNF_{\alpha}$) induces the expression of molecules on the endothelial cell surface, such as E-selectin and intercellular adhesion molecule-1 (ICAM-1), that increase neutrophil adhesiveness to endothelial cells (23,35). The presence of cytokines (IL-1, $TNF_{\alpha}$, and $TGF_{\beta}$) also stimulates endogenous endothelial production of IL-1, IL-8, and platelet activating factor (PAF) (22,35). Endothelial-derived PAF upregulates the CD1lb/CD18 complex, thereby increasing neutrophil adherence to the vessel wall and neutrophil responsiveness to chemotactic factors (35). IL-8 regulates transendothelial migration of neutrophils through the endothelial barrier (35). Endothelial cells are also known to produce several factors that inhibit neutrophil attachment, including adenosine, prostacyclin, and cyclic adenosine monophosphate (17,85,111). The balance between induction and suppression of neutrophil adherence is related to the physiologic or pathologic environment; further investigation is required to unravel the regulatory signals of these cellular interactions.

There are several specific factors produced by vascular endothelial cells that deserve further comment. The functionally intact endothelium releases EDRF when exposed to appropriate agonists (19,35). EDRF is indistinguishable from nitric oxide, has a short half-life (6 to 50 sec), and produces smooth muscle relaxation by increasing cyclic guanosine 3′,5′-monophosphate (GMP) (19,35). NO is synthesized from the conversion of L-arginine to citrulline by constitutive and inducible forms of NO synthase (35). Although it has been suggested that EDRF represents a number of nitroso compounds, NO appears to be the active form of EDRF (35,115). NO is a free radical and is rapidly inactivated by superoxide anions. It is readily diffusible adluminally and abluminally, where it has numerous physiologic effects, including relaxation of vascular smooth muscle in large and small arteries, veins, and microvessels, inhibition of platelet aggregation, and reduction in neutrophil activation and adherence to endothelial cell surfaces (19,35,85,97,99). NO may also scavenge reactive oxygen species and attenuate thrombin-induced PAF synthesis in endothelial cells (19,47). Infusion of L-arginine has been shown to reduce neutrophil infil-

tration, preserve endothelial function and enhance myocardial salvage in experimental models of regional ischemia and reperfusion (94,97).

The vasoregulatory role of endothelial cells is further illustrated by the isolation and purification of a 21-amino-acid peptide termed endothelin (ET), which has a vasoconstrictor potency 10 times that of angiotensin II (35,132,164). Three endothelins have been identified (ET-1, ET-2, ET-3), which are formed from an intermediate biological precursor, pre-endothelin, which then undergoes cleavage by a converting enzyme (35,163). Endothelial cells appear to be the most abundant source of ET-1; their production is stimulated by mechanical factors such as shear stress as well as by substances including thrombin, $TGF_{\beta}$, angiotensin II, and interleukin I (132,163). In situ hybridization revealed that cardiomyocytes are the source of ET in the setting of ischemia (149). Two distinct ET receptor subtypes have been identified: $ET_A$ which is selective for ET-1 and found in vascular smooth muscle; and $ET_B$ which is localized on endothelial cells and is a nonselective receptor (163). Activation of the $ET_A$ receptor results in an intense sustained vasoconstriction by increasing cellular calcium from intra- and intercellular sources (35). $ET_B$ activation mediates vasodilation by increasing NO and/or $PGI_2$ production and by activation of the calcium-activated $K^+$ channel (35). It is now recognized that a marked vascular variation in receptor distribution occurs in different species, and that $ET_B$ activation can elicit both a dilator and constrictor response (163). The predominant response to ET of most vascular beds including coronary arteries appears to be intense and prolonged vasoconstriction (149). Elevated plasma levels of ET have been found in experimental models of reperfusion and in patients undergoing thrombolysis (147,152,154). A several-fold increase in messenger RNA for ET-1 was found in rabbit myocardium undergoing 30 min of ischemia followed by 180 min of reperfusion (156). Similarly, a two-fold increase in *de novo* synthesis of ET-1 has been demonstrated in pigs reperfused after 90 min of regional ischemia (149).

Other vasoactive compounds produced by endothelial cells include prostacyclin, angiotensins, and EDHFs (2,35,85). At least some EDHFs are epoxyeicosatrienoic acids (21). Prostacyclin is synthesized from arachidonic acid and produces smooth muscle relaxation, disaggregates platelets, and acts as a profibrinolytic agent (2,17,35). Angiotensin I is synthesized by endothelial cells and is then converted to angiotensin II by angiotensin converting enzyme (ACE) in the cell. ACE also inactivates bradykinin, a peptide that stimulates the release of NO and prostacyclin (35).

The endothelium also plays a pivotal role in the myocardial metabolism of adenine nucleotides (143). Vascular endothelial cells, via their capacity to release and take up adenosine, are responsible for the maintenance of constant plasma levels of this nucleoside in the coronary circulation (15,143). Adenosine is a potent coronary arteriolar vasodilator and may be an important regulator of coronary blood flow (15). Adenosine disaggregates platelets and inhibits their release of thromboxane (1). Adenosine is also an important modulator of neutrophil function by markedly reducing superoxide anion generation and inhibiting adherence of activated neutrophils to cultured endothelial cells (32,33).

## Role of Neutrophils In Reperfusion Induced Microvascular Injury

Neutrophils play an important role in the acute inflammatory response to tissue injury and participate in reperfusion injury in the myocardium and other organs (44,74,108).

Neutrophil activation occurs early during myocardial ischemia, as demonstrated by the presence of numerous neutrophil chemotactic factors in cardiac lymph from the reperfused bed (39,41). Tissue injury appears to be a prerequisite for neutrophil infiltration, since only ischemic periods of longer than 40 min result in a 3–4 fold increase in neutrophil counts in the reperfused myocardium (66). The greatest extent of infiltration occurs during the first hour of reperfusion with preferential localization to the subendocardium (40). Studies using fluorescent videomicroscopy to visualize neutrophil adhesion to epicardial microvessels *in vivo* indicate that 60 min of regional ischemia followed by reperfusion significantly increases neutrophil adhesion to coronary microvessels compared to ischemia alone (139). Neutrophil accumulation increases within 10 min of reperfusion and peaks approximately 60 min after reperfusion (139). The time course of neutrophil adhesion and infiltration correlates with levels of chemotactic factors present in cardiac lymph (39,41). In contrast, neutrophil infiltration is observed only after 4 to 6 h in the permanent occlusion model, predominantly at the border of the ischemic zones (142). The essential initiating step in this accumulation involves adhesion of the neutrophil to vascular endothelial cells (74). This is followed by activation, diapedesis, and extravascular migration into surrounding myocytes.

Neutrophil activation and accumulation in reperfused tissue are caused by changes in both neutrophils and endothelial cells initiated by the presence of numerous inflammatory mediators present in reperfused myocardium. Activation of the complement cascade occurs during the early phase of ischemia even in the absence of reperfusion (120,131). The complement fraction $C1_q$ localizes to the ischemic myocardium *in vivo*, and C5a is found in cardiac lymph (39,131). Activated neutrophils contain an enzyme that cleaves C5a into an active chemoattractant fragment (158). Exposure of neutrophils to various chemoattractants derived from a variety of sources causes the cell to become more spherical and induces increased expression of CD1lb/CD18 receptor, which promotes adhesion, aggregation, and chemotaxis (75,80). The importance of the CD18 receptor in early localization of neutrophils in reperfused tissue is illustrated by the observation that monoclonal antibodies to the receptor significantly attenuate neutrophil infiltration during the first hour of reperfusion (40).

Ongoing neutrophil activation is mediated by a number of other chemotactic cytokines produced both by neutrophils (IL-1, TNFα) and other cell types such as IL-8 from endothelial cells (73,81). These compounds produce an increase in cytosolic calcium in the neutrophil, leading to activation of phospholipases and generation of arachidonate products from cyclooxygenase and lipoxygenase enzymes and the phospholipid PAF (92). Both leukotriene $B_4$ and PAF are potent chemoattractants; they enhance vascular smooth tone, potentiate platelet aggregation, promote endothelial permeability, and modulate proteolytic enzyme release (73). Activated neutrophils can also release phospholipid $A_2$ into the environment, thereby enhancing the production of eicosanoids and PAF by other cells (50,93,102). The importance of leukotrienes in the inflammatory response is suggested by the observation that lipoxygenase inhibitors attenuate neutrophil infiltration into reperfused myocardium and reduce infarct size (109).

Although the processes are complex, calcium appears to play a role as a second messenger in the secretion of cytotoxic substances from activated neutrophils (67). These substances include numerous potent proteolytic enzymes and reactive oxygen species. Exposure of neutrophils to chemoattractant factors such as leukotriene $B_4$ and C5a results in release of enzymes from azurophilic and specific granules via reverse endocytosis (67).

Neutrophil activation greatly enhances oxygen uptake by the cells (respiratory burst), resulting in the production of large quantities of reactive oxygen by NADPH oxidase (11). These species include superoxide anion, hydrogen peroxide, hydroxyl radical, hydrochlorous acid, and chloramines (70). Normal endothelial and myocardial cells are protected from oxidant injury by numerous endogenous enzymes that are depleted by ischemia (51,70). Both lysosomal enzymes, such as elastase, and reactive oxygen species have been shown to damage endothelial cell basement membranes *in vitro* (134,141). Neutrophil-derived oxidants may also inactivate antiproteases present in the plasma such as $\alpha_1$-antitrypsin (24). Neutrophil degranulation and free radical release may, therefore, permit unchecked activity of proteolytic enzymes on endothelial and myocyte membranes.

The recent identification of numerous adhesive molecules that regulate various interactions between endothelial cells and neutrophils has considerably increased our understanding of these key cellular elements in the acute inflammatory process. These molecules, which are expressed constitutively or over a variable time period by various modifiers (leukotrienes, complement, PAF, $TNF_\alpha$, IL-1), result in an orchestrated sequence of events involving neutrophils and endothelial cells at the site of inflammation. The initial phase of neutrophil contact is slowing or rolling of the cells in venules, followed by activation and firm adherence to the endothelial cells, and finally transmigration through endothelial cell junctions into the myocardium. Members of all three families of adhesion molecules (selectin, immunoglobulin, and integrin) play an important role in modulating the various stages of the acute inflammatory response. A detailed description of the molecules can be found in numerous review articles (16,89,96). Exaggerated and prolonged activation of this natural defense mechanism occurs following reperfusion of ischemic, but viable, myocardium resulting in lethal injury to endothelial and myocardial cells.

## Role of Endothelial Cells in Reperfusion Induced Microvascular Injury

### *Histological changes*

A marked disparity is noted in the progression of ultrastructural changes between myocytes and endothelial cells with comparable periods of permanent ischemia (6,62,90). Whereas myocytes in the subendocardium manifest changes of irreversible injury after 40 min of ischemia, only mild swelling of endothelial cells in small vessels is observed, suggesting that the latter cell is more resistant to ischemia (6). After 60 min of ischemia, 20% of endothelial cells manifest focal swelling with loss of pinocytotic vesicles, which increases to 40% after 90 to 180 min of ischemia. After more prolonged periods of ischemia (180 to 360 min), progressive changes are noted, which include margination of nuclear chromatin and mild swelling of cytoplasm with occasional vesicle formation. Longer durations of permanent ischemia are associated with capillary obstruction secondary to red and white cell accumulation and occasional endothelial cell protrusions (62). In contrast, reperfusion is associated with marked acceleration of vascular injury (18,58, 90). Reperfusion after 45 to 90 min of ischemia results in explosive endothelial swelling of the microvasculature. Histological changes range from mild capillary endothelial cell swelling, loss of pinocytotic vesicles, blebs, and cytoplasmic protrusions to breaks in endothelial cells, disruption of the basement membrane, and red cell and neutrophil plugging, which result in capillary obstruction (90). Reperfusion following ischemic times greater than 40 min in the canine model is also associated with a rapid increase in neutrophil infil-

tration (40,66). Intravascular neutrophils may "plug" up to 27% of the capillaries, associated with a decrease in regional blood flow (45). Endothelial cell damage associated with capillary obstruction by blood elements contributes to the continual decrease in blood flow to areas of ischemic myocardium even after the onset of reperfusion — the "no-reflow" phenomenon (3).

### *Functional changes*

Reperfusion is a key trigger responsible for inducing abnormalities in endothelial dependant relaxation of large and small coronary vessels after regional ischemia. Isolated coronary rings harvested after 60 min of ischemia exhibit normal vasodilatory response to acetylcholine, whereas this response is abolished when the vessels are reperfused for 60 min (151). Abnormalities of vascular permeability and vasodilatation occur with ischemic episodes which do not induce myocardial necrosis and are observed within 25 min of reperfusion (34,87,95). Abnormalities of endothelial dependent vasodilatory reserve are amplified with longer duration of ischemia in the setting of reperfusion (87). Similar changes are observed in small resistance vessels (arterioles) *in vitro* undergoing ischemia and reperfusion (122). The duration of vasodilatory abnormalities are dependent on the period of preceding ischemia; vascular reactivity normalizes within 2 h after 15 min of ischemia, whereas an abnormal response persists for 12 weeks following 60 min of ischemia (87,117).

Several studies support a key role for neutrophils in the pathogenesis of endothelial dysfunction. In this regard, the administration of different antineutrophil agents (Fluosol, adenosine, prostacyclin, antibodies to Mac-1) preserves endothelial vasodilatory responses after reperfusion (9,54,95). Although the exact mechanisms through which neutrophils mediate functional endothelial abnormalities remain unknown, generation of superoxide anion during the early stage of reperfusion may act as a precipitating cause since administration of superoxide dismutase (SOD) preserves endothelial vasodilatory reserve (150).

### *Concept of progressive microcirculatory failure — "Dynamic no-reflow"*

Kloner et al. in 1974 demonstrated incomplete return of microcirculatory blood flow 20 min after reperfusion in myocardium subjected to 90 min of ischemia (90). This entity is termed the "no-reflow" phenomenon and is postulated to occur in areas where myocytes have already undergone irreversible necrosis. Studies by Ambrosio et al., utilizing the same experimental model and perfusion markers, demonstrated that blood flow changes are dynamic and decrease progressively over time (3). The area of impaired perfusion increases 3-fold after 3.5 h of reperfusion compared with two minutes after reperfusion. Ultrastructural analysis reveals severe ischemic changes with plugging of capillary lumina by neutrophils (3). Studies using MRI confirm the role of no-reflow in reperfusion injury (129). In this regard, microvascular obstruction increases progressively to 30% at 48 h after reperfusion and is strongly correlated with malperfusion (<50% of baseline blood flow). MRI-assessed infarct size similarly increases over 48 h after reperfusion (30% relative increase as a percentage of the risk region) (129).

These studies demonstrate that the "no-reflow" phenomenon occurs in a biphasic fashion; an immediate phase and a late phase in which a progressive decrease in flow

occurs in the sub- and mid-myocardium for at least 48 h after reperfusion, associated with a progressive increase in infarct size. The observation that agents which attenuate vascular changes result in significant myocardial salvage strongly support the hypothesis of a direct causal relationship between additional microvascular damage and final infarct size (9,54, 150).

## Clinical Significance of Impaired Microvascular Perfusion

In the majority of patients, angioplasty or stent implantation successfully restores TIMI grade III blood flow in an occluded epicardial vessel, reduces mortality and infarct size, and improves ventricular remodeling and function. However, more recent studies underscore the importance of restoring and maintaining flow in the microvasculature. Numerous diagnostic techniques are now available to evaluate flow in the microvascular circulation. Both semi-quantitative techniques, such as myocardial blush grade (MBG), and quantitative methods, such as Doppler flow wire, can evaluate tissue perfusion (14,30). The latter is a more sensitive technique to predict left ventricular functional recovery because it measures the functional and structural integrity of the reperfusion bed (14). MRI with contrast is also a sensitive indicator of microvascular obstruction, with no-reflow regions appearing as dark hypo-enhanced zones surrounding hyper-enhanced zones (160). Myocardial contrast echocardiography (MCE), in which sonicated micro-bubbles are injected either intracoronary or intravenously, is another useful technique to evaluate the microcirculation (83). The advantage of both MRI and MCE is that they are non-invasive and can measure flow serially over time.

Studies using the aforementioned methods demonstrate the clinical prevalence and significance of impaired microperfusion (IMP), which occurs in 29 to 44% of reperfused patients irrespective of whether the reperfusion strategy is thrombolysis or mechanical (primary PTCA or stenting) (14,30,83). Numerous studies show that IMP is more prevalent with occlusion of the LAD with an incidence of 50 to 80% (14,86). This may be secondary to more severe ischemia in the anterior wall because no-reflow correlates directly with the degree of LV dysfunction at the time of reperfusion. There is a marked disparity between restoration of TIMI grade flow and preservation of tissue perfusion. The CADILLAC study ($n = 1301$) found that only 17.4% of patients with TIMI grade III flow had normal tissue perfusion utilizing MBG (30). While glycoprotein $II_b/III_a$ inhibitors are beneficial in preventing recurrent ischemic events and target vessel revascularization in the setting of mechanical reperfusion for acute MI, they do not reduce infarct size or improve tissue perfusion after PTCA or primary stenting (5,30).

IMP has major clinical implications. IMP correlates with infarct size, ventricular remodeling, and early and late mortality, and predicts early and late recovery of ventricular function (5,14,30,37,83,86,160). IMP evaluated by MRI is associated with larger infarcts, ventricular wall thinning, increased ventricular volumes and a higher incidence of cardiovascular events (160). IMP remains an independent prognostic indicator after controlling for infarct size (160). The “no-reflow” phenomenon has also been associated with ventricular arrhythmias, early congestive cardiac failure and cardiac rupture. Therefore, the development of devices or pharmacologic agents that prevent IMP following recanalization of a major epicardial artery has important clinical implications.

# RATIONALE FOR THE USE OF ADENOSINE AS A CARDIOPROTECTIVE AGENT FOLLOWING REPERFUSION OF THE ISCHEMIC MYOCARDIUM

## Basic Biochemistry and Pharmacology of Adenosine

Adenosine is an endogenous nucleoside produced in part from the degradation of adenosine triphosphate (ATP) (15). Adenosine functions as a "retaliatory metabolite," and the heart continually releases adenosine in relation to myocardial function (143). The endothelium plays a pivotal role in the myocardial metabolism of adenine nucleotides (63). Adenine tri-, di-, and monophosphates (ATP, ADP and AMP, respectively) are present in a 3-fold greater concentration and adenosine in 40-fold greater concentration in cultured endothelial cells compared with myocardial cells (85). Vascular endothelial cells thus maintain levels of adenosine in the coronary circulation during normoxia (63). In contrast, during ischemia, the major source of coronary adenosine is the myocyte (13). Myocardial ischemia results in the accumulation of ADP and AMP due to the inability of mitochondria to rephosphorylate ATP (84). Also, conversion of cAMP to AMP by phosphodiesterases may also provide a source of adenosine during ischemia. Adenosine formation occurs following ischemia primarily by dephosphorylation of intracellular AMP by endo-5′-nucleotidase and extracellular AMP by ecto-5′-nucleotidase (137). Hydrolysis of S-adenosylhomocysteine (SAH) by SAH hydrolase may also contribute to the pool of adenosine (137). Enzymes of adenosine formation are regulated by metabolic factors, such as ATP, ADP, and inorganic phosphate, and neurohumoral factors, such as norepinephrine (137). Inactivation of adenosine deaminase and adenosine kinase may also contribute to adenosine production. Transport systems facilitate the loss of both adenosine and adenine nucleotides from the ischemic myocyte, and adenosine deaminase deaminates adenosine to inosine in the interstitium (26). Further depletion of the adenine nucleotide pool and adenosine occurs with reperfusion because of rapid washout. Inosine is enzymatically converted to hypoxanthine, and xanthine oxidase metabolizes hypoxanthine to xanthine. Restoration of high energy phosphates following reperfusion of viable cells can occur via the nucleotide salvage pathways that are metabolically rapid or through a slower *de novo* synthetic pathway (116). Phosphoribosyl pyrophosphate (PRPP) is an essential component of the *de novo* pathway and is also a substrate in two of the three salvage pathways. A limited supply of PRPP following ischemia associated with rapid washout of adenine nucleotides during reperfusion results in slow replenishment of high energy phosphates (36).

Purinergic type I (P1) receptors located on the cell surface mediate most of the effects of adenosine (48,162). $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ comprise the family of P1 receptors, and all four receptors belong to the superfamily of G-protein-coupled receptors containing seven transmembrane spanning domains. Knockout models and over-expressing animals have been generated for most of the P1 receptors. These models and the development of highly specific agonists and antagonists have clarified the physiologic role of each P1 receptor in the cardiovascular system (162).

The $A_1$ and $A_3$ receptors are negatively linked to adenylate cyclase via $G_i$ whereas $A_{2A}$ and $A_{2B}$ receptors are positively coupled to adenylate cyclase via $G_s$. In addition, $A_1$ re-

ceptors, most likely via βγ G-proteins, activate ATP-dependent potassium channels ($K_{ATP}$), a signal transduction pathway that hyperpolarizes myocytes and reduces influx of calcium via voltage-gated calcium channels (48,162). Adenosine receptors reside on most cells in the body. Cardiomyocytes contain $A_1$, $A_{2A}$, and $A_3$ receptors, and coronary arteries express all four P1 receptors. Adenosine receptors also are abundant on circulating blood cell elements. Neutrophils contain $A_1$, $A_{2A}$, and $A_3$ receptors, platelets $A_{2A}$ receptors and mast cells $A_{2A}$, $A_{2B}$, and $A_3$ receptors (48,162). Also, adenosine receptors exist on a number of cell types involved in cellular immunity including monocyte/macrophages, natural killer cells, lymphokine-activated killer cells and T-lymphocytes (48,162).

## Adenosine Inhibits Mechanisms Involved in Reperfusion Injury (Fig. 1)

Numerous mechanisms underlie myocardial reperfusion injury including neutrophil-mediated myocardial and endothelial cell injury, generation of cytotoxic oxygen-derived free radicals, the "no-reflow" phenomenon, alterations in calcium homeostasis, and ongoing depletion of high-energy phosphate stores (18,55,58). The diverse physiological properties of adenosine, coupled with increased adenosine concentrations in the ischemic myocardium, suggest that it may play an important role in regulating and protecting the myocardium in the setting of ischemia followed by reperfusion. Adenosine may also have beneficial effects during the later phases of reperfusion by promoting vascular repair, via formation of new blood vessels, and by inhibiting ventricular remodeling.

### *Adenosine promotes preservation of microvascular blood flow*

Although the mechanisms responsible for microvascular injury with subsequent decrease in myocardial blood flow are complex and diverse, adenosine appears to be a crucial counter-regulatory compound in the maintenance of microcirculatory flow due to its numerous pharmacological actions. First, adenosine could decrease mechanical obstruction of capillary channels caused by neutrophil adherence and neutrophil-mediated cellular damage via activation of $A_{2A}$ receptors (32,33). Second, the potent arteriolar vasodilator properties of adenosine would oppose the effects of vasoconstrictor substances present in the vascular bed after reperfusion, such as endothelin, leukotrienes, and PAF (15). Vasodilatory actions are predominantly mediated through $A_{2A}$ receptors (NO-independent), although the $A_{2B}$ receptor may be involved in some vascular beds (NO-dependent) (29,133). Inhibition of superoxide anion-release from neutrophils by adenosine would prevent destabilization of NO, further enhancing vasodilation (32). Third, adenosine, via stimulation of $A_{2A}$ receptors, would reduce the release of vasoconstrictor substances produced by activated platelets and neutrophils (1,138). Furthermore, $A_1$ receptor-mediated inhibition of norepinephrine release from sympathetic nerve endings and reduced renin release would also decrease the vasoconstrictor burden on the reperfused bed (128). Finally, adenosine may hasten repletion of endogenous vasodilator and antiinflammatory compounds produced by endothelial cells by restoring the metabolic machinery of these cells through replenishment of ATP stores or by enhancing oxygen delivery through arteriolar vasodilatation (15,105).

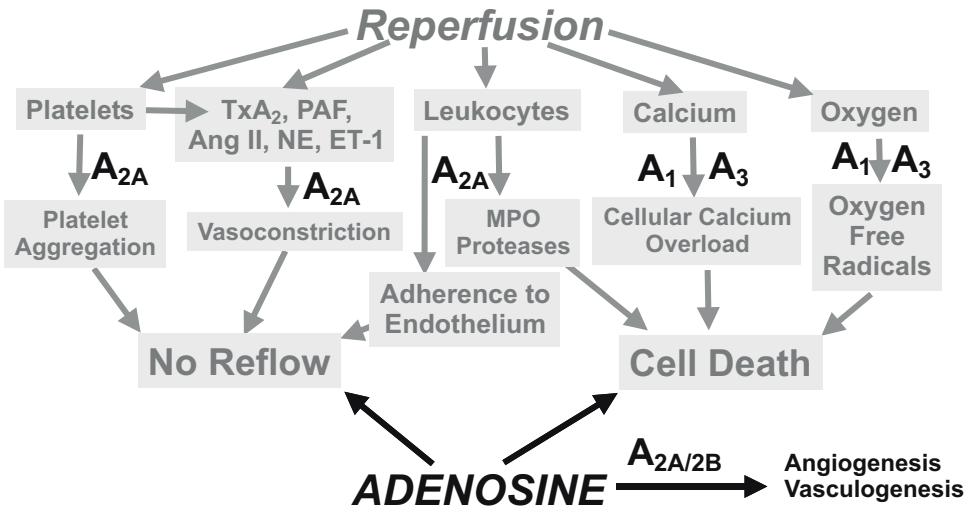


**FIG. 1.** This figure summarizes some of the multiple mechanisms involved in myocardial reperfusion injury and indicates how adenosine administered during the reperfusion period protects against reperfusion injury. Reperfusion introduces platelets, vasoconstrictors ($TxA_2$, thromboxane $A_2$; PAF, platelet activating factor; Ang II, angiotensin II; NE, norepinephrine; ET-1, endothelin-1), leukocytes, calcium and oxygen into the injured cardiac vascular tree and myocardium. Platelet aggregation and adhesion, vasoconstriction and adherence of leukocytes to endothelial cells (vascular plugging) contribute to the "no-reflow" phenomenon. Release of substances from activated leukocytes (MPO, myeloperoxidase; proteases) as well as cellular calcium overload and generation of oxygen free radicals contribute to cell death. $A_{2A}$ adenosine receptors inhibit platelet activation, mediate vasodilation to counteract vasoconstrictors and inhibit leukocyte activation. $A_1$ and $A_3$ receptors open $K_{ATP}$ channels, which hyperpolarizes myocytes and reduces cellular calcium influx. Also, $A_1$ and $A_3$ receptors may upregulate antioxidant enzymes. The net effect is that adenosine attenuates the "no-reflow" phenomenon and cell death. Also, via $A_{2A}$ and $A_{2B}$ receptors, adenosine promotes angiogenesis and vasculogenesis.

### *Adenosine inhibits neutrophils*

Adenosine is an important modulator of neutrophil function. Both adenosine and adenosine analogues markedly inhibit superoxide anion production by neutrophils through $A_{2A}$ receptor activation (32,169). In cultured endothelial cells, adenosine, via $A_{2A}$ receptors, reduces neutrophil adherence, by inhibiting upregulation of MAC1 receptors, and cytotoxicity (159). Although adenosine facilitates neutrophil chemotaxis *in vitro* via the $A_1$ receptor, *in vivo* studies show decreased numbers of neutrophils in the reperfused bed in animals receiving exogenous adenosine (9,31,114). These observations suggest that with pharmacological doses of adenosine, $A_{2A}$ receptor stimulation mediates the antineutrophil action of adenosine. The protective effects of adenosine on myocardial reperfusion injury may, therefore, be mediated in part by its effects on neutrophils. In addition to neutrophils, the ability of adenosine to reduce inflammation by inhibiting multiple cell types involved in cellular immunity may also importantly contribute to adenosine-mediated tissue protection (48,162). Increased levels of the pro-inflammatory cytokine $TNF_\alpha$ are found in the isolated heart subjected to ischemia and reperfusion (72). Activators of the $A_2$ receptor significantly inhibit $TNF_\alpha$ production by both tissue macrophages and myocytes, which may contribute to cardioprotective effects of adenosine following reperfusion (157).

### *Adenosine restores key metabolic substrates*

Adenosine initiates numerous metabolic events that could be beneficial in the setting of ischemia and reperfusion. Administration of exogenous adenosine restores ATP levels in viable but energy-deficient cells following myocardial ischemia. In this regard, adenosine has a high-affinity for nucleoside transporters and bypasses many of the preliminary reactions in the purine salvage pathways, thereby accelerating recovery of the intracellular adenine nucleotide pool (26,116). This hypothesis is confirmed in preparations of both regional and global ischemia in which adenosine with or without an adenosine deaminase inhibitor results in a rapid increase in ATP levels following reperfusion (56).

Adenosine may also maintain cell viability by increasing cellular uptake of glucose independent of its vasodilator action by enhancing anaerobic glycolysis (101,161). The $A_1$-receptor-mediated antilipolytic effect of adenosine stabilizes cellular membranes, thereby decreasing intracellular lactate production and acidosis (59). Myocardial ischemia is also associated with increased levels of endogenous catecholamines, thereby increasing myocardial oxygen consumption through stimulation of β-adrenoceptors. Adenosine may reduce oxygen consumption, thereby conserving high-energy phosphates, through its negative inotropic and chronotropic effects and by inhibiting norepinephrine release from sympathetic nerve endings through activation of $A_1$ receptors (118,128).

### *Adenosine inhibits production of oxygen-derived free radicals*

Adenosine possesses a number of physiological effects that may reduce free radical formation following ischemia. Adenosine reduces superoxide anion production by neutrophils *in vitro* via an interaction with the $A_{2A}$ receptor (33,169). Inhibition of norepinephrine release from sympathetic nerve endings ($A_1$-receptor-mediated) and reduced formation of thromboxane from platelets ($A_{2A}$-receptor-mediated) may also reduce free-radical generation via auto-oxidation of catecholamines or from arachidonate, thereby limiting the degree of lethal myocardial injury (1,128). Adenosine also decreases lipolysis ($A_1$-receptor-mediated) which could stabilize cellular membranes and prevent further lipid peroxidation (59). Therefore, the multiple effects of adenosine on free radical generation in reperfused tissue suggest that it would be useful in limiting free radical-induced reperfusion injury after regional ischemia.

### *Adenosine restores calcium homeostasis*

It is well known that activation of $A_1$ receptors opens $K_{ATP}$ channels in the myocardium (28). Because open $K_{ATP}$ channels hyperpolarize myocardial cells and consequently reduce calcium via voltage-regulated calcium channels, adenosine ameliorates reperfusion injury in part via this mechanism. In support of this conclusion, recent experiments indicate that: (i) protection from reperfusion injury by ischemic preconditioning is mediated by activation of $A_1$ receptors by endogenous adenosine (98), (ii) the protective effects of ischemic preconditioning are blocked by glibenclamide, an antagonist of $K_{ATP}$ channels (69), and (iii) aprikalim, a $K_{ATP}$ channel opener, reduces reperfusion injury (69). Of particular note is a recent study demonstrating that administration of the $K_{ATP}$ channel opener nicorandil just before reperfusion improves outcome in acute MI patients undergoing mechanical reperfusion therapy (82). In addition to opening $K_{ATP}$ channels, activation of $A_1$ receptors would inhibit catecholamine-induced activation of adenylate cyclase, which in turn would decrease intracellular levels of cAMP, reduce activation of protein kinase A,

decrease phosphorylation of the calcium slow channel, and attenuate the flux of calcium through this channel.

### *Adenosine promotes vascular repair and inhibition of ventricular remodeling*

Recent studies suggest that adenosine may play an important role in promoting vascular repair (vasculogenesis) and accelerating development of new blood vessels (angiogenesis) following vascular injury. Exogenous and endogenous adenosine promotes growth of cultured rat and porcine endothelial cells via stimulation of the $A_{2B}$ receptor (42). In the intact mouse, wound healing is accelerated secondary to recruitment of endothelial progenitor cells and local vessel formation (angiogenesis) with an $A_{2A}$ receptor agonist (106). Adenosine stimulates the secretion of angiogenic factors, such as IL-8, vascular endothelial group factor (VEGF) and basic fibroblast growth factor (bFGF) from microvascular endothelial cells (49). Adenosine stimulates mast cells to secrete IL-8, VEGF ($A_{2B}$) and angiopoietin-2 ($A_3$) in a cooperative fashion resulting in new capillary formation (49). Therefore, adenosine may play an important role in repairing injured endothelial cells in the microvasculature after reperfusion and preventing ventricular remodeling by promoting collateral blood flow.

Adenosine, through $A_{2B}$ receptors, inhibits vascular smooth muscle cell proliferation, migration and extracellular matrix production (43). Because most patients with acute MI undergo primary or deferred stenting, adenosine theoretically may limit intimal hyperplasia following balloon injury by inhibiting vascular smooth muscle cell responses.

### *Adenosine is the mediator of pre-and post-conditioning*

Ischemic pre-conditioning refers to the observation that a brief period of ischemia renders the myocardium more resistant to reversible or irreversible injury following a subsequent more prolonged episode of ischemia (110). There are two phases of pre-conditioning — an early phase that provides immediate protection lasting 1–2 h after the stimulus ("classical" or early pre-conditioning) and a late phase that develops 18–24 h later and lasts for days (155). While both types provide a permanent reduction in infarct size, their protective effects are lost when the ischemic time is greater than 60 min in the *in vivo* canine model (155). Post-conditioning is a recently described phenomenon where repetitive short bursts of ischemia following a prolonged ischemic insult results in a significant decrease in myocardial necrosis (173). Both phenomena are well described in a number of animal models of regional ischemia, after global ischemia in the isolated heart model and in cell culture models subjected to hypoxia (88).

Adenosine is an important endogenous cardioprotective agent that mediates the phenomena of pre- and post-conditioning. In this regard, $A_1$ receptor activation mimics the effects of pre-conditioning in numerous animal species, and $A_1$ antagonists attenuate preconditioning (8,145). Also, preconditioning is enhanced in transgenic animals over-expressing the $A_1$ receptor (107). Other studies suggest a role of the $A_{2A}$ and $A_3$ receptors alone or in conjunction with $A_1$ (165). Post-conditioning is well described in several species and is associated with delayed washout of endogenous adenosine in the isolated heart preparation with subsequent activation of $A_{2A}$ and $A_3$ receptors (88). Both phenomena appear to produce equal myocardial protection although some studies suggest that the two processes are additive (8,88,107,145,173).

Studies have partially elucidated the mechanisms by which adenosine mediates pre-conditioning. $A_1$ receptor stimulation activates protein kinase C (PKC) and thereby modulates the function of many proteins within cells by phosphorylation (27). $A_1$ receptor occupancy also opens cell surface and mitochondrial $K_{ATP}$ channels, which could protect ischemic cells by reducing action potential duration and calcium influx, both of which would conserve energy stores and might reduce calcium-induced injury (28). The important role of the $K_{ATP}$ channel is supported by the observations that glibencamide, a blocker of $K_{ATP}$ channels, abolishes the effect of pre-conditioning, and that pinacidil, a potassium channel opener, reproduces the infarct-size reduction of pre-conditioning (69).

Recent studies have focused on various kinases as participants in pre- and post-conditioning (12,76,77,125,171). Mitogen activated protein kinases (MAPKs) are a family of serine and threonine kinases and include survival kinases, such as p42 and p44 extracellular signal regulated kinases (ERKs), and death promoting kinases, such as p38 and c-Jun-terminal kinase (JNK) MAPK (12,76,77,125,171). Phosphatidylinositol 3-kinase (PI3K) is also a survival kinase which activates yet another kinase called Akt (also known as protein kinase B). The pro-survival kinases are up-regulated and activated in the setting of pre-conditioning, ischemia, and reperfusion. Activation of adenosine receptors has been consistently shown to stimulate survival kinases, thereby mediating a number of physiologic effects which promote cell survival. Firstly, they result in phosphorylation and inactivation of intracellular pro-apoptotic proteins (BIM, BAX, BAD, p53), either directly or via recruitment of p70 kinase (39). Secondly, they induce phosphorylation and inactivation of caspases 3 and 9 which are activated through death-dependent receptors or via receptor-independent pathways which involve release of cytochrome C and apoptosis-inducing factor (AIF) by mitochondria. Thirdly, they promote phosphorylation and activation of endothelial nitric oxide synthase which increases NO production in the cell. Fourthly, they regulate expression of genes associated with cell survival. Finally, activators of MAPKs have been shown to target a nonspecific, large-conductance pore on the inner mitochondrial membrane known as mPTP and prevent its opening. mPTP forms in response to ischemia, and its opening uncouples the mitochondria thereby killing the myocyte by depriving it of its primary energy source (38).

## EFFECTS OF ADENOSINE ON INFARCT SIZE IN EXPERIMENTAL MODELS OF REGIONAL ISCHEMIA

### Methodologic Concerns

Caution must be used when extrapolating the results of animal studies to humans. In this regard, there are differences between experimental acute occlusion of normal animal coronary arteries and that of chronically diseased human coronary vessels. There is variability in collateralization in different animal species and inter-individual variation in collateralization in humans. Unlike in humans, pre-conditioning responses are often absent in animal models of occlusion/reperfusion. Also, the speed of reperfusion is usually immediate and complete in animal models whereas in humans the timing and efficacy of reperfusion varies depending on the reperfusion strategy. Although the effect of

speed of reperfusion on infarct size is unknown, gradual reperfusion does enhance the infiltration of neutrophils into the reperfused zone.

Differences in experimental preparations from different laboratories may also influence the final results of an intervention. The failure in some studies to measure collateral blood flow, an important determinant of cell necrosis in the canine model, could lead to spurious results if the treated group by chance has a higher collateral blood flow (126). Open chest anesthetized preparations undergo accelerated cell necrosis as a result of high myocardial oxygen consumption and potential activation of neutrophils and the sympathetic nervous system when compared with conscious closed chest preparations. Other methodological variables include the type of experimental animal utilized, duration of ischemia and reperfusion, and the method of determination of irreversible injury. Because a number of therapeutic agents merely delay cell necrosis, it is essential to measure infarct size after the infarct is fully evolved. In the canine model, it appears that at least a 24-h period of reperfusion is required to adequately evaluate the effect of an agent or intervention (170). Finally, differences in dose, timing and duration of pharmacologic agents affect drug efficacy. The administration of an agent before and throughout the ischemic period would not allow for differentiation of whether the drug would be beneficial if initiated after the ischemic insult but either before or after reperfusion, the more clinically relevant considerations.

## Effects of Adenosine on Infarct Size in Animal Models of Myocardial Reperfusion

(Table 1)

### *Intracoronary adenosine limits infarct size*

The effects of adenosine on myocardial blood flow and infarct size in a closed chest canine preparation of reperfusion have been extensively investigated. Selective intracoronary administration of adenosine at 3.75 mg/min beginning just before reperfusion and continuing for 60 min thereafter produced a 75% reduction in infarct size expressed as a percentage of the risk region when compared with blood reperfused controls (114). The reduction in infarct size was associated with improvement in regional ventricular function in the ischemic zone. Regression analysis of infarct size and collateral flow showed a definite inverse relationship in control animals whereas in adenosine-treated animals infarct size was small irrespective of flow. The difference between regression lines was greater at lower collateral flow suggesting a greater benefit of treatment following severe ischemia (114).

### *The time-window of opportunity is between 120 and 180 min*

Additional studies utilizing intracoronary adenosine were performed to determine the time-window of opportunity for intervention with adenosine therapy in the canine model. Animals were subjected to 40 (Group I), 120 (Group II), and 180 min (Group III) of coronary occlusion followed by reperfusion with either adenosine (3.75 mg/min) or blood reperfusion alone (control) into the left main coronary artery for the first 60 min of reperfusion (10,57,153). In the 40- and 120-min, but not 180-min, groups, adenosine significantly reduced infarct size, assessed ≥24 h after reperfusion. These observations confirm and extend our previous findings where intracoronary adenosine reduced infarct size after

90 min of ischemia (114). These studies demonstrate that reperfusion-induced injury has a limited time course and that adenosine is only effective when given following 40, 90, and 120 min of ischemia but not after 180 min of ischemia. These finding have major implications for interpreting clinical trials with adenosine in acute MI and indicate the need to institute adenosine/reperfusion therapy in less than 180 min following the onset of symptoms, and preferably within the first 120 min.

### *Intravenous adenosine is also efficacious and reduces ultimate infarct size*

To determine the efficacy of intravenous (as opposed to intracoronary) adenosine, and to evaluate whether adenosine was reducing and not just delaying infarct size, intravenous adenosine (140 μg/kg/min) was administered to animals undergoing 90 min of regional ischemia beginning just before reperfusion and continuing for 150 min after reperfusion (121). Treated animals demonstrated a significant decrease in infarct size at 72 h ($35.3 \pm 4.3\%$ in controls vs. $17.1 \pm 4.3\%$ in treated animals, $P < 0.01$) and improved regional ventricular function utilizing a computer-calculated radial shortening method ($5.5 \pm 2\%$ in controls vs. $17.3 \pm 3.5\%$ in treated animals, $P < 0.01$). Adenosine did not significantly affect heart rate or blood pressure. Therefore, intravenous adenosine resulted in a sustained reduction of infarct size in the canine model. Other laboratories have confirmed these findings in open chest canine models although the route and doses utilized varied (20,168).

*TABLE 1. Preclinical studies on the effects of adenosine (administered in the peri-reperfusion period) on infarct size*

| Reference | Species | Ischemic time (min) | Reperfusion period (h) | Dose and route of administration | Infusion time (min) | Infarct size reduction | % reduction |
|---|---|---|---|---|---|---|---|
| Olafsson et al. (114) | Dog | 90 | 24 | 3.75 mg/min intracoronary | 60 | Yes | 75 |
| Velasco et al. (153) | Dog | 40 | 72 | 3.75 mg/min intracoronary | 60 | Yes | 63 |
| Forman et al. (57) | Dog | 120 | 24 | 3.75 mg/min intracoronary | 60 | Yes | 75 |
| Babbitt et al. (10) | Dog | 180 | 72 | 3.75 mg/min intracoronary | 60 | No | 0 |
| Pitarys et al. (121) | Dog | 90 | 72 | 140 μg/kg/min intravenous | 150 | Yes | 50 |
| Budde et al. (20) | Dog | 60 | 6, 24, 48 | 140 μg/kg/min intravenous | 120* | Yes | 50 |
| Zhao et al. (168) | Dog | 60 | 6 | 140 μg/kg/min into left atrium | 120 | Yes | 50 |
| Norton et al. (113) | Rabbit | 30 | 48 | 0.1, 0.3, 0.55 mg/min intravenous | 60 | Yes | 40 |
| Norton et al. (112) | Rabbit | 30 | 48 | 0.1, 0.01, 0.001 mg/min intravenous | 60 | Yes | 53 |

*Multiple doses given with reperfusion times of 24 and 48 h.

### *Adenosine is effective even in the setting of low collateral blood flow*

To evaluate the effect of adenosine in a model with inherently low collateral blood flow, various doses of intravenous adenosine, beginning just before reperfusion and continuing for 60 min, were administered to rabbits undergoing 30 min of occlusion of the left circumflex artery and 48 h of reperfusion (113). Low (0.1 mg/min), intermediate (0.3 mg/min) and high (0.5 mg/min) doses of adenosine significantly reduced histologically-determined infarct size as a percent of the perfusion bed (control: 52.0 ± 4.6%; low: 35.3 ± 4.1%; immediate: 31.7 ± 4.6%, high: 31.3 ± 4.6%). Both the immediate and high doses significantly reduced mean blood pressure. One possible explanation for the absence of a dose-response effect on infarct size is that the drug may be acting on circulating formed elements exposed to high levels of the nucleoside at the tip of the infusion catheter. These findings suggest that myocardial reperfusion injury plays a significant role in limiting myocardial salvage even when collateral blood flow is low.

### *The protective effects of adenosine are receptor mediated*

The protective effects of adenosine on myocardial reperfusion injury could be mediated through activation of several receptors or through a non-receptor mechanism such as replenishment of the ATP pool. To address this, the efficacy of adenosine at various doses was compared with that of a selective $A_1$ receptor agonist (cyclopentyladenosine) and a selective $A_{2A}$ receptor agonist (CGS21680) in the rabbit model (112). A significant reduction in infarct size was noted with all three doses of adenosine, intermediate and low doses of cyclopentyladenosine, and the high and intermediate doses of CGS21680. Furthermore, all three adenosine receptor agonists afforded equal degrees of protection. This study shows that intravenous infusion of a very low dose of adenosine enhances myocardial salvage and that this protection is receptor mediated. The contribution of each receptor type to myocardial protection was not determined in this study because it is conceivable that local concentrations of each agent may have been high at the infusion site thereby negating the selectivity of each agonist on formed elements such as neutrophils and platelets.

Numerous other laboratories have confirmed the cardioprotective effects of $A_1$ and $A_2$ receptor agonists on myocardial reperfusion injury in various models of regional ischemia (135,162,166). Also, overexpression of cardiac $A_1$ receptors in transgenic mice resulted in a marked protection from ischemic-reperfusion injury (104). Although the $A_3$ receptor plays a role in conjunction with the $A_1$ receptor in preconditioning, its role in reperfusion injury remains unclear. Specific $A_3$ receptor agonists exhibited cardioprotective effects on isolated myocytes, in the isolated perfused heart subjected to hypoxia, and in small animal models of ischemia (144,148). One study in the dog model showed that a specific $A_3$ agonist IB-MECA given just before reperfusion resulted in a 40% reduction in infarct size measured 3 h after reperfusion (7). In contrast, genetic deletion of the $A_3$ receptor significantly reduced infarct size (71). A plausible hypothesis is that excessive $A_3$ stimulation could enhance the inflammatory response through mast cell degranulation. The contribution of each receptor type in the pathogenesis of myocardial reperfusion injury will be difficult to define due to variability in receptor expression, affinity for specific receptor agonists and the variability of receptor types on cells of the same organ in different species.

### *Mechanisms of adenosine-mediated attenuation of reperfusion injury*

Numerous studies have shown that reperfusion accelerates structural and functional changes in the vasculature of the ischemic bed resulting in a progressive decrease in blood flow ("no-reflow" phenomenon) (3,9,34,54,55,87,95,117,122,150,151). Adenosine has been demonstrated to prevent the progressive decrease in blood flow to the inner two-thirds of the myocardial bed at both 3 and 24 h after reperfusion following 40–120 min of regional ischemia (9,57,114,153). Additional studies demonstrate that adenosine preserves endothelial dependent and independent vasodilatory reserve in animals subjected to 2 h of ischemia (9). Subsequent studies by Budde et al. show that endothelial dependent vasodilation is maintained up to 48 h after reperfusion (20). Ultrastructural analysis in animals subjected to 120 min or less of ischemia demonstrate extensive microvascular injury in capillaries in the subendocardium associated with luminal plugging by endothelial projections, neutrophils, platelets and red cells in control animals. Adenosine treatment markedly attenuates these changes with relative preservation of endothelial cells and only occasional obstruction of capillaries by cellular elements (9). These and other studies suggest that prevention of microvascular injury by adenosine may preserve reversibly injured myocytes following restoration of blood flow. The importance of the vascular compartment as a primary site for the cardioprotective effect of adenosine is supported by the observation that the administration of an adenosine mimetic confined to the intravascular space reduces infarct size by inhibiting neutrophil adherence to the endothelium (146).

In contrast to the findings when reperfusion is restored after 120 min of ischemia, infusion of adenosine after 180 min of ischemia followed by reperfusion does not increase blood flow to the inner two-thirds of the myocardium or prevent the "no-reflow" phenomenon (10). Similarly, ultrastructural changes are severe and similar in both control and treatment groups with endothelial swelling, cytoplasmic projections, presence of membranous vesicles and focal disruption, and plugging of capillary lumens by cellular elements. These findings suggest that microvascular injury may be irreversible in the inner two-thirds of the myocardium after 180 min of ischemia.

The mechanisms responsible for the progressive decrease in regional blood flow after the ischemia-reperfusion cycle are complex and not fully defined. Both mechanical factors, such as leukostasis, platelet aggregation and/or vascular disruption, as well as humoral mediators may be involved (45,57,154). The mechanisms through which adenosine is effective in ameliorating reperfusion injury are likely multifactorial. The antineutrophil effects of adenosine appear to play a major role in its protective action on the vasculature (32,159,169). Numerous studies, utilizing histological methods and by measuring myeloperoxidase activity, show that adenosine reduces neutrophil infiltration into the reperfused bed (9,114,168). Immunohistochemical studies reveal reduced density of CD18 positive neutrophils in treated animals (168). *In vitro*, adenosine reduces adherence of unstimulated neutrophils to isolated epicardial coronary arteries exposed to ischemia and reperfusion suggesting that adenosine augments the basal antineutrophil function of the endothelium (168). The mechanism does not appear to involve ICAM expression which is constitutively expressed and remains unchanged following 6 h of reperfusion (168).

*In vitro* studies show that adenosine and adenosine analogues markedly inhibit superoxide anion production by neutrophils through $A_{2A}$ activation (32,169). Adenosine also reduces neutrophil adherence and cytotoxicity to cultured endothelial cells via $A_{2A}$ receptors (169). Adenosine inhibits upregulation of CD116b/CD18) on FMLP-activated neu-

trophils via the $A_{2A}$ receptor (159). In animals, adenosine reduces apoptotic cell death, confirmed by the presence of cleavage of double stranded DNA on aggarose gels and TUNEL positive cells on myocytes, following regional ischemia (166,168). These findings in conjunction with preservation of endothelial cell function support the hypothesis that adenosine's cardioprotective effects are mediated by inhibition of neutrophil-induced vascular injury following reperfusion.

Adenosine may also prevent microvascular spasm by reversing the effects of numerous vasoconstrictive mediators released from damaged endothelial cells, activated platelets, and neutrophils in the reperfused bed. The isolation of a potent vasoconstrictive peptide from endothelial cell suggests that ET may play a role in microvascular hypoperfusion following relief of myocardial ischemia (132,163,164). In rabbits, 3 h of reperfusion increases by 2.6 fold the myocardial levels of ET RNA (156). While regional myocardial ischemia *in vivo* results in progressive and parallel increases in coronary sinus and aortic levels of ET, a further significant release in coronary sinus levels occurs during the early reperfusion period (154). Moreover, there is a significant correlation between mean reperfusion levels of ET in the coronary sinus and endocardial blood flow 3.5 h after reperfusion (154). Infusion of adenosine in known cardioprotective doses suppresses cardiac release of ET (152). Blockade of $ET_A$ and $ET_B$ receptors reduces infarct size in the rabbit, further supporting a role for ET in reperfusion injury (156). The detrimental effects of ET on the reperfused myocardium may involve mechanisms other than alterations in blood flow. ET produces intercellular alkalization and increases inositol phosphate production and intracellular calcium levels, all of which can be potentially detrimental to the survival of reperfused cells. Furthermore, ET may have an important role in the inflammatory response because it may mediate the effects of some cytokines. An additional effect of ET may be associated with its actions on ion fluxes and membrane potential changes such as its effects in calcium activated potassium channels (156).

Apoptosis is a distinct reperfusion-induced phenomenon which involves cleavage of genomic DNA by endonuclease (172). Inhibition of apoptosis by endonuclease inhibitors reduces infarct size suggesting that apoptotic cells may undergo secondary death during the later phases of reperfusion and contribute to final infarct size following regional ischemia (172). In the isolated perfused heart endothelial cell apoptosis precedes myocyte apoptosis suggesting that the vascular cells release pro-apoptotic mediators (136). Adenosine, through activation of $A_{2A}$ receptors, significantly reduces the number of apoptotic cells and is associated with modification of pro- and anti-apoptotic regulatory proteins (166). The reduction in apoptosis by adenosine may also be secondary to the modification of the release of cytokines, proteolytic enzymes and reactive oxygen species by acute and chronic inflammatory cells in the reperfused bed (172).

## CLINICAL TRIALS UTILIZING ADENOSINE IN ACUTE MI (Table 2)

Measurements of early and late mortality are the most powerful and direct endpoints in randomized trials assessing reperfusion therapy in acute MI. However, because timely reperfusion per se reduces infarct size and improves survival, large numbers of patients

are required to demonstrate statistically significant incremental reductions in mortality with adjunctive regimens. The inclusion of low-risk patients is another challenge to statistical power. Therefore most studies have incorporated surrogate endpoints of myocardial salvage and/or infarct size utilizing the radiopharmaceutical technetium-99m sestamibi, which correlate closely with pathologically determined risk regions and infarct size in the experimental model. Of note, the area at risk and final infarct size, as determined by sestamibi imaging, are significantly larger in anterior than non anterior MI (65). Clinical events and mortality are also lower with inferior infarctions, thus requiring large number of patients to evaluate efficacy of an adjunctive therapy. The interpretation of clinical studies of adjunctive agents such as adenosine following reperfusion therapy in acute MI thus depends on the breakdown of anterior vs. non-anterior MI, as well as other factors including myocardial salvage and final infarct size, baseline TIMI flow, time to reperfusion and the presence of collateral vessels to the infarct zone.

Garratt et al. performed the first clinical study of adenosine in acute MI (61). In this small pilot study, intravenous adenosine at 70 μg/kg/min was infused over 1 h with concomitant lidocaine in 35 patients with acute MI (57% anterior) undergoing primary PTCA. Control patients comprised a historical group (47 patients, 32% anterior) who had undergone primary PTCA. Although the primary endpoint was safety, Garratt et al. also measured myocardial salvage. Transient hypotension developed in three patients requiring lowering of the dose. Adenosine did not significantly affect heart rate nor did it precipitate advanced AV block. Although the risk region and timing of the late perfusion images were dissimilar, adenosine-treated patients exhibited significantly greater salvage ($30 \pm 21$ vs. $13 \pm 19\%$; $p < 0.001$) (61).

*TABLE 2. Clinical studies with adenosine in acute ST segment elevation myocardial infarction*

| Reference | Number of patients | Study type | Reperfusion strategy | Dose of adenosine | Infarct size (SPECT) | LV function | MACE |
|---|---|---|---|---|---|---|---|
| Garratt et al. (61) | 82 | Historical controls | PTCA | 70 μg/kg/min IV for 60 min | Reduced | — | — |
| Claeys et al. (25) | 279 | Historical controls | PCI | 60 or 90 μg/kg/min i.c. for 20 min | Reduced (EKG) | — | Decreased |
| AMISTAD I (100) | 236 | Prospective randomized | Lytics | 70 μg/kg/min i.v. for 3 h | Decreased (anterior) | — | Unchanged |
| Marzilli et al. (103) | 56 | Prospective randomized | PTCA | 4 mg i.c. | Decreased (CPK) | Improved | Decreased |
| ATTAC (123,124) | 608 | Prospective randomized | Lytics | 10 μg/kg/min i.v. for 6 h | — | Unchanged | *Decreased |
| AMISTAD II (91,130) | 2118 | Prospective randomized | PTCA or lytics | 50 or 70 μg/kg/min i.v. for 3 h | Decreased (70 dose) | — | $^{+}$Decreased |

**Post hoc* analysis of 181 patients with anterior infarction and decreased LV function; $^{+}$*post hoc* analysis of patients treated within 3.17 h of symptoms; CPK, creatine phosphokinase; EKG, electrocardiogram; i.c., intracoronary; i.v., intravenous; MACE, major adverse clinical events; PTCA, percutaneous coronary angioplasty; PCI, percutaneous coronary intervention; SPECT, single photon computer tomography.

In a retrospective study, Claeys et al. evaluated 79 patients presenting within 12 h of STEMI with 200 historical controls undergoing PCI (PTCA, stents) (25). Treated patients received a 20-min intracoronary infusion of adenosine of 90 μg/kg/min into the left coronary system or 60 μg/kg/min into the right system commencing just before PCI. Reperfusion injury was defined as persistent (>50% of initial value) ST elevation after PCI and infarct size expansion was evaluated with a QRS scoring system performed prior to PCI and at 7 weeks after intervention. Reperfusion injury was significantly reduced in the adenosine group (19 vs. 35%; $p = 0.004$) and this was reproducible in a subgroup of patients receiving stents and glycoprotein $II_b/III_a$ inhibitors. Infarct size remained unchanged in the adenosine group ($3.4 \pm 3.0$ and $3.5 \pm 3.1$), whereas it increased significantly in the control group ($3.1 \pm 2.7$ and $4.5 \pm 3.2$; $p = 0.003$). Although the study was underpowered to evaluate clinical events, major adverse events (death or infarction) occurred more frequently in patients with reperfusion injury (~14%) compared to those without (~2%).

The first prospective, multicenter open-label **A**cute **M**yocardial **I**nfarction **St**udy of **AD**enosine (AMISTAD-I) trial was performed in 236 patients with anterior and inferior infarctions presenting within 6 h of symptoms who were candidates for thrombolytic therapy (100). Adenosine (70 μg/kg/min) or placebo (saline) was infused for 3 h with coadministration of lidocaine in 72% of patients. Primary endpoints were infarct size measured by tc-99m single-photon emission computed tomography (SPECT) sestamibi imaging at $6 \pm 1$ days, with secondary endpoints being myocardial salvage index (available in 30% of patients) and in-hospital clinical outcomes (death, re-infarction, shock, CHF or stroke). A 33% relative reduction in infarct size (% of left ventricle) was present in the entire group (13 vs. 19.5%, $p = 0.03$ by univariate analysis, $p = 0.03$ by multivariate analysis), with anterior infarcts exhibiting a 67% reduction (15 vs. 45.5%: $p = 0.014$). Myocardial salvage was also significantly greater in anterior infarcts treated with adenosine (62 vs. 15% of the left ventricle, $p = 0.015$). In-hospital clinical outcomes occurred with similar frequency between the two treatment groups, though a trend was present toward more adverse clinical events in the patients with non anterior MI assigned to adenosine compared with placebo. Hypotension, bradycardia, heart block and ventricular arrhythmias were also slightly more common in adenosine treated patients with inferior infarcts.

Marzilli et al. performed a well-conceived, albeit small, study in 56 patients (50% anterior infarction) undergoing primary angioplasty within 2 h of the onset of symptoms (103). The investigators randomly administered an intracoronary bolus of adenosine (4 mg) or saline prior to angioplasty. Adenosine significantly decreased the "no-reflow" phenomenon, as assessed by TIMI flow 30 min after successful PTCA, significantly decreased CPK levels and adverse clinical effects, and significantly improved regional ventricular function as measured by echocardiography one week after reperfusion.

ATTAC (Attentuation of Cardiac Complications Trial) was a prospective large-scale, randomized placebo-controlled study in which a low dose of adenosine (10 μg/kg/min) was infused intravenously for 6 h in patients undergoing thrombolysis (123). The primary endpoint was global and regional ventricular function measured with echocardiography 4 days after hospitalization. The oversight committee prematurely discontinued ATTAC after 608 of the planned 1,000 patients had been randomized due to failure to show beneficial effects on the primary endpoint. However, the majority of patients had small infarcts (left ventricular ejection fraction greater than 40%), and a significant degree of stunning may still be present at 4 days, making interpretation of the results problematic. At 12 months, trends were present among 292 patients with anterior infarcts treated with adeno-

sine in all-cause mortality (8.4 vs. 15.3%; $p = 0.07$) and cardiovascular mortality (8.4 vs. 14.6%; $p = 0.08$). The authors subsequently performed a *post hoc* analysis in 181 patients with anterior infarcts and depressed left ventricular function on day four (wall motion score greater than 1.5) (124). This analysis showed significant differences in both all-cause mortality (2 vs. 12.1%; $p = 0.007$) and cardiovascular mortality (2 vs. 10.8%; $p = 0.01$) in the treated group. Thus, although the study was underpowered, beneficial clinical endpoints were demonstrated in the high-risk group with anterior infarction.

AMISTAD II was a double-blinded, placebo-controlled randomized study in 2118 patients with anterior STEMI (ST-segment elevation myocardial infarction) undergoing thrombolysis or primary angioplasty within 6 h of symptoms (130). Patients were randomized to either a 3-h infusion of adenosine (50 or 70 μg/kg/min) or placebo. The primary endpoint was the 6 month composite rate of death, new onset CHF developing after 24 h, or re-hospitalization for CHF. The major secondary endpoint was infarct size measured by technetium-99m sestamibi SPECT imaging in a 243 patient substudy. Patients tolerated adenosine well with a low incidence of adverse effects. There was no difference in the primary end point between placebo and the pooled adenosine dose groups (17.9 vs. 16.3%, $p = 0.43$). The composite rate was also similar in the low dose and high dose adenosine groups (16.5 vs. 16.1%). The failure to observe a significant clinical benefit was not surprising since the study was severely underpowered. A trend was present toward a smaller median infarct size in the pooled adenosine group compared with the placebo group (17 vs. 27%, $p = 0.074$). However, adenosine resulted in a marked reduction (55%) in infarct size in the high-dose group compared to placebo (11 vs. 27%, $p = 0.02$) whereas the low-dose group did not exhibit myocardial salvage (final infarct size 23 vs. 27%, respectively, $p = 0.41$). Moreover, infarct size and the occurrence of the primary composite clinical endpoint were significantly related; the median infarct size in patients developing an adverse clinical endpoint was 43% compared to 17% in patients without an adverse endpoint event ($p < 0.001$). In an abstract presentation, a subgroup analysis of the pooled-adenosine group who were *successfully* reperfused did show a significant reduction in the composite endpoint (4). Given the well described impact of reperfusion delays on myocardial salvage and the observation of a narrow time window for reperfusion injury in animal models, a *post hoc* analysis of the AMISTAD II trial has recently been published evaluating the effect of time to treatment on the efficacy of adenosine on clinical endpoints (91). In patients receiving reperfusion therapy within 3.17 h of symptoms adenosine compared to placebo significantly reduced 1-month mortality (5.2 vs. 9.2%, $p = 0.014$),6-month mortality (7.3 vs. 11.2%, $p = 0.03$), and the occurrence of the 6-month composite clinical endpoints of death, inhospital CHF or rehospitalization for CHF at 6 months (12.0 vs. 17.2%, $p = 0.022$). These findings extend the observations of the original trial and other smaller clinical studies and strongly support the utilization of intravenous adenosine as adjunctive therapy in anterior STEMI treated with reperfusion strategies within 3–4 h of symptoms.

## PHARMACOKINETICS AND ADVERSE EFFECTS

Adenosine functions as a local hormone and is found in numerous tissues and organs throughout the body. Cardiac vascular levels during myocardial ischemia are derived pre-

dominately from myocytes when adenosine undergoes simple diffusion into the interstitial fluid space with subsequent "washout" into the vascular system. During normoxia endothelial cells release small amounts of adenosine directly into the vascular compartment. Intravascular adenosine has an extremely short half-life (1–2 sec in humans) due to rapid uptake by endothelial cells and red blood cells orchestrated by an active nucleoside transport system. Intracellular adenosine is then rephosphorylated to ATP or deaminated to inosine. Since adenosine is an endogenous compound and has a rapid plasma half-life, no cytotoxic or safety issues have arisen with intravenous infusions. The frequency of adverse effects in the Adenoscan Mulicenter Registry Trial evaluating the effects of a six minute infusion of adenosine at 140 μg/kg/min in patients with suspected coronary artery disease and in the AMISTAD II trial where patients with anterior STEMI received a 3-h infusion of 50 or 70 μg/kg/min are shown in Tables 3 and 4. In both studies the infusions were safe and the infusion protocol completed in the majority of patients. Intolerable adverse effects rapidly resolved with a decrease or termination of the infusion.

*TABLE 3. Frequency of adverse effects in the ADENOSCAN Multicenter Trial Registry (n = 9256)*

| | Number of patients | % |
|---|---|---|
| Flushing | 3377 | 36.5 |
| Dyspnea | 3260 | 35.2 |
| Chest pain | 3207 | 34.6 |
| Abdominal discomfort | 1352 | 14.6 |
| Headache | 1318 | 14.2 |
| TNJ discomfort | 1078 | 11.6 |
| Dizziness | 783 | 8.5 |
| AV block | 450 | 4 |
| Arrhythmia | 309 | 3.3 |
| Hypotension | 163 | 1.8 |
| Bronchospasm | 12 | 0.1 |

AV block, second and third degree atrioventricular block; TNJ, throat, neck, and jaw.

*TABLE 4. Frequency of adverse events in the clinical trial of adenosine as an adjuct to reperfusion in the treatment of myocardial infarction (AMISTAD II)*

| Adverse events | Placebo $n = 692$ | Adenosine 50 μg/kg/min $n = 690$ | Adenosine 70 μg/kg/min $n = 702$ |
|---|---|---|---|
| Hypotension (%) | 14.00 | 19.40 | 18.40 |
| Bradycardia (%) | 2.30 | 2.70 | 2.70 |
| Ventricular tachycardia (%) | 3.60 | 1.90 | 4.30 |
| Second degree AV block (%) | 0.01 | 0.01 | 0.03 |
| Third degree AV block (%) | 0.00 | 0.01 | 0.04 |
| Nausea/vomiting (%) | 6.90 | 7.10 | 7.80 |
| Premature drug discontinuation(%) | 3.60 | 6.40 | 5.10 |

## CONCLUSIONS

The optimal treatment of choice for most patients with STEMI is catheter-based mechanical reperfusion performed either as a primary procedure or in conjunction with thrombolytic therapy. While successful reperfusion reduces cardiac mortality, decreases infarct size and improves ventricular function, studies in high risk patients demonstrate less favorable outcomes with suboptimal myocardial salvage and high mortality. Understanding the deleterious effects of reperfusion injury in these patients may allow for the development of adjunctive therapies to enhance left ventricular recovery and further improve clinical outcomes in this high risk group. Numerous experimental investigations have demonstrated that adenosine has multiple favorable effects that may mitigate reperfusion injury, including inhibition of neutrophil-mediated vascular damage (thus abrogating the "no-reflow" phenomenon), and mediation of pre- and post-conditioning. In experimental models of ischemia <180 min, both intracoronary and intravenous administration of adenosine has resulted in a marked reduction in infarct size. Clinical studies have demonstrated a significant reduction of infarct size in patients with anterior MI reperfused within 6 h treated with intravenous high dose adenosine. Though the studies performed to date have been of insufficient size to demonstrate an improvement in clinical outcome with adenosine with an intention to treat analysis, such a relationship may be expected based on the strong association between infarct size and adverse events noted in the AMISTAD II trial. This hypothesis has recently been validated by the *post hoc* analysis where a significant reduction in early and late mortality and composite clinical endpoint of death or CHF at 6 months was observed in adenosine patients treated within 3.17 h of symptoms (91).

Cardiologists should no longer consider myocardial reperfusion injury as purely a laboratory phenomenon; rather they should conceptualize reperfusion injury as a significant contributor to final infarct size. In contrast to other agents which have shown disappointing effects in man, adenosine is the only agent which has consistently reduced infarct size in patients with anterior MI. *Post hoc* analysis of AMISTAD II trial demonstrates that infarct size reduction is also associated with a significant reduction in mortality and improvement in event–free survival. These observations support the utilization of a 3 h infusion of adenosine at 70 μg/kg/min as adjunctive therapy in patients with anterior STEMI undergoing reperfusion therapy within 3 to 4 h of the onset of symptoms.

**Abbreviations.** AMISTAD, Acute Myocardial Infarction STudy of ADenosine; ATP, adenosine triphosphate; CGS21680, 2-*p*-(carboxyethyl)phenethyl-5′-N-carboxamidoadenosine; CHF, congestive heart failure; EDRF, endothelium-derived relaxing factor; ET, endothelin; fMLP, formyl-Met-Leu-Phe; IB-MECA, 1-deoxy-1-[6-[(3-iodophenyl)methyl]amino]-*9H*-purin-9-yl]-*N*-methyl-β-D-ribofuranuronamide; IL, interleukin; MI, myocardial infarction; NO, nitric oxide; PAF, platelet activating factor; PCI, percutaneous coronary intervention; PTCA, Percutaneous Transluminal Coronary Angioplasty; TIMI, Thrombosys In Myocardial Infarction; TNF, Tumor Necrosis Factor; SPECT, Single Photon Computer Tomography; STEMI, ST-segment Elevation Myocardial Infarction.

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