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COMMENTARY

Efficacy of preconditioning should be gauged by reduction of infarction

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Cardioprotection has become the 'holy grail' of the cardiovascular community: everyone is looking for it, but no one has quite found a clinically acceptable approach. Although the concepts of cardiac protection and salvage of ischemic myocardium date back to the early 1970s to the seminal investigations of Maroko, Braunwald, and their colleagues (Maroko *et al*., 1971), suggested approaches yielded inconsistent results until the most improbable report of Murry *et al*. in 1986 that brief periods of myocardial ischemia somehow prepared the myocardium to better withstand a subsequent, more prolonged ischemic insult. This preconditioning phenomenon has been demonstrated in multiple animal species, and the trigger of the infarct-sparing action is no longer exclusively brief ischemia, but also multiple pharmacologic agents including adenosine, bradykinin, opioids, and free radicals (Cohen & Downey, 2001). Despite nearly 15 years of intensive investigation, the identity of the end-effector of protection is unknown, and the signaling pathway leading to protection has only been partially elucidated. It is generally believed that binding of cell surface receptors by ligands released by ischemic cells initiates a complex signaling cascade perhaps involving transactivation of surface growth factors (Krieg *et al*., 2002), activation of membrane-bound phosphatidylinositol 3-kinase (Tong *et al*., 2000), phosphorylation of protein kinase B (PKB; also known as Akt), stimulation of nitric oxide synthesis, and consequent activation of guanylyl cyclase, leading to the production of protein kinase G (PKG) (Oldenburg *et al*., 2004). The latter, either directly or through phosphorylation of other intermediates, opens mitochondrial K_{ATP} channels, causing increased production of small quantities of reactive oxygen species which act as signaling elements to activate a kinase cascade including protein kinase C (PKC) and tyrosine kinases (possibly those leading to the activation of p38 MAP kinase) (Cohen & Downey, 2001). Other components are uncertain.

The earliest demonstration of the profound biologic effect of preconditioning was the ability to decrease the infarct size (Murry *et al*., 1986), and this end point continues to be the most robust, and probably the one with the greatest clinical significance. However, many surrogate end points have also been used, including reperfusion arrhythmia formation and postischemic recovery of the left ventricular function. One imagines that other end points were sought because of the more demanding and more time-consuming process of quantitation of the risk zone and extent of infarction. Measurement of postischemic return of function as an end point is quite popular in small animals and is important to cardiovascular surgeons, as their patients' hearts resume function after ischemic arrest for revascularization procedures. Postischemic myocardial depression may in part be related to infarction, but is generally caused by a transient insult called stunning, which is acknowledged to result from free radical damage (Bolli & Marbán, 1999). However, it is becoming increasingly evident that postischemic cardiac function is not an optimal measure of the effectiveness of preconditioning, and may even be misleading (Gelpi *et al*., 2002; Lochner *et al*., 2003), and preconditioning may have little effect on stunning (Jenkins *et al*., 1995). In this issue of the journal, Sato *et al*. (2004) have studied the ability of minoxidil, a well-known agent for the treatment of hypertension and alopecia, to open sarcolemmal and mitochondrial K_{ATP} channels of ventricular cardiomyocytes, and to preserve the postischemic function of guinea-pig ventricular myocardium. Thus, Sato has studied the effect of minoxidil on myocardial stunning. To their credit, these authors do not claim that they are studying preconditioning, but rather only cardioprotection. The distinction between preconditioning and stunning as a form of cardioprotection is not merely a semantic one. Although recovery of postischemic cardiac function may have notable consequences in the recovery room, it is the degree of infarction that will determine long-term cardiac dysfunction, symptoms, and prognosis. Hence infarct size should be the gold standard for judging cardioprotection.

Investigation of the signaling pathway of preconditioning has involved the use of tool drugs to activate or inhibit ion channels, kinases, production of reactive oxygen species, etc. Probing the involvement of K_{ATP} channels has depended in part on the use of openers such as diazoxide and pinacidil, and closers as glibenclamide and 5-hydroxydecanoate. Recent concerns over the selectivity of diazoxide and 5-hydroxydecanoate have prompted the search for other tool drugs, which can manipulate these channels. Sato *et al*. (2004) have demonstrated that mitochondrial K_{ATP} channels are 25 times more sensitive to minoxidil than sarcolemmal channels, and, therefore, propose that it be used to study the involvement of mitochondrial K_{ATP}. Although introduction of this newer tool drug is welcome, enthusiasm must be tempered by the same considerations which are now prompting some to question the unique activity of diazoxide against mitochondrial K_{ATP}. Selectivity of all pharmacologic agents fades with time, and it

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is premature to conclude that minoxidil's selectivity will be better than diazoxide's. In fact, part of Sato's conclusion that low-dose minoxidil is a potent opener of mitochondrial channels is based on the ability of HMR 1098, a putatively selective sarcolemmal channel closer, to have no effect on minoxidil's action. But the selectivity of HMR compounds has been questioned (Birincioglu *et al*., 1999; Gross, 2000). Therefore, minoxidil may suffer from the same uncertainties as diazoxide.

Hence, the infarct-sparing effect of an intervention should be the critical end point for studies of cardioprotection, unless stunning is being specifically evaluated. Also, tool drugs are useful, indeed mandatory, for the study of signaling pathways. As we gain more experience with these agents, it is increasingly evident that no one drug has unassailable specificity. Therefore, it is recommended that, when available, multiple agents with differing molecular structures, but similar specificities, be examined.

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