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Role of Adenosine in the Treatment of Myocardial Stunning

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Summary. **Adenosine is an endogenous nucleoside produced from the breakdown of adenosine triphosphate (ATP) that possesses a number of complex cellular and metabolic effects that could ameliorate postischemic contractile dysfunction (myocardial stunning). Potential mechanisms include the repletion of high-energy phosphate stores, reduced myocardial oxygen consumption, a decrease in oxygen-derived free radicals, restoration of calcium homeostasis, and an increase in regional myocardial blood flow. Experimental studies have shown that adenosine can reduce myocardial stunning with or without a concomitant increase in the total myocardial ATP stores. Adenosine may be a useful pharmacologic strategy in the prevention and treatment of ventricular dysfunction following episodes of regional or global ischemia, although further studies are needed to clarify the precise cellular mechanisms involved.**

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Key Words. **adenosine, adenosine triphosphate, regional blood flow, free radicals, calcium homeostasis, stunning**

Myocardial stunning is defined as postischemic dysfunction of myocytes that have not undergone irreversible cell injury [1,2]. The concept of stunning was initially described after brief periods of regional ischemia (5–15 minutes), emphasizing that it occurred in the setting of reversibly injured cells [2,3]. This definition has been broadened to include longer periods of ischemia, since stunning may also occur in reversibly injured cells in the border zones surrounding areas of infarcted myocardium [4,5]. Therefore, early reperfusion of an evolving myocardial infarction may result in prolonged left ventricular dysfunction due to gradual recovery of viable myocardium in the outer two thirds of the ventricular wall overlying an area of subendocardial infarction. Regional myocardial ischemia in patients following exercise-induced angina, after prolonged episodes of coronary vasospasm, and following percutaneous coronary balloon angioplasty may also produce myocardial stunning [6,7]. Finally, the observation that isolated perfused hearts subjected to global ischemia or hypoxia manifest stunning suggests that ventricular dysfunction may also occur in patients undergoing cardiopulmonary bypass during cardiac surgery [8,9]. This observation is supported by the finding that many patients require inotropic support during the early postoperative period following surgery.

Metabolic Consequences of Ischemia

The limited energy reserves in the myocardium coupled with the high-energy requirements mandate a constant supply of oxygen and substrates to myocardial cells to maintain normal metabolic and contractile function [10,11]. The normal myocardium preferentially utilizes free fatty acids that are metabolized via β-oxidation to yield 72 molecules of adenosine triphosphate (ATP) [11]. High-energy phosphate (HEP) stores in the myocardium are limited and exist predominantly in the form of ATP and creatine phosphate (CP) [10]. Myocardial ischemia is associated with the cessation of oxidative phosphorylation such that alternate metabolic pathways are activated in an attempt to maintain cellular levels of HEP. The low levels of citrate, ATP, and CP present in ischemic tissue in conjunction with increased concentrations of inorganic phosphates results in activation of various metabolic steps involved in anaerobic glycolysis, resulting in enhancement of this pathway as an alternate source of HEP [12]. This mechanism is relatively inefficient, producing less than 10% of the ATP required for normal myocardial function and therefore is unable to sustain myocardial viability for prolonged periods [10]. Furthermore, continued ischemia results in progressive acidosis due to the accumulation of protons, for example, $NADPH_2$ and lactic acid, with eventual inhibition of glycolysis as an alternate energy source [12]. Small quantities of ATP are also produced during myocardial ischemia via dephosphorylation of CP. The

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limited stores of CP in conjunction with inefficiency of anaerobic glycolysis results in rapid depletion of HEP during ischemia such that 80% of creatine phosphate is lost within minutes of regional ischemia and greater than half of ATP by 15 minutes [13].

Although the energy requirements of myocardial cells are decreased during ischemia, ATP continues to be utilized to maintain essential metabolic functions of the cells, such as removal of sodium and replenishment of calcium in the sarcoplasmic reticulum [14]. The inability of the mitochondria to rephosphorylate ATP results in the accumulation of adenosine diphosphate (ADP) and adenosine monophosphate (AMP) [13]. AMP is metabolized via 5-nucleotidase, predominantly found in the membranous fraction of the cell, to adenosine, which is then deaminated to inosine both in the myocardial cell and the interstitium [15]. Once AMP is degraded to adenosine, nucleoside transport systems facilitate the loss of nucleoside molecules, such as adenosine and inosine, from ischemic myocardial cells [16]. Inosine is catabolized to hypoxanthine and xanthine by enzymes localized predominantly in endothelial cells. It has been proposed that the xanthine oxidase pathway may produce large amounts of the superoxide anion with the introduction of oxygen at reperfusion [17].

Restoration of HEP following reperfusion of viable cells can occur via nucleotide salvage pathways that are metabolically rapid or through a slow de novo synthetic pathway (Figure 1) [18]. 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 [19]. Experimental studies have shown that following a 15-minute coronary occlusion, ATP levels may remain significantly depressed, even after 3 days of reperfusion [19].

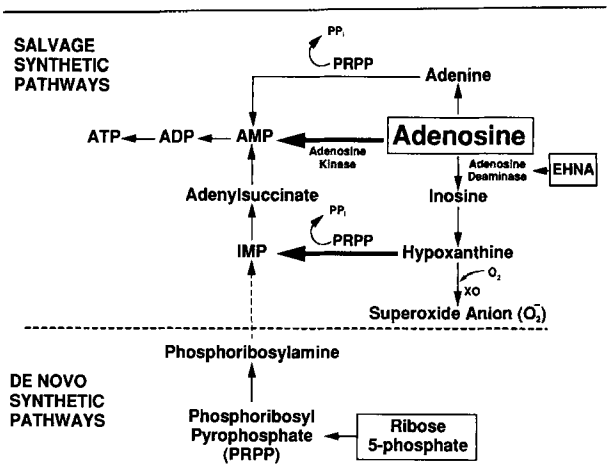

Fig. 1. *Flow diagram illustrating salvage and de novo synthetic pathways in the production of ATP. The salvage pathways that are kinetically most active are thought to be adenosine and hypoxanthine. Adenosine would result in more rapid restoration of high-energy stores, since it can immediately be converted to adenosine monophosphate (AMP) by adenosine kinase. In contrast, the administration of inosine would require more biochemical pathways and would be dependent on phosphoribosyl pyrophosophate (PRPP) as a substrate that may be depleted following ischemia and reperfusion. A limited supply of PRPP would also result in the conversion of hypoxanthine to oxygen-derived free radicals through the xanthine oxidase pathway. Repletion of ATP through de novo synthesis is slow, also requiring a number of intermediary metabolites and the presence of PRPP. EHNA = erythro-9-[2-hydroxy-3-nonyl] adenine; IMP = inosine monophosphate; XO = xanthine oxidase.*

Rationale for Use of Adenosine

Although the exact mechanisms of myocardial stunning remain to be elucidated, numerous mechanisms have been proposed. These include depletion of intracellular HEP stores, generation of cytotoxic oxygen-derived free radicals, alterations in calcium homeostasis, and a reduction in microcirculatory blood flow of the reperfused bed [1,2]. Adenosine is an endogenous nucleoside produced from the degradation of ATP and may be a useful agent in ameliorating myocardial stunning, since it acts on many of the postulated mechanisms. The potential pathways implicated in the pathogenesis of myocardial stunning and the effect of adenosine on these mechanisms is illustrated in Figure 2. The effects of adenosine may be mediated predominantly by extracellular membrane purinergic receptors or through a nonreceptor mechanism such as replenishment of the myocardial nucleotide pool [20]. Extracellular receptors are classified as A_1 or A_2, and are found predominantly in the myocardium and coronary vasculature, respectively. Regulatory G proteins are involved in the signal transduction for both receptors, resulting either in activation (A_2) or inhibition (A_1) of adenylate cyclase [20]. A_1 receptor activation may also open potassium channels independent of adenylate cyclase [21].

Replenishment of ATP stores

Numerous experimental studies utilizing both in vivo and in vitro models of regional and global ischemia have demonstrated a rapid depletion of HEPs, particularly creatine phosphate and ATP [13,19]. Replenishment of ATP stores may take days, depending on

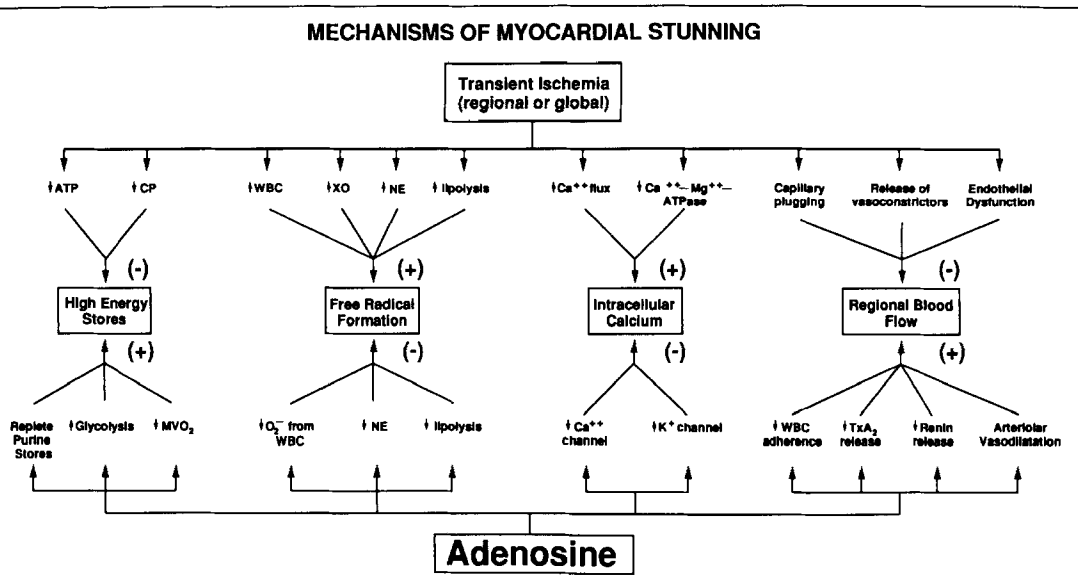

Fig. 2. *Schematic diagram of potential mechanisms of action of adenosine in reducing myocardial stunning. Transient regional or global ischemia followed by reperfusion results in depletion of myocardial high-energy stores, increased production of oxygen-derived free radicals via neutrophils, the xanthine oxidase pathway, autooxidation of catecholamines, and products of lipid peroxidation. Free-radical-mediated injury may also contribute to abnormalities in calcium homeostasis. A reduction in subendocardial blood flow associated with reduced vasodilatory reserve is also observed in the stunned myocardium. Endogenous release of adenosine during ischemia may preserve cell viability by enhancing anaerobic glycolysis and decreasing myocardial oxygen consumption. Reperfusion results in a rapid washout of various adenine nucleosides, including adenosine. Administration of adenosine could accelerate the recovery of contractile function through a number of mechanisms. Adenosine may enhance intracellular ATP levels via the salvage pathway while conserving utilization through its negative inotropic effect. Adenosine could also reduce free-radical-mediated injury by inhibiting superoxide anion release from neutrophils and decreasing catecholamine release and lipid peroxidation. Adenosine also blocks calcium-dependent channels and therefore may restore intracellular calcium homeostasis. Finally, adenosine may enhance oxygen delivery and washout of "toxic" metabolites through arteriolar vasodilatation and anti-platelet and antineutrophil effects. ATP = adenosine triphosphate; ATPase = adenosine triphosphatase; Ca^{++} = calcium ions; CP = creatine phosphate; Mg^{++} = magnesium ions; MVO_2 = myocardial oxygen consumption; NE = norepinephrine; O_2^- = superoxide anion; TXA_2 = thromboxane A_2; WBC = white blood cell.*

the duration and severity of the ischemic episode [19,22]. Recovery of adenine nucleotides occurs in two phases in ischemic cells and is determined by the availability of essential substrates required for the salvage and de novo synthetic pathways [18]. Adenosine would appear to have several advantages over the administration of inosine in restoring ATP levels. First, adenosine would bypass many of the preliminary reactions in the salvage pathways, thereby accelerating its conversion to AMP through the enzyme adenosine kinase. (Figure 1) The conversion of inosine to ATP requires several steps and is dependent on PRPP as a coenzyme [18]. Second, inosine has a lower affinity for the nucleoside transporter than adenosine, and therefore its entry into the myocyte would be slower than adenosine [16]. Finally, the limited supply of PRPP in reperfused tissue would result in the degradation of inosine to xanthine, thereby increasing the potential production of O_2^- via xanthine oxidase [17].

Reduction of myocardial oxygen consumption

Myocardial ischemia is associated with an increase in endogenous catecholamines, which increases myocardial oxygen consumption through the stimulation of beta-adrenoreceptors [23]. Adenosine has been shown to reduce norepinephrine release from sympathetic nerve endings, primarily through its action on the A_1 receptor [24]. The chronotropic and dromotropic effects of A_1 stimulation on the conducting system would also result in a decrease in myocardial oxygen consumption [25]. Adenosine could therefore have beneficial effects on myocardial stunning by delaying HEP depletion during ischemia through a decrease in oxygen consumption.

Improvement in metabolic substrate of the ischemic cell

Continual viability of the ischemic myocardial cell is dependent on the maintenance of minimal energy pro-

duction required to regulate essential metabolic functions. Intracellular levels of potassium and calcium are controlled by Na^+/K^+-ATPase and a calcium ATPase, which require ATP as an energy source [10]. Owen et al. have shown that, during low-flow ischemia, enhanced glucose uptake provides a critical supply of ATP to prevent ischemic contracture [9]. Adenosine increases cellular uptake of glucose independent of its vasodilatory action and stimulates glycolytic flux in both normoxic and anoxic myocardium [26,27]. Adenosine also inhibits lipolysis, which may stabilize cellular membranes and decrease intracellular lactate and subsequent acidosis [28]. Therefore, adenosine nucleotides could have a beneficial effect on myocardial stunning secondary to improvement in the metabolic substrate of the previously ischemic myocardium.

Reduction of oxygen-derived free radicals

Numerous studies support the hypothesis that oxygen-derived free radicals play an important role in the pathogenesis of myocardial stunning [29]. First, the addition of free radicals to in vivo and in vitro preparations reduces contractile function [30,31]. Second, increased quantities of free radicals have been demonstrated in global and regional ischemic models utilizing electron paramagnetic resonance spectroscopy and spin trapping agents [32,33]. Finally, improvement in contractile function in the experimental preparation has invariably been shown by the addition of free-radical scavenging enzymes or by agents that inhibit the formation of free radicals [29]. The exact source of free radicals remains to be determined, but they could be produced from activated neutrophils via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, cyclooxygenase and lipoxygenase pathways, autooxidation of catecholamines, autocatalytic lipid peroxidation, and via intramyocardial sources such as xanthine oxidase and the mitochondrial electron transport chain [17,34,35].

Adenosine possesses a number of physiological effects that may reduce free-radical formation following ischemia. Adenosine has been shown to reduce superoxide anion (O_2^-) production by neutrophils in vitro via an interaction with the A_2 receptor [36]. Inhibition of norepinephrine release from sympathetic nerve endings and reduced formation of thromboxane from platelets may also reduce free-radical generation via autooxidation of catecholamines or from arachidonate byproducts [24,37]. Another potential source of free radicals is the production of O_2^- when hypoxanthine is converted to xanthine via the enzyme xanthine oxidase following the introduction of oxygen at reperfusion [17]. By reducing ATP degradation, adenosine may decrease the production of O_2^- after reperfusion, thereby limiting the degree of myocardial stunning. Adenosine also decreases lipolysis, which could stabilize cellular membranes and prevent further lipid peroxidation [28]. Therefore, the multiple effects of adenosine on free-radical generation in reperfused tissue suggests that it would be useful in limiting free-radical induced ventricular dysfunction after ischemia.

Alleviation of calcium overload

Calcium overload has been proposed as a possible cause of myocardial stunning. Cytosolic free calcium increases after 9–10 minutes of ischemia and precedes the loss of plasma membrane structural integrity [38,39]. Sarcoplasmic reticulum obtained from stunned myocardium demonstrates alterations in calcium transport and a decrease in activation of calcium and magnesium-ATPase [40]. In a Langendorf preparation of global ischemia, a shift in maximal calcium-activated pressure has been observed, which may reflect either decreased myofilament calcium sensitization or a decrease in calcium transients [41]. Selective administration of calcium antagonists into the reperfused bed has been shown to improve ventricular function at doses that do not alter systemic hemodynamics or regional myocardial blood flow [42]. The exact mechanisms whereby calcium induces contractile dysfunction remain unknown, but enhanced calcium entry at reperfusion could activate phospholipases or increase the generation of reactive oxygen species [17]. Stimulation of A_1 receptors by adenosine could reduce calcium overload following ischemia, either by inhibiting potassium-dependent calcium uptake or by impeding further calcium entry through blockade of calcium-dependent channels [43,44].

Prevention of abnormalities of coronary vasodilatory reserve

Another postulated mechanism for myocardial stunning is abnormalities in microvascular blood flow. Regional myocardial ischemia of 10–15 minutes is associated with an increase in resting coronary vascular resistance and a prolonged impairment of vasodilatory reserve to endothelial-dependent and -independent agonists [45,46]. Augmentation of coronary blood flow with papaverine and dipyridamole has been shown to significantly reduce postischemic ventricular dysfunction [47]. The mechanisms remain unknown, but they may be due to alleviation of heterogenous microvascular flow secondary to vasospasm or mechanical plugging, to increased myofiber stretch, or to washout of "toxic" metabolites formed during ischemia. Irrespective of the mechanism, adenosine would improve contractile function through its potent coronary arteriolar vasodilatory action.

Mimicking an endogenous protective mechanism—is adenosine the mediator of preconditioning?

Preconditioning refers to the observation that brief episodes of repetitive ischemia renders the myocardium resistant to lethal cell injury from a subsequent ischemic episode [48]. Preconditioning has been shown to slow the rate of ATP depletion and anaerobic glycolysis, resulting in a reduction in intracellular lactate accumulation and relative ultrastructural preservation of myocardial cells [49]. Depletion of glycogen stores by anoxia increases the contractile function of isolated globally ischemic rat hearts, and this effect was reversed by the addition of lactate [50]. The administration of adenosine or an adenosine A_1 agonist mimics the protective effect of preconditioning on infarct size and is abolished with adenosine receptor blockers [51]. Intravenous adenosine also significantly improves regional contractility after reperfusion in the intact dog subjected to 90 minutes of ischemia [52]. These observations support the hypothesis that endogenous adenosine may also be a protective mediator against myocardial stunning.

Treatment of Myocardial Stunning with Adenosine

In the previous section, the rationale for the use of adenosine as a therapeutic modality in reducing myocardial stunning was explored. Since this represents a new area of research activity, the role of some of these possible mechanisms has not been examined. However, the purpose of this section is to review what is known about the effect of adenosine or "adenosine-like" agents on contractile function pertaining to the state of ATP stores and regional myocardial blood flow. Although the beneficial effects of adenosine may also be mediated by improvement in the metabolic substrate of the ischemic cell, restoration of calcium homeostasis, and a reduction in oxygen free radicals, further studies are needed to clarify these mechanisms.

Effect of exogenous administration of adenine nucleotides on ATP stores and myocardial function

Since the exact mechanism of myocardial stunning remains to be elucidated, current pharmacologic therapy is empiric. The observation that both regional and global ischemia was associated with a rapid depletion of HEPs, including ATP, suggests that a deficiency of energy supply was the cause of contractile dysfunction following ischemia. The administration of adenosine, inosine, and drugs that increase endogenous adenosine levels by inhibiting its degradation, such as erythro-9-[2-hydroxy-3-nonyl] adenine (EHNA), have been shown to accelerate ATP repletion after both regional and global ischemia [53–57]. Infusions of ribose that increase the level of 5-phosphoribosyl-1-pyrophosphate, a major limiting factor for the biosynthesis of adenine nucleotides, or 5 amino-4-imidazolcarboxamide riboside (AICAR), which bypasses the initial metabolic processes in the salvage pathways, have also been shown to increase myocardial ATP levels [22,58]. Mauser et al. compared the effect of infusions of adenosine, AICAR, and ribose in the intact dog undergoing 45 minutes of regional ischemia and 3 hours of reperfusion [59]. The most striking increase in adenine nucleotide synthesis was observed with adenosine (90-fold increase), which resulted in significantly higher myocardial ATP levels 3 hours after reperfusion. Although ribose and AICAR produced moderate increases in nucleotide synthesis, they failed to increase ATP levels since their incorporation into the nucleotide pool was much slower than adenosine [59]. A significant increase in ATP in reperfused myocardium has also been shown in both the intact and isolated globally ischemic heart with adenosine alone, adenosine in combination with the adenosine deaminase inhibitor EHNA, and EHNA combined with the adenine nucleotide transport blocker p-nitrobenzylthioinosine (NBMPR) [53,54,60]. In contrast to the study of Mauser et al., ribose administered to both the isolated and intact preparation of global ischemia in the rat has also been shown to significantly increase ATP levels [18,58].

Some studies have shown a correlation between repletion of ATP levels and improvement of contractile function of the stunned myocardium. Bolling et al. found that administration of adenosine in the cardioplegic solution in isolated rabbit hearts undergoing 120 minutes of global ischemia significantly repleted ATP, and this was associated with an improvement in both systolic and diastolic functional parameters of ventricular function [55]. Foker et al. reported that the combination of adenosine and EHNA restored ATP levels to 88% of the preischemic levels in dogs undergoing 20 minutes of ischemia followed by 30 minutes of cardiopulmonary bypass, and this was associated with an improved cardiac output while the animals were on bypass [54]. Ribose has also been shown to increase ATP levels and to improve function in globally ischemic hearts [18,58].

Replenishment of total myocardial ATP stores appears not to be mandatory to improve myocardial stunning, since contractility can be restored with the administration of inotropic agents [61,62]. Przyklenk and Kloner observed that superoxide dismutase and

catalase improved myocardial stunning in the open-chest dog model, and this was not accompanied by an increase in myocardial ATP stores [63]. Some studies have shown that adenosine can exert beneficial effects on postischemic contractile function independent of replenishment of the total myocardial adenine nucleotide pool. One possibility is that adenosine may have replenished the mitochondrial ATP pool, which, although only comprising 15–20% of the total cellular pool, is essential for ATP synthesis [64]. This is supported by the study of Asmarkis et al., who showed that repetitive short episodes of ischemia resulted in prolonged depletion of mitochondrial ATP [64]. Alternatively, adenosine may have modified the functions of various cells and metabolic processes thought to play an important role in the pathogenesis of myocardial stunning.

Effects of adenosine on myocardial blood flow

The beneficial effects of adenosine may also be due to improvement in regional myocardial blood flow. Dauber et al. showed that 15 minutes of regional ischemia resulted in increased microvascular permeability and impaired endothelial-dependent relaxation in isolated coronary rings from dogs undergoing 15 minutes of regional ischemia [46]. Bolli et al. reported an increase in coronary vascular resistance and impaired vasodilatory response in the microvasculature following 15 minutes of ischemia in the intact dog [45]. Stahl et al. noted that selective enhancement of coronary blood flow with numerous vasodilators significantly improved ventricular function after repetitive short periods of regional ischemia [47]. Therefore, the potent coronary arteriolar vasodilatory effects of adenosine may account for its beneficial effects on myocardial stunning. This is supported by the study of Ledingham et al. that addition of adenosine in the reperfusate of isolated rat hearts undergoing 3 hours of hypothermic ischemic arrest increased ventricular function and coronary blood flow without a concomitant increase in intramyocardial ATP stores [8]. The mechanisms whereby increased regional myocardial blood flow improve ventricular function remain speculative but may be related to washout of various cytotoxic compounds known to accumulate during ischemia, such as hydrogen ions and lactic acid and/or enhancement of the metabolic substrate of viable cells by enhanced oxygen delivery.

Conclusions

The exact pathogenetic mechanisms responsible for myocardial stunning remain to be elucidated. Experimental studies support a role for the use of adenine nucleosides in the therapy of myocardial stunning. Restoration of high energy stores by adenosine has been associated with an improvement in regional contractile function in some studies. Amelioration of myocardial stunning has also been shown with adenosine in the absence of repletion of total myocardial ATP stores. Adenosine possesses a number of complex cellular and metabolic effects, which could be beneficial in the setting of ischemia and reperfusion. Further studies are needed to define the precise cellular effects of adenosine in myocardial stunning. However, experimental observations suggest that adenine nucleosides, especially adenosine, may be a useful pharmacologic strategy in the prevention and treatment of ventricular dysfunction in humans following episodes of regional or global ischemia.

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