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COMMENTARY

Cardioprotection with adenosine: 'a riddle wrapped in a mystery'

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Review of the published literature on adenosine and cardioprotection could lead one to paraphrase the famous words of Sir Winston Churchill (Radio broadcast, 1 October 1939 (in reference to Russia)) and conclude: *'I cannot forecast to you the action of adenosine. It is a riddle wrapped in a mystery inside an enigma'*. That is, although it is well-established that adenosine can render cardiomyocytes resistant to lethal ischemia/reperfusion-induced injury, new and intriguing insights continue to emerge as to the mechanisms by which adenosine might limit myocardial infarct size.
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Historical perspective

Widespread interest in the cardioprotective properties of adenosine was fueled by compelling evidence from Downey and co-workers that adenosine plays a pivotal role in the phenomenon of ischemic preconditioning (PC). Specifically, the authors proposed that breakdown of ATP during brief antecedent episodes of PC ischemia/reperfusion, the resultant increase in interstitial adenosine concentration, and subsequent stimulation of adenosine receptors on the myocytes' surface, serve as a trigger for the profound reduction of infarct size seen in preconditioned hearts *versus* controls (Liu *et al*., 1991). Support for this hypothesis was provided by numerous studies demonstrating that the favorable effects of preconditioning could be mimicked by treatment with adenosine receptor agonists given in lieu of brief PC ischemia and, conversely, abrogated by adenosine receptor antagonists given during the PC stimulus (reviewed in Przyklenk & Kloner, 1998; Yellon & Downey, 2003). Adenosine has even, in some reports, been implicated to protect against lethal 'reperfusion injury', with adenosine agonists and antagonists given during sustained ischemia or immediately before reflow (rather than preischemia, as per the preconditioning paradigm) shown to attenuate and exacerbate infarct size, respectively (Toombs *et al*., 1992; Zhao *et al*., 1993; Xu *et al*., 2000).

If adenosine is cardioprotective, then administration of exogenous adenosine *per se* should, logically, limit infarct size. However, in contrast to the agonist–antagonist approach, attempts to achieve cardioprotection with intravenous (i.v.) or intracoronary infusions of adenosine have yielded mixed results. Although some studies have reported significant reduction of infarct size with exogenous adenosine (Olafsson *et al*., 1987; Toombs *et al*., 1992; Lasley *et al*., 1995), failures have been attributed to both its confounding hemodynamic effects and, more notably, its short biologic half-life. That is, efforts to increase adenosine concentrations in the interstitium by i.v. administration of the nucleoside are hampered by the well-recognized, rapid uptake and metabolism of adenosine in endothelial cells and erythrocytes (Lasley *et al*., 1998). An alternative strategy conceived to circumvent these problems was explored by Whittaker *et al*., who sought to investigate the cardioprotective properties of adenosine *via* direct intramyocardial injection. Direct injection of adenosine did, indeed, limit infarct size; however, interpretation of these data was complicated by the observation that placebo injection of saline alone was equally protective (Whittaker *et al*., 1996). Moreover, the reduction of infarct size seen with intramyocardial injections was blocked by gadolinium chloride, thereby implicating the involvement of stretch-activated ion channels, rather than (or, possibly, 'in parallel' to) adenosine receptor stimulation (Whittaker *et al*., 1996).

The mystery deepens

Manintveld and co-workers have, in the current issue of the journal, added yet another layer of complexity to the adenosine-cardioprotection story, and provide evidence of adenosine-induced reduction of infarct size achieved without an increase in myocardial interstitial adenosine concentrations (Manintveld *et al*., 2005). Specifically, rats were assigned to receive a 15 min i.v. infusion of adenosine ($200\,\mu g\,kg^{-1}\,min^{-1}$) followed by 10 min of washout, 15 min of PC ischemia + 10 min of reflow, or no intervention (controls) and, after the treatment phase, all animals underwent a sustained, 1 h ischemic insult. PC ischemia was associated with an expected, robust increase in interstitial adenosine concentration (assessed by microdialysis) while, in contrast, interstitial adenosine levels remained unchanged with i.v. adenosine treatment. Nonetheless, both PC and i.v. adenosine-treated groups displayed a significant, 35% reduction of infarct size *versus* controls (Manintveld *et al*., 2005).

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If adenosine was not delivered to the interstitium by i.v. infusion – thereby presumably precluding myocardial adenosine receptor stimulation as the trigger for the cardioprotection seen in this group – how did i.v. adenosine limit infarct size? Insight into this apparent paradox was provided by the authors' observations that the benefits of i.v. adenosine were abrogated by the nitric oxide synthase (NOS) inhibitor LNNA and attenuated by the ganglionic blocker hexamethonium, thereby leading Manintveld and co-workers to conclude that reduction of infarct size with i.v. adenosine is dependent upon NO, is initiated at least in part at extra-cardiac sites, and is mediated by activation of a neurogenic pathway (Manintveld *et al*., 2005).

These data provide new perspectives regarding the mechanisms by which adenosine may protect the heart. However, the results also raise multiple, as-yet unanswered questions. For example: *What is the specific locus and source of the NO production?* Although, as suggested by Manintveld and co-workers, coronary endothelium-derived NO may play a pivotal role, no conclusions can be drawn as to the site of NO production. In addition, the use of LNNA, a nonselective NOS inhibitor, does not allow for the definitive identification of the NOS isoform(s) that may be involved. Second: *What are the signaling pathways that participate in the apparent adenosine-initiated upregulation of NOS and release of NO?* Although not addressed in the current study, one hypothesis that may merit future investigation is the potential contribution of PI3-kinase/Akt signaling, a paradigm that has recently been described for adenosine receptor-mediated stimulation of NO production in isolated rat cardiomyocytes (Xu *et al*., 2005). Third: *What are the remote, extracardiac sites involved in the cardioprotection seen with IV adenosine?* The results obtained with hexamethonium support the involvement of a neurogenic mechanism and thus, by inference, activation of a protective stimulus at extracardiac sites. However, as acknowledged by the authors, the current study design does not provide insight into this issue. Finally, the concept that cardioprotection seen with i.v. adenosine is initiated in part at extracardiac sites raises the obvious question: *What is the mechanistic relationship between the reduction of infarct size seen with i.v. adenosine and the cardioprotection evoked by 'preconditioning at a distance'* (i.e. the phenomenon whereby brief ischemia applied in a remote coronary vascular bed or in distant organs can protect virgin myocardium from a sustained ischemic insult (Przyklenk *et al*., 1993; 2003))? That is, beyond the common theme of adenosine-mediated stimulation of afferent nerves, shown previously by the authors' laboratory to contribute to the favorable effects of brief antecedent mesenteric artery occlusion (Gho *et al*., 1996; Liem *et al*., 2002), is infarct size reduction by i.v. adenosine and 'preconditioning at a distance' achieved by the same mediators and signaling pathways?

It could be argued that one hallmark of a meritorious study is that, in exploring one question, new and equally stimulating queries arise. A second quote from Churchill (Speech at the Lord Mayor's Day Luncheon, London, 10 November 1942) seems fitting to describe the current contribution of Manintveld and co-workers to our knowledge of adenosine-induced cardioprotection: *'Now is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning'*.

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