



# Can we target metabolism and ion flux as a therapy for coronary artery occlusion?

Michael Marber<sup>1</sup>, Gary Lopaschuk<sup>2</sup>  
<sup>1</sup>Department of Cardiology, St. Thomas' Hospital, London UK  
<sup>2</sup>Cardiovascular Research Group, Faculty of Medicine,  
 University of Alberta, Edmonton, Alberta, Canada

Correspondence: Professor Michael S. Marber, Department of Cardiology, KCL, St Thomas' Hospital, Lambeth Place Road, London SE1 7EH, UK. e-mail: mike.marber@kcl.ac.uk

This issue of *Heart and Metabolism* is dedicated to a discussion of interventions that preserve myocardium following coronary artery occlusion by influencing the changes in metabolism and ion fluxes that accompany ischemia and reperfusion. For such interventions to have a clinical impact they must slow the rate at which myocardium dies following coronary artery occlusion, and/or must limit lethal events occurring when infarct-related artery patency is restored, so-called 'reperfusion injury'. The underlying premise is that either intra- or postischemic protection saves myocardium, maintaining contractile reserve, thereby limiting maladaptive postinfarction remodeling. The net effect of such protective interventions would be to limit increases in postinfarction end-systolic volume, the most powerful predictor of subsequent mortality.<sup>1</sup> The question therefore is, are there any metabolic or ionic interventions that are able to reduce mortality?

## Agents that only slow necrosis

The first compelling problem is that discussed by Drs Sack and Yellon. In the experimental laboratory there are a number of interventions that can slow the rate of myocardial death following coronary occlusion. However, most investigators find that these treatments, which include adenosine and sodium proton exchange inhibitors, must be given before the moment of coronary artery occlusion. One reason why this is necessary is that the amount of collateral support to the ischemic

zone is uncertain, and therefore to ensure that the protective substance is in contact with the myocardium it has to be delivered by ante-grade flow. Another reason is that these interventions only slow the rate of necrosis, they do not resurrect dead myocytes, and must therefore be present when myocardial necrosis is threatened rather than established. In the clinical arena, where presentation with chest pain and ST-segment elevation is triggered by coronary occlusion, there are few circumstances where such interventions are practical. As Drs Avkiran, Cohen, and Downey point out, it is exactly these sorts of issues that may explain why the GUARDIAN<sup>2</sup> and AMISTAD<sup>3</sup> trials, though negative overall, suggested that treatment with a sodium proton exchange inhibitor or adenosine may still benefit certain subgroups.

## Agents that limit reperfusion injury

The concept of reperfusion injury is one that we have always found difficult to grasp. Unfortunately, despite decades of research it remains controversial, with one camp believing that reperfusion merely unmasks myocytes that died during ischemia, and the other camp believing that reperfusion kills myocytes that were still alive at the end of ischemia. The most convincing way to unravel the contribution of reperfusion is to find interventions that limit ultimate infarct size only when given during reperfusion. Unfortunately, most such interventions have yielded dichotomous results, with some investigators finding they

do protect and others finding they do not. Recently, however, the observation that the process of apoptosis may continue during reperfusion has provided a target for late intervention. This allows strategies that are closely allied to the treatment of patients presenting with ST elevation, namely reperfusion therapy.

In this issue Drs Sack and Yellon point out that insulin may have antiapoptotic properties thereby enabling it to limit infarct size even when given during reperfusion. Together with Drs Avkiran, Cohen, and Downey they argue it is exactly these properties of insulin that enabled the small pilot ECLA trial of glucose, insulin, and potassium (GIK) to succeed<sup>4</sup> where larger trials such as AMISTAD and ESCAMI failed. Moreover, in the clinical study described in this issue by Drs van Campen, Klein, and Visser, GIK was also shown to improve left ventricular function during the reperfusion phase of myocardial infarction, enabling the detection of viable but stunned or hibernating myocardium. This is a significant finding since it is exactly under these circumstances that dobutamine extends necrosis in animal models.<sup>5</sup>

Hence it is the cheap metabolic strategy of GIK, lacking patent protection, that receives the greatest support from our contributors. The GIK hypothesis is currently being tested in the larger, noncommercial, ECLA 2 full-scale trial. We urge those of you who are interested to visit <http://www.ecla.org.ar>.

## Summary

In patients with acute coronary artery occlusion and ST elevation there is no proven intervention other than reperfusion that definitely reduces the volume of myocardium that undergoes infarction. Further clinical investigation is necessary to determine whether metabolic and other interventions are cardioprotective at reperfusion. Whilst these studies are ongoing, keep taking the aspirin,  $\beta$ -blockers, ACE inhibitors, and statins! ■

## REFERENCES

1. White HD, Norris RM, Brown MA, Brandt PWT, Whitlock RML, Wild CJ. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. *Circulation* 1987;76:44–51.
2. Theroux P, Chaitman BR, Danchin N, et al. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations — main results of the GUARDIAN trial. *Circulation* 2000;102:3032–3038.
3. Mahaffey KW, Puma J, Barbagelata NA, et al. Adenosine as an adjunct to thrombolytic therapy for acute myocardial infarction — results of a multi-center, randomized, placebo-controlled trial: the Acute Myocardial Infarction Study of Adenosine (AMISTAD) trial. *J Am Coll Cardiol* 1999;34:1711–1720.
4. Díaz R, Paolasso A, Piegas LS, et al. Metabolic modulation of acute myocardial infarction — The ECLA Glucose-Insulin-Potassium Pilot Trial. *Circulation* 1998;98:2227–2234.
5. Schulz R, Rose J, Martin C, Brodde OE, Heusch G. Development of short-term myocardial hibernation. Its limitation by the severity of ischemia and inotropic stimulation. *Circulation* 1993;88:684–695.

# Different strategies for cardiac protection: metabolic, ionic, signaling

Metin Avkiran

Centre for Cardiovascular Biology and Medicine, King's College, London,  
and The Rayne Institute, St Thomas' Hospital, London, UK

Correspondence: Dr Metin Avkiran, Centre for Cardiovascular Biology and Medicine,  
The Rayne Institute, St Thomas' Hospital  
Lambeth Palace Road, London SE1 7EH, UK, e-mail: metin.avkiran@kcl.ac.uk

## Introduction

Cardiovascular disease remains a leading cause of death worldwide. For example, cardiovascular mortality, arising principally from ischemic heart disease, accounts for 40% of all deaths before the age of 74 years in European countries.<sup>1</sup> Despite the availability of preventative treatments, patients often present at an advanced stage of coronary artery disease, with atherosclerotic plaques that are liable to cause abrupt coronary occlusion, myocardial infarction, and death. Currently, the most effective method of reducing mortality in patients who suffer such an abrupt coronary occlusion is to achieve rapid reperfusion, by thrombolysis or mechanical disruption of the occlusive coronary thrombus and plaque. However, it is well established that for reperfusion to be of optimal benefit (by salvaging ischemic myocardium from necrosis and thereby maintaining ventricular function), it has to be achieved rapidly after the onset of ischemia. Unfortunately, many patients with acute myocardial infarction do not receive the full benefit of reperfusion because they suffer from delays in reporting to hospital and/or receiving appropriate treatment.<sup>2</sup> Furthermore, there is evidence that the phenomenon of 'reperfusion-induced injury' may detract from the undoubted benefits of coronary flow restoration.<sup>3</sup>

There are currently no clinically proven therapies that are used widely to enhance the myocardium's tolerance to ischemia and thereby extend the time-window for tissue salvage by reperfusion, or to further enhance the benefits of reperfusion by attenuating reperfusion-induced injury. If available, such therapies would be expected to be of value not only in the management of acute myocardial infarction but also in cardiac surgery, where

the heart is often subjected to periods of global ischemia and reperfusion. In the surgical setting, these may find application as adjuvant therapies and be used in conjunction with existing cardioprotective strategies, such as cardioplegic arrest or intermittent ventricular fibrillation, to enhance postischemic myocardial viability and function.

From the above, it is apparent that the development of effective treatments for the direct protection of ischemic and reperfused myocardium should provide cardiologists and cardiac surgeons with novel therapeutic options and would be expected to have a beneficial impact on the mortality and morbidity associated with ischemic heart disease. The objective of this article is to highlight several approaches to cardiac protection that have received attention in recent years, some of which have already reached the stage of clinical evaluation. These approaches have aimed to improve myocardial tolerance to ischemia and improve its viability and function after reperfusion, by manipulating cellular metabolism or ion transport and by understanding (and subsequently exploiting) the signaling mechanisms that regulate myocardial susceptibility to injury, in particular those that are responsible for the powerful endogenous protection afforded by adaptive phenomena such as ischemic preconditioning (*Figure 1*).

## Metabolic approaches

Under normal, aerobic conditions, the myocardium can utilize a variety of substrates (eg, free fatty acids, glucose, lactate) to produce the energy required to maintain its viability and function, in the form of adenosine triphosphate (ATP), primarily through oxidative metabolism. In contrast, in ischemic

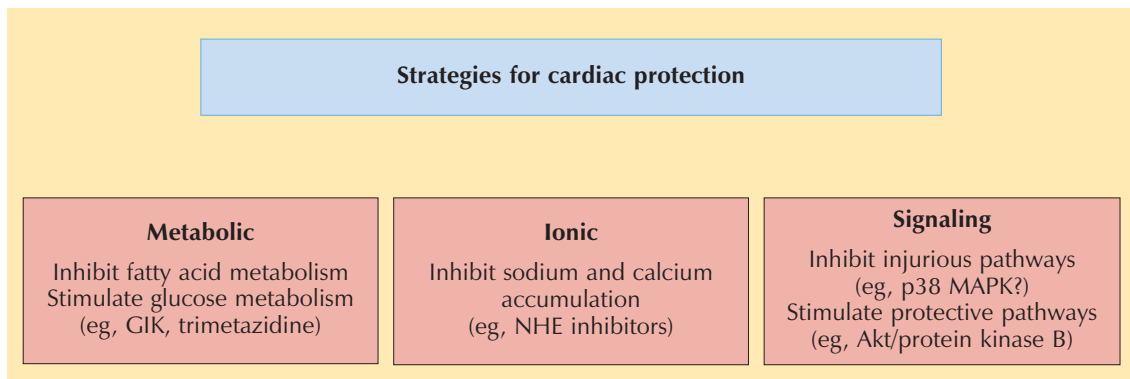


Figure 1. Potential approaches to cardiac protection. GIK, glucose-insulin-potassium; NHE, sodium/hydrogen exchanger; p38 MAPK, p38 mitogen-activated protein kinase.

myocardium, the metabolism of glucose to lactate by anaerobic glycolysis becomes the main source of ATP generation.<sup>4</sup>

As reviewed recently,<sup>5,6</sup> in myocardium subjected to ischemia and reperfusion, significant functional benefit may be obtained by using interventions that inhibit fatty acid metabolism and/or stimulate glucose metabolism, in particular glucose oxidation. This may be achieved by the administration of glucose and insulin, a concept which has received renewed attention<sup>7</sup> following encouraging clinical findings with glucose-insulin-potassium (GIK) treatment, from a metaanalysis<sup>8</sup> and a prospective, randomized clinical trial,<sup>9</sup> in patients with acute myocardial infarction. Interestingly, recent experimental data suggest that GIK therapy may also help maintain systolic and diastolic ventricular function following transient ischemia that does not produce irreversible myocardial injury.<sup>10</sup> A likely mechanism for the benefits of GIK therapy is reduced free fatty acid metabolism (through decreases in both circulating fatty acids and myocardial fatty acid uptake), although other mechanisms such as increased glycolytic ATP synthesis may also contribute.<sup>7</sup> Alternatively, glucose oxidation may be increased by pharmacological stimulation of the rate-limiting enzyme in this process, the pyruvate dehydrogenase complex.<sup>5</sup> For example, dichloroacetate, which stimulates pyruvate dehydrogenase activity, has been shown to enhance glucose oxidation and thereby increase cardiac work and efficiency during reperfusion of iso-

lated hearts subjected to global ischemia.<sup>11</sup> However, a low potency and short in vivo half-life limit the clinical utility of dichloroacetate.<sup>5</sup>

Another approach to alter the balance between fatty acid oxidation and glucose oxidation beneficially is to inhibit key enzymes in the former process. The antianginal drug trimetazidine has been shown recently to inhibit mitochondrial long-chain 3-ketoacyl coenzyme A thiolase, a critical enzyme in fatty acid oxidation, and thereby increase glucose oxidation.<sup>12</sup> Presumably through such an effect on myocardial metabolism, trimetazidine has also been shown to attenuate the intracellular acidosis and the intracellular accumulation of sodium that develop in isolated hearts during ischemia, and to improve the postischemic recovery of systolic and diastolic function.<sup>13</sup> It is clear, therefore, that myocardial injury and dysfunction during ischemia and reperfusion may be attenuated through a variety of interventions targeted at myocardial metabolism.

### Ionic approaches

Ischemia is associated with a disruption of myocardial ionic homeostasis, which results in the intracellular accumulation of sodium and calcium and the extracellular accumulation of potassium. These ionic disturbances, some of which (in particular the intracellular accumulation of calcium, or 'calcium overload') may

be further exacerbated during early reperfusion, have been linked causally with the unfavorable consequences of ischemia and reperfusion, such as arrhythmias, contractile dysfunction, and myocardial necrosis.<sup>14</sup> As such, ion translocating proteins have been favored targets for putative cardioprotective agents. One ion translocating protein which has received particular attention in recent years is the sodium/hydrogen exchanger (NHE), which is activated during ischemia and reperfusion<sup>15</sup> and is believed to contribute to the intracellular accumulation of sodium, and consequently calcium, in myocardial cells (Figure 2).<sup>16</sup>

Since the mid-1990s, several new drugs that selectively target the cardiac NHE and inhibit its activity have been developed and shown to attenuate severe arrhythmias, limit the extent of myocardial necrosis (ie, infarct size), and preserve myocardial contractile function in a variety of models of ischemia and reperfusion.<sup>16,17</sup> Interestingly, relative to

ischemic preconditioning, the magnitude of the protection afforded by NHE inhibition appears to be comparable or, if the ischemic period is prolonged, even greater.<sup>18-20</sup> One of the new NHE inhibitors, cariporide, has also been tested clinically in two recent studies. Rupprecht et al<sup>21</sup> have obtained data from a cohort of 100 patients with acute anterior myocardial infarction, which suggested that cariporide could preserve tissue viability and contractile function when used as in conjunction with direct coronary angioplasty. These encouraging findings need to be confirmed by larger clinical studies. Th eroux et al<sup>22</sup> have determined the effects of cariporide in a much larger, combined phase-II/phase-III study (the GUARDIAN [Guard During Ischemia Against Necrosis] study), which involved patients in a variety of high-risk ischemic situations. The results of this study indicated no significant benefit of cariporide in patients with unstable angina or non-ST-

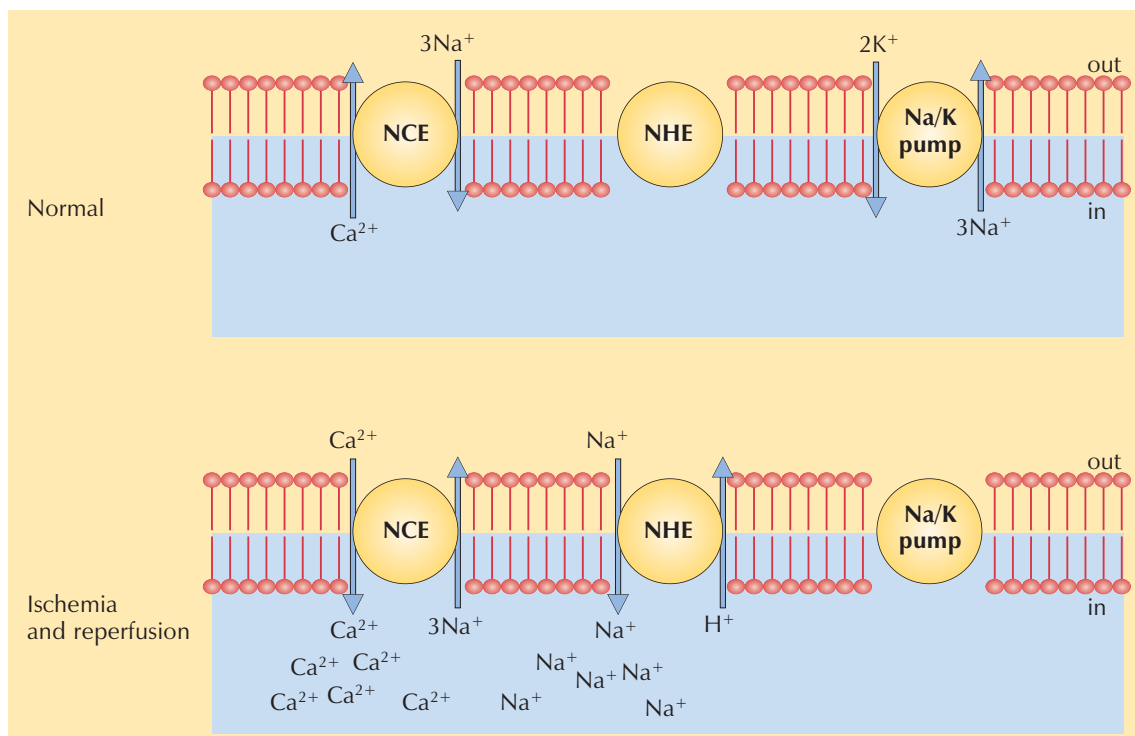


Figure 2. Putative mechanism of calcium overload during myocardial ischemia and reperfusion. Increased activity of the sodium/hydrogen exchanger (NHE), primarily as a result of intracellular acidosis, occurs concomitantly with inhibition of the sodium/potassium pump (Na/K pump) and leads to the intracellular accumulation of sodium. This in turn produces calcium influx through reverse-mode sodium/calcium exchange (NCE).

elevation myocardial infarction and those undergoing a high-risk percutaneous coronary intervention.<sup>22</sup> However, in patients undergoing high-risk coronary artery bypass graft surgery, there was a significant reduction in the primary endpoint (incidence of death or myocardial infarction), with the highest dose of cariporide (12.1% [89/734] vs. 16.2% [120/743] in the placebo group,  $P = 0.03$ ).<sup>22</sup> These data suggest that the experimental promise of NHE inhibition may be fulfilled clinically, at least in the setting of coronary artery bypass graft surgery.

The interest in the intracellular accumulation of sodium and calcium as potential causal factors in ischemia and reperfusion-induced injury has also led to recent studies with pharmacological inhibitors of other ion translocating proteins, such as the sodium/calcium exchanger<sup>23</sup> and the sodium/bicarbonate symporter,<sup>24</sup> in cellular models of simulated ischemia. However, there is inadequate whole heart and in vivo data with such agents and they have not reached the stage of clinical evaluation.

## Signaling

Until recently, for many cardiovascular investigators, 'signaling' was something they occasionally did while driving. However, the burgeoning interest in the mechanisms that underlie the powerful cardioprotective adaptation that is initiated by ischemic preconditioning<sup>25</sup> has encouraged an intense interest in the intracellular signaling pathways that operate in myocardial cells, particularly those that are mediated through protein phosphorylation reactions catalyzed by enzymes known as protein kinases.

There appears to be general agreement that protein kinase C plays a pivotal role in initiating the adaptive signaling mechanisms that underlie ischemic preconditioning.<sup>26–28</sup> However, distal components of the relevant signaling pathways have not been definitively identified. For example, considerable confusion surrounds the role p38 mitogen-activated protein kinase (p38 MAPK) in preconditioning,<sup>29</sup>

since p38 MAPK has been suggested to be a critical mediator of both the preconditioning response<sup>30,31</sup> and ischemic injury.<sup>32–34</sup>

The identity of the 'end effector' of ischemic preconditioning also remains unclear. Although the mitochondrial ATP-sensitive potassium channel had been suggested to serve such a function,<sup>35</sup> more recent evidence indicates a more proximal, triggering role for this channel in the activation of the relevant signaling pathways.<sup>36</sup>

Interest in the signaling pathways that determine myocardial susceptibility to injury during ischemia and reperfusion has also included those pathways that may regulate myocyte loss through programmed cell death, or apoptosis. In this context, experiments in a variety of models have suggested that activation of antiapoptotic pathways, such as those mediated via extracellular signal-regulated kinase (also known as p42/p44 MAPK)<sup>37</sup> and Akt/protein kinase B,<sup>38,39</sup> may provide functional benefit during ischemia and reperfusion, by preserving myocardial viability. Indeed, it has been suggested that activation of such antiapoptotic pathways by growth factors may present a promising new approach to the attenuation of reperfusion-induced injury.<sup>40</sup>

It is likely that research into the intracellular signaling pathways that mediate ischemic preconditioning and those that modulate myocardial susceptibility to injury and dysfunction during ischemia and reperfusion (either by mimicking or independently of preconditioning) will continue unabated for some years to come. The hope is that such work will identify critical pathways which subsequently can be exploited to therapeutic benefit, through either pharmacological or genetic manipulation.

## Conclusion

Exciting new data continue to emerge from the search for metabolic, ionic, and signaling strategies for the protection of the myocardium during ischemia and reperfusion. Although these strategies have been discussed under separate headings in this article, it should be

noted that the cellular processes manipulated by such strategies impact upon each other. For example, altered myocardial metabolism can undoubtedly modulate cellular ionic balance (eg, by altering the activity of ion translocating proteins) and signal transduction (eg, by regulating protein phosphorylation reactions). Similarly, activation or inhibition of targeted signaling molecules can produce responses by positively or negatively affecting the activities of metabolic enzymes or ion translocating proteins. Greater understanding of the crossregulatory mechanisms that operate between distinct cellular processes and the consequences of their manipulation during ischemia and reperfusion is likely to lead the way in the development of effective new cardioprotective therapies which will fulfill an important void in the physicians' arsenal in the battle against ischemic heart disease. ■

## REFERENCES

- Sans S, Kesteloot H, Kromhout D. The burden of cardiovascular diseases mortality in Europe: Task Force of the European Society of Cardiology on Cardiovascular Mortality and Morbidity Statistics in Europe. *Eur Heart J*. 1997;18:1231–1248.
- Cannon CP. Time to treatment of acute myocardial infarction revisited. *Curr Opin Cardiol*. 1998;13:254–266.
- Hearse DJ, Bolli R. Reperfusion induced injury: manifestations, mechanisms, and clinical relevance. *Cardiovasc Res*. 1992;26:101–108.
- Depre C, Vanoverschelde J-LJ, Taegtmeyer H. Glucose for the heart. *Circulation*. 1999;99:578–588.
- Lopaschuk GD. Treating ischemic heart disease by pharmacologically improving cardiac energy metabolism. *Am J Cardiol*. 1998;82:14K–17K.
- Taegtmeyer H, King LM, Jones BE. Energy substrate metabolism, myocardial ischemia, and targets for pharmacotherapy. *Am J Cardiol*. 1998;82:54K–60K.
- Apstein CS. Glucose-insulin-potassium for acute myocardial infarction: remarkable results from a new prospective, randomized trial. *Circulation*. 1998;98:2223–2226.
- Fath-Ordoubadi F, Beatt KJ. Glucose-insulin-potassium therapy for treatment of acute myocardial infarction. *Circulation*. 1997;96:1152–1156.
- Díaz R, Paolasso EC, Piegas LS, et al. Metabolic modulation of acute myocardial infarction: the ECLA Glucose-Insulin-Potassium Pilot Trial. *Circulation*. 1998;98:2227–2234.
- Zhu P, Lu L, Xu Y, Greyson C, Schwartz GG. Glucose-insulin-potassium preserves systolic and diastolic function in ischemia and reperfusion in pigs. *Am J Physiol Heart Circ Physiol*. 2000;278:H595–H603.
- Liu B, Clanachan AS, Schulz R, Lopaschuk GD. Cardiac efficiency is improved after ischemia by altering both the source and fate of protons. *Circ Res*. 1996;79:940–948.
- Kantor PF, Lucien A, Kozak R, Lopaschuk GD. The antianginal drug trimetazidine shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long-chain 3-ketoacyl coenzyme A thiolase. *Circ Res*. 2000;86:580–588.
- El Banani H, Bernard M, Baetz D, et al. Changes in intracellular sodium and pH during ischaemia-reperfusion are attenuated by trimetazidine: comparison between low-flow and zero-flow ischaemia. *Cardiovasc Res*. 2000;47:688–696.
- Pierce GN, Czubryt MP. The contribution of ionic imbalance to ischemia/reperfusion-induced injury. *J Mol Cell Cardiol*. 1995;27:53–63.
- Avkiran M. Cardiac sarcolemmal Na<sup>+</sup>/H<sup>+</sup> exchanger activity in ischaemia: potential regulatory factors. *Eur Heart J*. 1999;1(suppl):K11–K17.
- Avkiran M. Rational basis for use of sodium-hydrogen exchange inhibitors in myocardial ischemia. *Am J Cardiol*. 1999;83:10G–18G.
- Karmazyn M, Gan XT, Humphreys RA, Yoshida H, Kusumoto K. The myocardial Na<sup>+</sup>-H<sup>+</sup> exchange: structure, regulation, and its role in heart disease. *Circ Res*. 1999;85:777–786.
- Shipolini AR, Yokoyama H, Galiñanes M, Edmondson SJ, Hearse DJ, Avkiran M. Na<sup>+</sup>/H<sup>+</sup> exchanger activity does not contribute to protection by ischemic preconditioning in the isolated rat heart. *Circulation*. 1997;96:3617–3625.
- Gumina RJ, Buerger E, Eickmeier C, Moore J, Daemmgen J, Gross GJ. Inhibition of the Na<sup>+</sup>/H<sup>+</sup> exchanger confers greater cardioprotection against 90 minutes of myocardial ischemia than ischemic preconditioning in dogs. *Circulation*. 1999;100:2519–2526.
- Avkiran M. Protection of the myocardium during ischemia and reperfusion: Na<sup>+</sup>/H<sup>+</sup> exchange inhibition versus ischemic preconditioning. *Circulation*. 1999;100:2469–2472.
- Rupprecht H-J, vom Dahl J, Terres W, et al. Cardioprotective effects of the Na<sup>+</sup>/H<sup>+</sup> exchange inhibitor cariporide in patients with acute anterior myocardial infarction undergoing direct PTCA. *Circulation*. 2000;101:2902–2908.
- Théroux P, Chaitman BR, Danchin N, et al. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations: main results of the GUARDIAN trial. *Circulation*. 2000;102:3032–3038.
- Ladilov Y, Haffner S, Balsler-Schäfer C, Maxeiner H, Piper HM. Cardioprotective effects of KB-R7943: a novel inhibitor of the reverse mode of

- Na<sup>+</sup>/Ca<sup>2+</sup> exchanger. *Am J Physiol Heart Circ Physiol*. 1999;276:H1868–H1876.
24. Schäfer C, Ladilov YV, Siegmund B, Piper HM. Importance of bicarbonate transport for protection of cardiomyocytes against reoxygenation injury. *Am J Physiol Heart Circ Physiol*. 2000;278:H1457–H1463.
  25. Downey JM, Cohen MV. Mechanisms of preconditioning: correlates and epiphenomena. In: Marber MS, Yellon DM, eds. *Ischaemia: preconditioning and adaptation*. Oxford: BIOS Scientific Publishers; 1996:21–34.
  26. Zhao J, Renner O, Wightman O, et al. The expression of constitutively active isoforms of protein kinase C to investigate preconditioning. *J Biol Chem*. 1998;273:23072–23079.
  27. Ping P, Takano H, Zhang J, et al. Isoform-selective activation of protein kinase C by nitric oxide in the heart of conscious rabbits: a signaling mechanism for both nitric oxide-induced and ischemia-induced preconditioning. *Circ Res*. 1999;84:587–604.
  28. Marber MS. Ischemic preconditioning in isolated cells. *Circ Res*. 2000;86:926–931.
  29. Ping P, Murphy E. Role of p38 mitogen-activated protein kinases in preconditioning: a detrimental factor or a protective kinase? *Circ Res*. 2000;86:921–922.
  30. Weinbrenner C, Liu GS, Cohen MV, Downey JM. Phosphorylation of tyrosine 182 of p38 mitogen-activated protein kinase correlates with the protection of preconditioning in the rabbit heart. *J Mol Cell Cardiol*. 1997;29:2383–2392.
  31. Dana A, Skarli M, Papakrivopoulou J, Yellon DM. Adenosine A<sub>1</sub> receptor induced delayed preconditioning in rabbits: induction of p38 mitogen-activated protein kinase activation and Hsp27 phosphorylation via a tyrosine kinase- and protein kinase C-dependent mechanism. *Circ Res*. 2000;86:989–997.
  32. Saurin AT, Martin JL, Heads RJ, et al. The role of differential activation of p38-mitogen-activated protein kinase in preconditioned ventricular myocytes. *FASEB J*. 2000;14:2237–2246.
  33. Ma XL, Kumar S, Gao F, et al. Inhibition of p38 mitogen-activated protein kinase decreases cardiomyocyte apoptosis and improves cardiac function after myocardial ischemia and reperfusion. *Circulation*. 1999;99:1685–1691.
  34. Mackay K, Mochly-Rosen D. An inhibitor of p38 mitogen-activated protein kinase protects neonatal cardiac myocytes from ischemia. *J Biol Chem*. 1999;274:6272–6279.
  35. Gross GJ, Fryer RM. Sarcolemmal versus mitochondrial ATP-sensitive K<sup>+</sup> channels and myocardial preconditioning. *Circ Res*. 1999;84:973–979.
  36. Pain T, Yang X-M, Critz SD, et al. Opening of mitochondrial K<sub>ATP</sub> channels triggers the preconditioned state by generating free radicals. *Circ Res*. 2000;87:460–466.
  37. Yue T-L, Wang C, Gu J-L, et al. Inhibition of extracellular signal-regulated kinase enhances ischemia/reoxygenation-induced apoptosis in cultured cardiac myocytes and exaggerates reperfusion injury in isolated perfused heart. *Circ Res*. 2000;86:692–699.
  38. Fujiyo Y, Nguyen T, Wencker D, Kitsis RN, Walsh K. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. *Circulation*. 2000;101:660–667.
  39. Miao WF, Luo ZY, Kitsis RN, Walsh K. Intracoronary, adenovirus-mediated Akt gene transfer in heart limits infarct size following ischemia-reperfusion injury in vivo. *J Mol Cell Cardiol*. 2000;32:2397–2402.
  40. Yellon DM, Baxter GF. Reperfusion injury revisited: is there a role for growth factor signaling in limiting lethal reperfusion injury? *Trends Cardiovasc Med*. 1999;9:245–249.

# Can pharmacologic treatment initiated shortly before reperfusion salvage ischemic myocardium?

Michael V. Cohen<sup>1,2</sup>, James M. Downey<sup>1</sup>

<sup>1</sup>Department of Physiology, University of South Alabama, College of Medicine, Mobile, AL, USA

<sup>2</sup>Department of Medicine, University of South Alabama, College of Medicine, Mobile, AL, USA

Correspondence: Dr Michel V. Cohen, Department of Physiology, MSB 3050, University of South Alabama, College of Medicine, Mobile, AL 36688, USA. Tel: +1 334 460 6812, e-mail: mcohen@usmail.usouthal.edu

Coronary artery disease is still the leading cause of death in the United States. Because it is now well accepted that the formation of an intracoronary thrombus is the final step in the pathogenesis of coronary occlusion, early reperfusion therapy is acknowledged to be a very effective means of salvaging ischemic myocardium. The introduction of thrombolytic agents for the treatment of acute myocardial infarction has strikingly altered the approach to acute coronary thrombosis and has reduced mortality. The current recommendation is reperfusion within a period not exceeding 6 h after onset of chest pain. Reperfusion may be effected either pharmacologically with a thrombolytic agent or mechanically with angioplasty or bypass surgery.

However, it must be realized that despite the on-call availability of interventional catheterization teams in major clinical centers, there is an unavoidable delay until the coronary artery can be opened; and if thrombolysis is chosen, this process still may take 1–2 h. When myocardium is deprived of arterial blood delivering oxygen, reperfusion delays translate into necrosis. Thus, while reperfusion therapy has reduced the amount of infarction, the latter has not been eliminated. Therefore, there has been an intense effort to develop strategies that will preserve viability of ischemic tissue in the period before reperfusion can be established.

There is also evidence that the very process which rescues ischemic myocardium, ie, reperfusion, may also be the cause of additional damage to the tissue, the so-called reperfusion injury. When ischemic myocardium is reperfused, its appearance rapidly changes from that of normal tissue to one

characterized by obvious necrosis. It is unclear whether this reflects additional necrosis resulting from reperfusion or whether it is simply the expression of irreversible damage incurred during ischemia. Many causes of this reperfusion injury have been suggested: oxygen free radicals, increases in intracellular  $Ca^{++}$  content, neutrophil recruitment, complement activation, disturbed endothelial function leading to the no-reflow phenomenon, impaired cellular energetics, and damage to the extracellular collagen matrix. Unfortunately, reperfusion injury has been difficult to prove, and establishment of a mechanism has been elusive.

There are a number of established interventions which utilize the mechanism of ischemic preconditioning to salvage ischemic myocardium.<sup>1</sup> Unfortunately, the majority of clinically acceptable agents require pretreatment to be effective. Obviously the need for pretreatment limits this form of protection to patients undergoing revascularization surgery or possibly angioplasty, but would not be suitable for patients admitted with acute myocardial infarction.

## Sodium-hydrogen exchange blockers: theory and experimental data

Hence attention has focused on limitation of reperfusion injury, since such interventions could be administered after the onset of ischemia. This would be practical in patients with myocardial infarction in whom reperfusion is being considered. In the myocardial cell, acidosis exerts potent depressive effects on cardiac function by interfering with excita-

tion-contraction coupling. Accordingly, there are two major alkalinizing exchangers in the cell that protect against acidosis: the sodium/hydrogen exchanger (NHE) and the  $\text{Na}^+/\text{HCO}_3^-$  symporter. There are at least six distinct NHE isoforms, although NHE1 is ubiquitous and is the predominant one expressed in the heart.<sup>2</sup> An increase in intracellular protons activates the NHE.  $\text{Na}^+$  exchanges for  $\text{H}^+$  in a 1:1 stoichiometric relationship until the intracellular pH is restored and then the exchanger is again inhibited. The steep  $\text{Na}^+$  gradient across the membrane supplies energy for extruding protons. During ischemia, excess protons are produced as mitochondrial oxidation is replaced with anaerobic glycolysis. As protons accumulate in the cell, the NHE extrudes  $\text{H}^+$  in favor of  $\text{Na}^+$  in an attempt to restore pH. Because  $\text{Na}^+, \text{K}^+$ -ATPase is inhibited during ischemia,  $\text{Na}^+$  increases as a byproduct of this process. Due to the presence of an  $\text{Na}^+/\text{Ca}^{++}$  exchanger, some  $\text{Na}^+$  is transported back out of the ischemic cell, but at the expense of increasing intracellular  $\text{Ca}^{++}$ . The latter is felt to be particularly injurious to the cell because of activation of calcium-dependent proteases and enzymes generating free radicals. Acidification of the extracellular fluid may limit NHE during ischemia. At the time of reperfusion, however, the pH of the extracellular fluid is quickly normalized. Accordingly, there should be an increased exchange of extracellular  $\text{Na}^+$  for the accumulated intracellular  $\text{H}^+$ . The former is then exchanged for  $\text{Ca}^{++}$ , resulting in accumulation of potentially damaging  $\text{Ca}^{++}$  ions which can uncouple oxidative phosphorylation in mitochondria,<sup>3</sup> activate phospholipases leading to membrane damage,<sup>3</sup> produce abnormalities of myofilament contraction and relaxation,<sup>3</sup> and initiate after-depolarizations leading to arrhythmias.<sup>4</sup> Indeed, direct ion measurements<sup>5</sup> and NMR studies<sup>6,7</sup> have confirmed these ion shifts in intact hearts. Hence, it was reasoned that if the NHE could be blocked at reperfusion, cellular  $\text{Ca}^{++}$  overload could be attenuated, and perhaps cellular damage mitigated.

The first NHE blockers, the potassium-sparing diuretic amiloride and its derivatives, were

indeed found to be protective in experimental animals. They preserved postischemic ventricular function<sup>3,5,6,8-10</sup> and diminished myocardial enzyme release.<sup>8-11</sup> Although protection was greatest when the drug was administered before ischemia,<sup>3,5,6,8-12</sup> in many studies protection was still evident even when treatment was delayed until just before reperfusion.<sup>5,9,10,12</sup> Unfortunately, these drugs were not specific for NHE. The newer NHE antagonists, however, are both potent and specific. One of these is cariporide. In experimental animals it decreases ischemic and reperfusion arrhythmias,<sup>13-16</sup> decreases enzyme release following ischemia,<sup>13</sup> diminishes infarct size,<sup>15,17-20</sup> and limits apoptosis.<sup>16,21</sup> These cardioprotective effects were clearly seen when cariporide was infused intravenously before ischemia,<sup>13-19,21</sup> and again, in some studies, protection was also seen when infusion was delayed until after the onset of ischemia<sup>20</sup> and even until shortly before reperfusion.<sup>15,19</sup>

### NHE clinical trials: GUARDIAN and ESCAMI

Because of the solid theory behind the cardioprotective effect of NHE blockers and the impressive experimental data obtained with cariporide, a clinical trial, GUARDIAN (GUARD During Ischemia Against Necrosis), was organized.<sup>22</sup> This was an international, combined phase-II/phase-III dosing, safety, and efficacy trial in which 11,590 subjects with acute coronary syndromes at risk for myocardial infarction were enrolled. There were three clinical arms: (1) unstable angina or non-ST-segment elevation myocardial infarction; (2) high-risk angioplasty; and (3) urgent, repeat, or high-risk coronary revascularization surgery. Patients were randomized to receive either intravenous placebo or 20, 80, or 120 mg cariporide tid. Drug infusion was initiated shortly before the procedure or as soon as the clinical diagnosis was made and was generally continued for 2–7 days. Primary endpoints were the incidence of myocardial infarction and mortality at 36 days after randomization, while secondary endpoints were

the appearance of events related to left ventricular dysfunction such as heart failure and shock at 6 months, and extent of infarction assessed by peak levels of CK-MB.

The results were disappointing. For all patients there was no benefit of cariporide over placebo for primary endpoints. Only one of the nine cariporide subgroups showed diminished risk. There was no effect at any dose in the unstable angina subgroup. A 23% decrease in risk in the angioplasty group treated with 20 mg tid barely missed significance ( $P = 0.06$ ), but there was no effect at the higher doses. There was also no effect of 20 and 80 mg doses in the surgical group. Only at 120 mg tid was the 25% decrease in risk of myocardial infarction and death following surgery significant ( $P = 0.027$ ). Mortality by itself was unaffected. Q-wave myocardial infarctions were decreased in all groups. Non-Q-wave infarctions were diminished by 47% in the surgical subjects treated with 120 mg tid ( $P = 0.005$ ), but not in the other two surgical subgroups. An encouraging note was the absence of any significant clinical adverse effects.

There are clearly some tantalizing observations. Because there was decreased risk in the surgical subgroup treated with the highest dose of 120 mg tid, it is reasonable to suspect that the other subjects were underdosed. The drug appeared to be safe at the doses used, so higher doses could be explored. A likely explanation for these results is that the most significant portion of the sodium-hydrogen exchange takes place during ischemia as opposed to reperfusion. Thus, only groups that had the luxury of pretreatment would have benefitted. Furthermore it is unlikely that the clinical group with unstable angina would have derived any significant benefit from cariporide because of the absence of prolonged ischemia and subsequent reperfusion. So the question of the ultimate value of NHE blockers is still unanswered. Interestingly ESCAMI (Evaluation of the Safety and Cardioprotective Effects of Eniporide in Acute Myocardial Infarction), a phase-II clinical trial with another selective NHE blocker eniporide, also showed no beneficial clinical effects in

patients with acute myocardial infarction, and Merck KGaA has decided to discontinue development of eniporide for this indication.

### Adenosine at reperfusion – clinical trial: AMISTAD

Adenosine has also been tested for its ability to reduce clinical reperfusion injury. Pretreatment with adenosine is known to protect the heart through the preconditioning mechanism.<sup>1,23</sup> However, its effect on reperfusion injury is controversial. Whereas some investigators including ourselves have been unable to demonstrate any effect of adenosine or selective adenosine receptor agonists on infarct size when administered shortly before reperfusion,<sup>24–26</sup> others have.<sup>27–29</sup> Proponents of an adenosine effect suggest this purine can replenish high-energy phosphate stores in endothelial cells and myocytes, inhibit oxygen free radical production, inhibit neutrophil activity and accumulation, and improve microvascular function. On the basis of these latter data a clinical trial AMISTAD (Acute Myocardial Infarction Study of Adenosine) was organized.<sup>30</sup> This was a small, open-label trial of thrombolysis in patients with myocardial infarction and acute ST-segment elevation. Patients were randomized to either placebo or intravenous adenosine (70 µg/kg per min for 3 h) starting shortly before or after infusion of the thrombolytic agent. The primary endpoint was infarct size measured with Tc-99m sestamibi SPECT 5–7 days after enrollment. A secondary endpoint was a composite of in-hospital clinical outcomes (mortality, reinfarction, shock, heart failure, stroke).

Two hundred thirty-six patients were enrolled and the results were mixed but provocative. For all patients, the 33% relative decrease in infarct size following treatment with adenosine was not significant ( $P = 0.085$ ); but when infarctions were subdivided into anterior and nonanterior, differences became apparent. The 67% relative decrease in infarct size in those with anterior infarction treated with adenosine was significant ( $P = 0.014$ ), whereas infarct size was not affected

by the presence of adenosine in those with other than anterior infarction. On the negative side, there was a nonsignificant excess of deaths, reinfarctions, heart failure, and cardiogenic shock in patients treated with adenosine. The composite endpoint was reached in 18% of the adenosine group and 14% of the placebo group (odds ratio 1.43).

Thus there was a likely benefit of adenosine in reducing infarct size only in patients with anterior infarction. Perhaps the larger size of the anterior infarctions gave adenosine a better chance to yield a positive effect. However, the small size of the groups raises the specter of a type II statistical error. It is also unsettling that there was a tendency for those subjects treated with adenosine to have more adverse clinical events. A very small subset of the patients had baseline perfusion scans to measure the size of the risk area. Only in these patients could actual myocardial salvage be estimated. Clearly, a measure of salvage would be a better gauge of an antiinfarct effect than determination of absolute infarct size. Medco has undertaken a phase-III trial of adenosine in acute myocardial infarction, AMISTAD II, in which approximately 2100 patients will be treated in 300 centers in the US and Canada. Data should be available next year.

It is certainly much more difficult to prove efficacy of a treatment in man than in experimental animals. Clinical trials require hundreds and even thousands of patients and are costly. But this translational research is critical if we are to continue to improve clinical strategies for the care of patients with acute coronary syndrome. Adjunctive reperfusion therapy seems to be a good idea, but we have yet to identify the ideal agent. ■

## REFERENCES

1. Cohen MV, Baines CP, Downey JM. Ischemic preconditioning: from adenosine receptor to  $K_{ATP}$  channel. *Annu Rev Physiol*. 2000;62:79–109.
2. Wakabayashi S, Shigekawa M, Pouyssegur J. Molecular physiology of vertebrate  $Na^+/H^+$  exchangers. *Physiol Rev*. 1997;77:51–74.
3. Myers ML, Mathur S, Li G-H, Karmazyn M. Sodium-hydrogen exchange inhibitors improve postischemic recovery of function in the perfused rabbit heart. *Cardiovasc Res*. 1995;29:209–214.
4. Thandroyen FT, Morris AC, Hagler HK, et al. Intracellular calcium transients and arrhythmia in isolated heart cells. *Circ Res*. 1991;69:810–819.
5. Tani M, Neely JR. Role of intracellular  $Na^+$  in  $Ca^{2+}$  overload and depressed recovery of ventricular function of reperfused ischemic rat hearts: possible involvement of  $H^+-Na^+$  and  $Na^+-Ca^{2+}$  exchange. *Circ Res*. 1989;65:1045–1056.
6. Pike MM, Luo CS, Clark MD, et al. NMR measurements of  $Na^+$  and cellular energy in ischemic rat heart: role of  $Na^+-H^+$  exchange. *Am J Physiol*. 1993;265:H2017–H2026.
7. Xiao X-H, Allen DG. Activity of the  $Na^+/H^+$  exchanger is critical to reperfusion damage and preconditioning in the isolated rat heart. *Cardiovasc Res*. 2000;48:244–253.
8. Karmazyn M. Amiloride enhances postischemic ventricular recovery: possible role of  $Na^+-H^+$  exchange. *Am J Physiol*. 1988;255:H606–H615.
9. Meng H-P, Pierce GN. Protective effects of 5-(N,N-dimethyl)amiloride on ischemia-reperfusion injury in hearts. *Am J Physiol*. 1990;258:H1615–H1619.
10. Otani H, Kato Y, Ko T, et al. Effects of amiloride and an analogue on ventricular arrhythmias, contracture and cellular injury during reperfusion in isolated and perfused guinea pig heart. *Jpn Circ J*. 1991;55:845–856.
11. Scholz W, Albus U, Linz W, Martorana P, Lang HJ, Schölkens BA. Effects of  $Na^+/H^+$  exchange inhibitors in cardiac ischemia. *J Mol Cell Cardiol*. 1992;24:731–740.
12. Yasutake M, Ibuki C, Hearse DJ, Avkiran M.  $Na^+/H^+$  exchange and reperfusion arrhythmias: protection by intracoronary infusion of a novel inhibitor. *Am J Physiol*. 1994;267:H2430–H2440.
13. Scholz W, Albus U, Counillon L, et al. Protective effects of HOE642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. *Cardiovasc Res*. 1995;29:260–268.
14. Xue YX, Aye NN, Hashimoto K. Antiarrhythmic effects of HOE642, a novel  $Na^+-H^+$  exchange inhibitor, on ventricular arrhythmias in animal hearts. *Eur J Pharmacol*. 1996;317:309–316.
15. Garcia-Dorado D, González MA, Barrabés JA, et al. Prevention of ischemic rigor contracture during coronary occlusion by inhibition of  $Na^+-H^+$  exchange. *Cardiovasc Res*. 1997;35:80–89.
16. Humphreys RA, Haist JV, Chakrabarti S, Feng Q, Arnold JMO, Karmazyn M. Orally administered NHE1 inhibitor cariporide reduces acute responses to coronary occlusion and reperfusion. *Am J Physiol*. 1999;276:H749–H757.
17. Klein HH, Bohle RM, Pich S, Lindert-Heimberg S, Wollenweber J, Nebendahl K. Time delay of cell death by  $Na^+/H^+$ -exchange inhibition in regionally ischemic, reperfused porcine hearts. *J Cardiovasc Pharmacol*. 1997;30:235–240.
18. Miura T, Ogawa T, Suzuki K, Goto M, Shimamoto

- K. Infarct size limitation by a new Na<sup>+</sup>-H<sup>+</sup> exchange inhibitor, Hoe 642: difference from preconditioning in the role of protein kinase C. *J Am Coll Cardiol*. 1997;29:693–701.
19. Linz W, Albus U, Crause P, et al. Dose-dependent reduction of myocardial infarct mass in rabbits by the NHE-1 inhibitor cariporide (HOE 642). *Clin Exp Hypertens*. 1998;20:733–749.
  20. Klein HH, Bohle RM, Pich S, et al. Time-dependent protection by Na<sup>+</sup>/H<sup>+</sup> exchange inhibition in a regionally ischemic, reperfused porcine heart preparation with low residual blood flow. *J Mol Cell Cardiol*. 1998;30:795–801.
  21. Chakrabarti S, Hoque ANE, Karmazyn M. A rapid ischemia-induced apoptosis in isolated rat hearts and its attenuation by the sodium-hydrogen exchange inhibitor HOE 642 (cariporide). *J Mol Cell Cardiol*. 1997;29:3169–3174.
  22. Théroux P, Chaitman BR, Danchin N, et al. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations: main results of the GUARDIAN trial. *Circulation*. 2000;102:3032–3038.
  23. Liu GS, Thornton J, Van Winkle DM, Stanley AWH, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A<sub>1</sub> adenosine receptors in rabbit heart. *Circulation*. 1991;84:350–356.
  24. Goto M, Miura T, Iliodoromitis EK, et al. Adenosine infusion during early reperfusion failed to limit myocardial infarct size in a collateral deficient species. *Cardiovasc Res*. 1991;25:943–949.
  25. Thornton JD, Liu GS, Olsson RA, Downey JM. Intravenous pretreatment with A<sub>1</sub>-selective adenosine analogues protects the heart against infarction. *Circulation*. 1992;85:659–665.
  26. Vander Heide RS, Reimer KA. Effect of adenosine therapy at reperfusion on myocardial infarct size in dogs. *Cardiovasc Res*. 1996;31:711–718.
  27. Olafsson B, Forman MB, Puett DW, et al. Reduction of reperfusion injury in the canine preparation by intracoronary adenosine: importance of the endothelium and the no-reflow phenomenon. *Circulation*. 1987;76:1135–1145.
  28. Pitarys CJ II, Virmani R, Vildibill HD Jr, Jackson EK, Forman MB. Reduction of myocardial reperfusion injury by intravenous adenosine administered during the early reperfusion period. *Circulation*. 1991;83:237–247.
  29. Zhao Z-Q, Nakamura M, Wang N-P, et al. Administration of adenosine during reperfusion reduces injury of vascular endothelium and death of myocytes. *Coron Artery Dis*. 1999;10:617–628.
  30. Mahaffey KW, Puma JA, Barbagelata NA, et al. Adenosine as an adjunct to thrombolytic therapy for acute myocardial infarction. Results of a multicenter, randomized, placebo-controlled trial: the Acute Myocardial Infarction Study of Adenosine (AMISTAD) Trial. *J Am Coll Cardiol*. 1999;34:1711–1720.

# Glucose-insulin-potassium imaging: the past and the future?

C.M.C. van Campen, Lucas J. Klein, Frans C. Visser

Department of Cardiology, Vrije Universiteit Medical Center, Amsterdam, The Netherlands

Correspondence: Dr C.M.C. van Campen, Department of Cardiology, Vrije Universiteit Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands. Tel: +31 20 4442244, fax: +31 20 4442446, e-mail: cardiol@azvu.nl

## Introduction

Recently, renewed interest has been shown in the use of glucose-insulin-potassium (GIK) infusion in acute myocardial infarction. A meta-analysis of acute myocardial infarction studies in the prethrombolytic era showed a reduction in mortality by GIK.<sup>1</sup> Also, a pilot study in South America showed a significant reduction in in-hospital mortality in patients treated with GIK and reperfusion therapy.<sup>2</sup> In experimental myocardial infarction and ischemia studies, GIK preserved oxidative metabolism,<sup>3</sup> reduced infarct size,<sup>4</sup> and improved recovery of left ventricular function.<sup>5,6</sup>

The exact mechanism behind the potential beneficial effect of GIK in acute myocardial infarction is unclear. Proposed explanations include reduction in plasma free fatty acid levels, optimization of calcium handling, stimulation of Na,K-ATPase, and improvement of glucose availability, with effects on intracellular ATP levels.<sup>7</sup>

Because GIK and dobutamine may share a similar mechanism of action, we hypothesized that GIK infusion improves left ventricular function and detects viable tissue to a similar extent as dobutamine. We therefore studied the use of GIK in comparison with dobutamine in patients with recent myocardial infarction.

## Patients and methods

Twenty patients with acute myocardial infarction were enrolled in the study. Myocardial infarction and its complications were treated in a standard fashion. In the subacute phase, patients underwent low-dose dobutamine (LDD) and GIK echocardiography on the same day. Exclusion criteria were severe ventricular

arrhythmias, atrial fibrillation, pacemaker rhythm, overt heart failure, severe primary valvular disease, and insulin-dependent diabetes mellitus.

Patients underwent LDD echocardiography in the morning and GIK echocardiography in the afternoon. This order was fixed to prevent a possible carryover effect of GIK.

## Echocardiography

A 2D echocardiogram was obtained including the parasternal long- and short-axis views, and the apical 2-, 3-, and 4-chamber long-axis views, while simultaneously monitoring cardiac rhythm.

## LDD echocardiography

Echocardiograms were obtained at baseline and at a dose of 15 µg/kg per min. None of the patients experienced significant side effects such as serious ventricular arrhythmias or chest pain.

## GIK echocardiography

Patients were studied during a hyperinsulinemic-euglycemic clamp as described previously.<sup>8</sup> In brief, cannulas were introduced into the left and right antecubital veins. One cannula was used for GIK infusion, and the contralateral cannula was used for blood sampling. Twenty units of insulin (Human Velosulin, 100 U/mL; Novo Nordisk, Alphen a/d Rijn, The Netherlands) were added to 50 mL 0.65% NaCl and infused at a constant rate of 100 mU/kg per h. Glucose infusion (500 mL 20% glucose with 20 mL 14.9% KCl to prevent hypokalemia) was started at a rate of 6 mg/kg per min and was adjusted to maintain normoglycemia, based on instantly determined plasma glucose levels, using a GlucoTouch (Lifes-

can, Beerse, Belgium) apparatus, adjusted for whole blood samples.

Echocardiography was performed prior to and at 60 min of GIK infusion. Thereafter the infusion was stopped. None of the patients had signs or symptoms of heart failure during or after the study.

### Echocardiographic data analysis

The LDD and GIK echocardiograms were scored by two independent observers, unaware of the clinical data of patients and type of intervention (LDD or GIK). Echocardiograms at baseline and after intervention were reviewed side by side. The LDD and GIK studies of the same patient were analyzed on two separate occasions, at least 1 month apart, and in random order. In case of disagreement, consensus was obtained by combined reading.

The left ventricle was divided into 13 segments (six basal, six distal, and one apical segment) as described previously.<sup>9</sup> Each segment was scored on a four-point scale assessing both inward wall motion and wall thickening: 0 = normal contraction; 1 = hypokinesia (decreased endocardial excursion and systolic wall thickening); 2 = akinesia (absence of endocardial excursion and systolic wall thickening); and 3 = dyskinesias (paradoxical outward movement during systole). This resulted in the wall motion score. Contractile reserve was considered present if the score of a dysfunctional segment at baseline decreased at least one point during LDD or GIK infusion. Dyskinetic segments at baseline had to show at least hypokinesia to have contractile reserve (decrease of the score by 2).<sup>10</sup> In a similar manner, functional recovery during follow-up was identified by comparing the score of the dysfunctional baseline segments with the scores during follow-up.

### Results

Twenty patients were enrolled in the study. The mean age was  $60 \pm 15$  years. There were

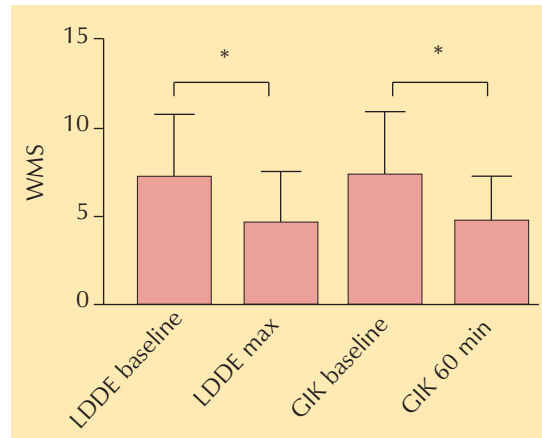


Figure 1. Improvement of regional left ventricular function expressed by wall motion score (WMS) before and after LDD echocardiography and GIK infusion. \* $P < 0.05$ .

17 male and three female patients. The location of the myocardial infarction was anterior in nine patients (45%) and inferior in 11 patients (55%). Four patients had had a previous myocardial infarction. Nine patients were treated conservatively, and 11 with revascularization therapy (including three PTCA procedures). All patients were treated with aspirin or Coumadin derivatives, and 85% of the patients were treated with  $\beta$ -blockers. The time between the myocardial infarction and the echocardiography protocol was  $6.1 \pm 2.7$  days.

Figure 1 shows the improvement of regional function: the wall motion score improved from  $7.15 \pm 3.65$  to  $4.70 \pm 2.77$  ( $P < 0.0001$ ) during LDD echocardiography, and from  $7.30 \pm 3.66$  to  $4.65 \pm 2.64$  ( $P < 0.0001$ ) during GIK infusion. Table 1 shows the agreement between LDD and GIK echocardiography (to detect contractile reserve). During LDD echocardiography, 50 dysfunctional segments showed contractile reserve whereas 57 did not. During GIK echocardiography, 53 showed contractile reserve and 55 did not. Of the segments with contractile reserve, none showed a biphasic response. Overall agreement was 87%, with a kappa value of 0.75.

Figure 2 shows an example of improvement of left ventricular function during LDD and GIK infusion.

Table 1. Agreement between LDD and GIK echocardiography to detect contractile reserve.

	LDD echocardiography			
	Normal	CR	No CR	Total
GIK echocardiography				
Normal	150	2	0	152
CR	2	43	8	53
No CR	1	5	49	55
Total	153	50	57	260

CR, contractile reserve.

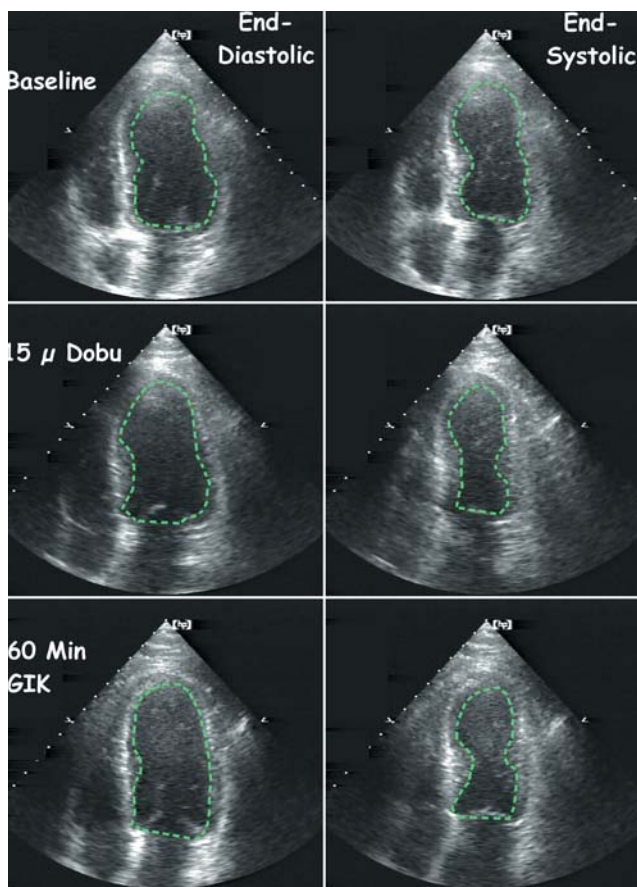


Figure 2. Example of global left ventricular function improvement. The upper example is the end-diastolic and end-systolic frame during baseline. The middle example is the end-diastolic and end-systolic frame during dobutamine 15  $\mu\text{g}/\text{kg}$  per min, and the lower example is the end-diastolic and end-systolic frame after 1 h of GIK infusion.

## Discussion

This study shows that GIK infusion results in improvement of left ventricular function, and that GIK echocardiography can detect contractile reserve soon after myocardial infarction. These effects are not different from those during LDD stimulation. In addition, GIK administration was safe.

Our data are in line with those of previous studies. In studies of experimental cardiac ischemia, GIK exerted beneficial effects on regional and global left ventricular function peri- and post-ischemia.<sup>5,6,11,12</sup> One study attributed the salutary effect of GIK to insulin alone.<sup>12</sup> In patients undergoing cardiac surgery, GIK infusion resulted in higher postoperative cardiac indices.<sup>13,14</sup> A comparison of GIK and LDD infusion in postoperative patients showed an increase in cardiac index and stroke work index during both interventions, but the rate-pressure product and the tension-time index increased only in the dobutamine group, suggesting that myocardial oxygen consumption was not changed during GIK.<sup>15</sup> Furthermore, whole body oxygen consumption was increased with LDD whereas it was unchanged in GIK infusion.

LDD echocardiography is a well-established technique to assess contractile reserve,<sup>16</sup> and can reliably predict left ventricular function improvement in patients with acute myocardial infarction<sup>17-19</sup> irrespective of the treatment strategy. Also, in patients with chronic ischemic left ventricular dysfunction, LDD echocardiography can predict improvement of function after revascularization.<sup>16,20</sup>

The present study shows a high agreement of GIK echocardiography with LDD echocardiography to detect contractile reserve, with a kappa value of 0.75. The diagnostic value of LDD echocardiography we observed is in line with that of previously published studies.<sup>17-19</sup>

The positive effects of GIK have been attributed to several mechanisms. These include enhanced availability of glucose to the cell, reduction of plasma free fatty acid levels, effects on  $\text{Na}^+, \text{K}^+$ -ATPase, decrease in myocardial oxygen consumption, and improved  $\text{Ca}^{2+}$  handling.<sup>7</sup> As a result of GIK

infusion, the availability of substrate for glycolysis is enhanced in ischemic cells, with a possibility for, albeit low, anaerobic ATP production.<sup>21</sup> The ATP produced by glycolysis is preferentially used to maintain membrane functions, such as ATP-sensitive K<sup>+</sup> channels<sup>22</sup> and the sarcolemmal Ca<sup>2+</sup> pump,<sup>23</sup> but may also become available for contraction. The functional impairment observed in postischemic myocardium is related to cellular Ca<sup>2+</sup> overload.<sup>23</sup> In this view, GIK infusion with subsequent enhancement of glycolysis may reduce the Ca<sup>2+</sup> overload present in postischemic myocardial cells. This reduction may be responsible for the enhanced contractility of dysfunctional myocardium observed in the present study.

### Methodological considerations

The dosage of GIK used in this study (100 mU/kg per h insulin) was higher than in GIK interventions in acute myocardial infarction.<sup>1,2</sup> It is, however, an accepted and widely used dosage in diagnostic FDG imaging.<sup>8</sup> In addition, it has been observed that low-dose GIK infusion is not effective after acute myocardial infarction.<sup>24</sup>

The order of the LDD and GIK echocardiography was fixed in this study and performed on the same day. The order was fixed because the duration of the positive effects of GIK is not known, in contrast to the short-lasting effects of dobutamine. To avoid bias, the observers were blinded to the intervention strategy (LDD or GIK), and echocardiograms were reviewed in a totally random order. The studies were performed on the same day to avoid influence of spontaneous recovery after the acute ischemic event. The use of echocardiography to compare LDD and GIK interventions rules out possible methodological differences between two different imaging modalities.

GIK echocardiography proved to be safe in this patient group, as no patients experienced significant side effects of GIK infusion (eg, signs of heart failure or ischemia, deterioration of serum potassium levels, or severe hypoglycemia).

### Summary

**We clearly demonstrated that GIK infusion results in improvement of left ventricular function, and that the improvement is related to the improvement of function of viable segments. Moreover, the magnitude of improvement is similar to that of dobutamine infusion. Therefore GIK can be used as an alternative to dobutamine both for functional improvement and for the detection of viable tissue.**

**Its major advantage over dobutamine is that GIK does not increase oxygen consumption<sup>25</sup> and the risk of ventricular arrhythmias. ■**

### REFERENCES

1. Fath-Ordoubadi F, Beatt KJ. Glucose-insulin-potassium therapy for treatment of acute myocardial infarction: an overview of randomized placebo-controlled trials. *Circulation*. 1997;96(4):1152–1156.
2. Diaz R, Paolasso EA, Piegas LS, et al. Metabolic modulation of acute myocardial infarction. The ECLA (Estudios Cardiológicos Latinoamérica) Collaborative Group. *Circulation*. 1998;98(21):2227–2234.
3. Opie LH, Bruyneel K, Owen P. Effects of glucose, insulin and potassium infusion on tissue metabolic changes within first hour of myocardial infarction in the baboon. *Circulation*. 1975;52(1):49–57.
4. Maroko PR, Libby P, Sobel BE, et al. Effect of glucose-insulin-potassium infusion on myocardial infarction following experimental coronary artery occlusion. *Circulation*. 1972;45(6):1160–1175.
5. Weissler AM, Altschuld RA, Gibb LE, Pollack ME, Kruger FA. Effect of insulin on the performance and metabolism of the anoxic isolated perfused rat heart. *Circ Res*. 1973;32(1):108–116.
6. Zhu P, Lu L, Xu Y, Greyson C, Schwartz GG. Glucose-insulin-potassium preserves systolic and diastolic function in ischemia and reperfusion in pigs. *Am J Physiol*. 2000;278(2):H595–H603.
7. Apstein CS. Glucose-insulin-potassium for acute myocardial infarction: remarkable results from a new prospective, randomized trial. *Circulation*. 1998;98(21):2223–2226.
8. Knuuti MJ, Nuutila P, Ruotsalainen U, et al. Euglycemic hyperinsulinemic clamp and oral glucose load in stimulating myocardial glucose utilization during positron emission tomography. *J Nucl Med*. 1992;33(7):1255–1262.
9. Kan G, Visser CA, Koolen JJ, Dunning AJ. Short and long term predictive value of admission wall

- motion score in acute myocardial infarction. A cross sectional echocardiographic study of 345 patients. *Br Heart J*. 1986;56(5):422–427.
10. Bax JJ, Cornel JH, Visser FC, et al. Prediction of recovery of myocardial dysfunction after revascularization: comparison of fluorine-18 fluorodeoxyglucose/thallium-201 SPECT, thallium-201 stress-reinjection SPECT and dobutamine echocardiography. *J Am Coll Cardiol*. 1996;28(3):558–564.
  11. Cardillo C, Kilcoyne CM, Nambi SS, Cannon RO III, Quon MJ, Panza JA. Vasodilator response to systemic but not to local hyperinsulinemia in the human forearm. *Hypertension*. 1998;32(4):740–745.
  12. Doenst T, Richwine RT, Bray MS, Goodwin GW, Frazier OH, Taegtmeier H. Insulin improves functional and metabolic recovery of reperfused working rat heart. *Ann Thorac Surg*. 1999;67(6):1682–1688.
  13. Coleman GM, Gradinac S, Taegtmeier H, Sweeney M, Frazier OH. Efficacy of metabolic support with glucose-insulin-potassium for left ventricular pump failure after aortocoronary bypass surgery. *Circulation*. 1989;80(3 pt 1):191–196.
  14. Lazar HL, Philippides G, Fitzgerald C, Lancaster D, Shemin RJ, Apstein C. Glucose-insulin-potassium solutions enhance recovery after urgent coronary artery bypass grafting. *J Thorac Cardiovasc Surg*. 1997;113(2):354–360.
  15. Hiesmayr M, Haider WJ, Grubhofer G, et al. Effects of dobutamine versus insulin on cardiac performance, myocardial oxygen demand, and total body metabolism after coronary artery bypass grafting. *J Cardiothorac Vasc Anesth*. 1995;9(6):653–658.
  16. Bax JJ, Wijns W, Cornel JH, Visser FC, Boersma E, Fioretti PM. Accuracy of currently available techniques for prediction of functional recovery after revascularization in patients with left ventricular dysfunction due to chronic coronary artery disease: comparison of pooled data. *J Am Coll Cardiol*. 1997;30(6):1451–1460.
  17. Knudsen AS, Darwish AZ, Norgaard A, Gotzsche O, Thygesen K. Time course of myocardial viability after acute myocardial infarction: an echocardiographic study. *Am Heart J*. 1998;135(1):51–57.
  18. Leclercq F, Messner-Pellenc P, Moragues C, et al. Myocardial viability assessed by dobutamine echocardiography in acute myocardial infarction after successful primary coronary angioplasty. *Am J Cardiol*. 1997;80(1):6–10.
  19. Smart SC, Sawada S, Ryan T, et al. Low-dose dobutamine echocardiography detects reversible dysfunction after thrombolytic therapy of acute myocardial infarction. *Circulation*. 1993;88(2):405–415.
  20. La Canna G, Alfieri O, Giubbini R, Gargano M, Ferrari R, Visioli O. Echocardiography during infusion of dobutamine for identification of reversible dysfunction in patients with chronic coronary artery disease. *J Am Coll Cardiol*. 1994;23(3):617–626.
  21. Cave AC, Ingwall JS, Friedrich J, et al. ATP synthesis during low-flow ischemia: influence of increased glycolytic substrate. *Circulation*. 2000;101(17):2090–2096.
  22. Weiss JN, Lamp ST. Glycolysis preferentially inhibits ATP-sensitive K<sup>+</sup> channels in isolated guinea pig cardiac myocytes. *Science*. 1987;238(4823):67–69.
  23. Jeremy RW, Koretsune Y, Marban E, Becker LC. Relation between glycolysis and calcium homeostasis in postischemic myocardium. *Circ Res*. 1992;70(6):1180–1190.
  24. Ceremuzynski L, Budaj A, Czepiel A, et al. Low-dose glucose-insulin-potassium is ineffective in acute myocardial infarction: results of a randomized multicenter Pol-GIK trial. *Cardiovasc Drugs Ther*. 1999;13(3):191–200.
  25. Rogers WJ, Russell RO Jr, McDaniel HG, Rackley CE. Acute effects of glucose-insulin-potassium infusion on myocardial substrates, coronary blood flow and oxygen consumption in man. *Am J Cardiol*. 1977;40(3):421–428.

# A new look at insulin as a potential cardioprotective agent

Michael N. Sack<sup>1</sup>, Derek M. Yellon<sup>2</sup>

<sup>1</sup>The Hatter Institute for Cardiology Research, MRC Inter-University Cape Heart Group, University of Cape Town Medical School, South Africa;

<sup>2</sup>The Hatter Institute for Cardiovascular Studies, UCL Hospitals and Medical School, London, UK

Correspondence: Professor Derek M. Yellon, The Hatter Institute and Centre for Cardiology, University College London Hospitals and Medical School, Grafton Way, London WC1E 6DB, UK.  
Tel: +44 20 7380 9888, fax: +44 20 7388 5095, e-mail: hatter-institute@ucl.ac.uk

## Introduction

The concept that the metabolic cocktail, glucose-insulin-potassium (GIK), may protect ischemic cardiomyocytes was initially introduced by Sodi-Pallares et al in 1962.<sup>1</sup> The rationale for the use of this metabolic therapy was further delineated by Opie, in 1970, when he described two chief mechanisms, ie, the promotion of cardiac glycolysis and the inhibition of free fatty acids (FFA) in the serum.<sup>2</sup> A number of early clinical studies using this metabolic cocktail yielded promising results and a subsequent metaanalysis suggested that GIK therapy might have an important role in reducing in-hospital mortality after acute myocardial infarction.<sup>3</sup> Two subsequent randomized, controlled clinical studies have been published. In the first of these, the Estudios Cardiológicos Latinoamerica (ECLA) study,<sup>4</sup> subjects who underwent reperfusion strategies showed a reduction in in-hospital mortality of 66% ( $2P = 0.008$ ) when GIK was coadministered with the reperfusion therapy. In contrast, a Polish study by Ceremuzynski et al<sup>5</sup> did not show any beneficial effect of low-dose GIK therapy. Apstein and Opie reviewed the different GIK doses in these two studies<sup>6</sup> and suggested that the ECLA study dose of GIK was consistent with the previous studies that showed benefit of high-dose GIK therapy (see reference 7 for review).

Acceptance of the benefits and subsequent use of this metabolic cocktail have not been forthcoming despite almost four decades since the therapy was proposed. The reasons for the lack of enthusiasm are probably multifactorial and include both the lack of large clinical studies and a poor understanding of the basic

mechanisms of how this metabolic cocktail acts. Although the progress towards a large clinical study using GIK is uncertain, recent research in our laboratories has begun to delineate a possible novel hypothesis whereby the insulin component of the GIK cocktail may promote the cardioprotective effects of GIK. These studies are described below in conjunction with the clinical data obtained from the ECLA study.

## GIK at reperfusion (more practical in the clinical arena!)

As the majority of GIK trials were performed in the prethrombolytic era, it was assumed that the benefit of this therapy may be less applicable in the current aggressive thrombolytic era. However, as was shown in the ECLA study, the only statistically significant reduction in mortality was in acute myocardial infarction in patients who received concomitant reperfusion treatment.<sup>4</sup> Interestingly, in the 1-year follow-up data, only the subjects who had received the high-dose GIK therapy had a statistical survival advantage over the control group.<sup>4</sup> The decision to use a high-dose GIK regimen was based on the pioneering dose-response studies of Rackley's group,<sup>8</sup> who determined the GIK infusion rates which would result in the maximal suppression of FFA levels, as well as the maximal myocardial glucose uptake. Concurrent to the ECLA clinical study, Jonassen and coworkers,<sup>9</sup> in an experimental study using rats, compared the efficacy of administering GIK prior to an ischemic insult or at the moment of reperfusion following the ischemic insult. Interesting-

ly, here GIK was demonstrated to be equally effective in reducing the final infarct size whether administered during the entire ischemia/reperfusion period or solely during the reperfusion period alone. Moreover, when GIK was administered at reperfusion, the early reperfusion FFA and glucose levels were unchanged compared with vehicle-treated controls. This was significantly different from the FFA and glucose levels in the animals treated with GIK throughout the ischemia/reperfusion period.<sup>9</sup> Taken together, the ECLA clinical study and this experimental study suggest that GIK may mediate a reperfusion cardioprotective effect. Moreover, the experimental data questioned the exclusivity of the glucose/FFA hypothesis concerning GIK's cardioprotective effects!

### Reperfusion injury and the potential effects of GIK

Although reperfusion is a prerequisite for tissue salvage following a myocardial infarction, there is a price to pay in terms of distinct reperfusion-associated pathologies (see reference 10 for review). One postulated aspect of this pathology is the development of reperfusion-induced myocyte loss beyond that sustained as a consequence of ischemia alone. In this regard it has recently been suggested that, in addition to necrosis, a component of cell death not previously considered in reperfusion injury, ie, programmed cell death or apoptosis, may play a biologically significant role.<sup>11</sup> Under experimental conditions, an increase in apoptosis has been observed in cardiac reperfusion models, suggesting that the deleterious effects of reperfusion are, at least in part, due to apoptosis.<sup>12,13</sup> Taking these data into consideration, we have suggested that GIK, or a component therein (insulin), may antagonize apoptosis during reperfusion and hence result in cardioprotection.<sup>11</sup> Recent experimental evidence has suggested that insulin can indeed attenuate such apoptotic processes in the brain.<sup>14</sup> Collectively, these data suggested to us that insulin may be the appropriate candidate in the GIK cock-

tail which could promote a cardioprotective effect at reperfusion, via an effect which may be independent of the original 'GIK hypothesis'.

### Insulin, the chief mediator of reperfusion protection in the GIK cocktail

To test initially the hypothesis that insulin is the major protagonist of cardioprotection when administered at the time of reperfusion, we studied insulin's putative cardioprotective effects in ischemia and reoxygenation experiments in rat neonatal cardiomyocyte experiments. The administration of insulin (0.3 mU/mL) at the moment of reoxygenation enhanced myocardial cell viability by 20% compared with vehicle-treated control cardiomyocytes ( $P < 0.001$ ).<sup>15</sup> To evaluate the putative role of insulin in the attenuation of reperfusion apoptosis, markers of apoptosis were ascertained in these experiments. Consistent with the cell viability data, insulin administration at reoxygenation reduced apoptosis by approximately 20–30% compared with vehicle-treated control cardiomyocytes.<sup>15</sup> As insulin is thought to confer anti-apoptotic effects via the activation of tyrosine kinase and phosphatidyl 3-kinase (PI3-kinase)-mediated cell signaling pathways, we used pharmacologic inhibitors of these signaling transduction pathways. In these experiments we demonstrated that the cardioprotective and antiapoptotic effects of insulin were completely abolished by tyrosine kinase and PI3-kinase inhibitors.<sup>15</sup> Furthermore, we have recently confirmed the cardioprotective effects of insulin at reperfusion in the isolated rat heart using infarct size as the endpoint.<sup>16</sup> In these studies we showed that early administration of insulin during reoxygenation/reperfusion appears to be an effective modality to reduce reoxygenation/reperfusion injury in the myocardium, in part via the attenuation of ischemia/reoxygenation-induced apoptosis. Moreover, the cardioprotective and antiapoptotic effects of insulin appear to be mediated via tyrosine kinase and PI3-kinase signaling pathways.

## Conclusion

For a number of years clinical data have supported a role of GIK in reducing morbidity and mortality following myocardial infarction. The encouraging data from the ECLA study suggested that this cardioprotective effect of GIK is achieved when reperfusion therapy is administered. The recent experimental data from our and other laboratories suggest that this reperfusion effect of GIK may be independent of the glucose/FFA hypothesis of GIK's cellular protective effects. Our subsequent experimental data have further advanced the mechanisms underlying this reperfusion cardiac cell protection, ie, the effect seems to be mediated by insulin alone and this effect may be via the attenuation of the known programmed cell death associated with reperfusion injury.<sup>10</sup>

Finally, our data begin to delineate the signal transduction pathways which may promote insulin-mediated cell survival effects. Thus we are encouraged in that the laboratory-based understanding of how GIK may promote cell survival is being actively pursued. However, to paraphrase Apstein and Taegtmeyer,<sup>17</sup> the need to reevaluate the clinical utilization of GIK is both timely and could result in an effective and affordable addition to the therapeutic armamentarium in the prevention of myocardial reperfusion injury. We hope the call for a larger, randomized, controlled, clinical study will be seriously considered in the very near future. ■

## REFERENCES

1. Sodi-Pallares D, Testelli M, Fishelder F. Effects of an intravenous infusion of a potassium-insulin-glucose solution on the electrocardiographic signs of myocardial infarction. *Am J Cardiol.* 1962;9:166–181.
2. Opie LH. The glucose hypothesis: relation to acute myocardial ischemia. *J Mol Cell Cardiol.* 1970;1:107–114.
3. Fath-Ordoubadi F, Beatt KJ. Glucose-insulin-potassium therapy for treatment of acute myocardial infarction: an overview of randomized placebo-controlled trials. *Circulation.* 1997;96:1152–1156.
4. Diaz R, Paolasso EA, Piegas LS, et al, on behalf of the ECLA Collaborative Group. Metabolic modulation of acute myocardial infarction. The ECLA Glucose-Insulin-Potassium Pilot Trial. *Circulation.* 1998;98:2227–2234.
5. Ceremuzynski L, Budaj A, Czepiel A, et al. Low-dose glucose-insulin-potassium is ineffective in acute myocardial infarction: results of a randomized multicenter Pol-GIK trial [see comments]. *Cardiovasc Drugs Ther.* 1999;13:191–200.
6. Apstein CS, Opie LH. Glucose-insulin-potassium (GIK) for acute myocardial infarction: a negative study with a positive value. *Cardiovasc Drugs Ther.* 1999;13:185–189.
7. Apstein CS. Glucose-insulin-potassium for acute myocardial infarction: remarkable results from a new prospective, randomized trial [editorial]. *Circulation.* 1998;98:2223–2226.
8. Stanley AW Jr, Moraski RE, Russell RO, et al. Effects of glucose-insulin-potassium on myocardial substrate availability and utilization in stable coronary artery disease. Studies on myocardial carbohydrate, lipid and oxygen arterial-coronary sinus differences in patients with coronary artery disease. *Am J Cardiol.* 1975;36:929–937.
9. Jonassen AK, Aasum E, Riemersma RA, Mjos OD, Larsen TS. Glucose-insulin-potassium reduces infarct size when administered during reperfusion. *Cardiovasc Drugs Ther.* 2000;14:615–623.
10. Yellon DM, Baxter GF. Reperfusion injury revisited: is there a role for growth factor signaling in limiting lethal reperfusion injury? *Trends Cardiovasc Med.* 1999;9:245–249.
11. Yellon DM, Baxter GF. Protecting the ischaemic and reperfused myocardium in acute myocardial infarction: distinct dream or near reality? *Heart.* 2000;83:381–387.
12. Gottlieb RA, Burleson KO, Kloner RA, Babior BM, Engler RL. Reperfusion injury induced apoptosis in rabbit cardiomyocytes. *J Clin Invest.* 1994;94:1621–1628.
13. Fliss H, Gattinger D. Apoptosis in ischemic and reperfused rat myocardium. *Circ Res.* 1996;79:949–956.
14. Ryu BR, Ko HW, Jou I, Noh JS, Gwag BJ. Phosphatidylinositol 3-kinase-mediated regulation of neuronal apoptosis and necrosis by insulin and IGF-I. *J Neurobiol.* 1999;39:536–546.
15. Jonassen AK, Brar BK, Mjos OD, Sack MN, Latchman DS, Yellon DM. Insulin administered at reoxygenation exerts a cardioprotective effect in myocytes by a possible anti-apoptotic mechanism. *J Mol Cell Cardiol.* 2000;32:757–764.
16. Jonassen AK, Brar BK, Mjos OD, Sack MN, Latchman DA, Yellon DM. Insulin modifies myocyte apoptosis and reduces myocardial infarct size when administered at reperfusion: a novel mechanism of protection. *Br J Pharmacol.* 1999;126(suppl):201P.
17. Apstein CS, Taegtmeyer H. Glucose-insulin-potassium in acute myocardial infarction: the time has come for a large, prospective trial [editorial; comment]. *Circulation.* 1997;96:1074–1077.

# Cardioprotection: a promising role for metabolic agents

Marc Pini  
Paris, France

## Rationale for metabolic cardioprotective intervention

Despite significant progress in recent years, coronary artery disease remains a major health problem worldwide. The therapeutic approach to the patient with acute myocardial infarction or unstable angina has radically changed in recent times. The current strategy is to consider reperfusion therapy, either catheter-based or fibrinolytic (for myocardial infarction only), and to initiate it as soon as possible. Appropriate therapy with aspirin, anticoagulants or  $\beta$ -blockers is prescribed to the majority of patients, since these have been proved through randomized clinical trials to reduce mortality. The pathophysiological mechanism is the acute rupture of an atherosclerotic plaque in an epicardial coronary artery, exposing endothelial tissue to a thrombogenic response and leading to severe obstruction of the vessel. While the vessel is considered to be the main target, there are multiple abnormalities in myocardial energy metabolism that contribute to a poor prognosis, especially in diabetic patients, despite the progress in revascularization procedures. Cardiac metabolism has remained for many years the lost child, despite accumulating evidence of its benefits in maintaining myocardial viability in acute coronary syndromes.

The metabolic approach to treating coronary heart disease is based on the concept that cardiac energy substrates can be manipulated to optimize ATP production and enhance cardiac output. The efficacy of this metabolic modulation in acute coronary syndromes has been confirmed in two randomized trials. The DIGAMI (Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction) study aimed to evaluate the impact on mortality of a glucose-insulin infusion followed by multidose insulin treatment in dia-

betic patients with acute myocardial infarction.<sup>1</sup> A total of 620 diabetic patients were studied and the glucose-insulin infusion produced a significant reduction in mortality at 1-year follow-up (relative mortality reduction 29%,  $P = 0.027$ ).

The ECLA (Estudios Cardiológicos Latina America) study was conducted in 407 nondiabetic patients with suspected acute myocardial infarction, who received either a glucose-insulin-potassium infusion (GIK) or placebo.<sup>2</sup> At 1-year follow-up, a statistically significant reduction in mortality (relative risk 0.34, 95% CI 0.15–0.78,  $2P = 0.008$ ) was observed in the GIK group vs. placebo in patients who had undergone reperfusion with fibrinolysis.

Although a large-scale trial is necessary to reliably determine the precise benefits of metabolic modulation, these studies have highlighted the potential benefits of metabolic cardiac protection.

## Cardioprotection with metabolic agents

Kantor et al<sup>3</sup> have recently clarified the precise mechanism of action of Vastarel 20 mg, the first in a new class of metabolic agents known as 3-KAT inhibitors. Vastarel 20 mg selectively inhibits a mitochondrial enzyme — the long-chain 3-ketoacyl CoA thiolase (3-KAT) — thereby shifting cardiac metabolism away from fatty acid  $\beta$ -oxidation and towards glucose oxidation, thus optimizing energy production in the myocardial cell (*Figure 1*).<sup>4</sup> The cardioprotective effect of Vastarel 20 mg was first demonstrated in experimental studies which confirmed that it restores myocyte viability and ATP content in cardiomyocytes exposed to hypoxia,<sup>5</sup> preserves the electrical activity of the myocardial cell under ischemic conditions,<sup>6</sup> reduces ischemic contracture,

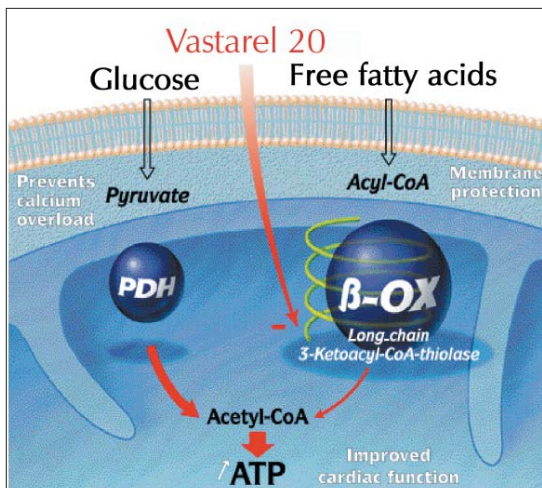


Figure 1. Mechanism of action of Vastarel 20. PDH, pyruvate dehydrogenase; β-OX, β-oxidation.

and improves functional recovery.<sup>7</sup> In vivo animal studies have demonstrated the additive beneficial effects of Vastarel 20 mg. Pretreatment with Vastarel 20 mg before coronary ligation followed by reperfusion led to a reduction in mean ST shift, a decrease in the size of the border area, and a significant reduction in the extent of infarction.<sup>8,9</sup>

These encouraging results have been confirmed in several clinical studies. During CABG, pretreatment with Vastarel 20 mg for 3 weeks, followed by the addition of a Vastarel 20 mg solution to cardioplegic solutions, resulted in a significant limitation of myocardial injury.<sup>10</sup>

In addition to thrombolysis, pretreatment with Vastarel 20 mg in 81 patients with anterior myocardial infarction recently produced a significant reduction of ventricular arrhythmias ( $P < 0.05$ ), a smaller creatine kinase peak ( $P = 0.012$ ), and a smaller end-systolic volume ( $P = 0.037$ ) compared with pretreatment with placebo.<sup>11</sup> During coronary angioplasty, intracoronary injection of Vastarel 20 mg led to a marked reduction in ECG signs of ischemia and anginal pain resulting from balloon inflation-induced ischemia during angioplasty.<sup>12,13</sup>

Despite a higher initial value, an earlier and more marked return to baseline ST-segment was observed in the Vastarel 20 mg group ( $P = 0.014$ ) in the LIST (Limitation of Infarct Size by Trimetazidine Trial) (Figure 2).

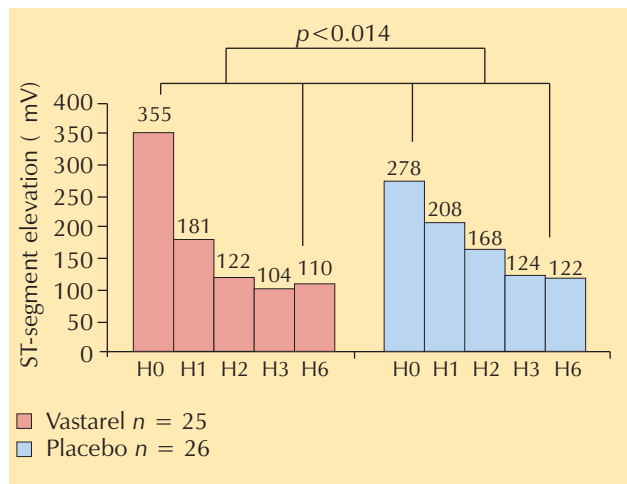


Figure 2. Vectorcardiographic results obtained in the LIST study.

These results suggest an earlier and probably more complete myocardial reperfusion with Vastarel 20 mg. Although randomized large-scale studies are needed to confirm these promising results, the concept of metabolic intervention in acute coronary syndromes appears to be effective and is expected to improve the prognosis of patients with coronary artery disease. ■

## REFERENCES

- Malmberg K, Ryden L, Efendic S, et al, on behalf of the DIGAMI Study Group. Randomized trial of insulin-glucose infusion followed by subcutaneous insulin treatment in diabetic patients with acute myocardial infarction (DIGAMI study): effects on mortality at 1 year. *J Am Coll Cardiol.* 1995;26:57–65.
- Díaz R, Paolasso EA, Leopoldo S, et al, on behalf of the ECLA (Estudios Cardiológicos Latina America) Collaborative Group. Metabolic modulation of acute myocardial infarction. The ECLA Glucose-Insulin-Potassium pilot trial. *Circulation.* 1998;98:2227–2234.
- Kantor PF, Lucien A, Kozak R, Lopaschuk GD. The antianginal drug trimetazidine shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long-chain-3-ketoacyl coenzyme A thiolase. *Circ Res.* 2000;86:580–588.
- Allibardi S, Chierchia SL, Margonato V et al. Effects of trimetazidine on metabolic and functional recovery of posts ischemic rat hearts. *Cardiovasc Drugs Ther.* 1998;12(6):543–549.

5. Honore E, Adamantidis M, Challice CE, Dupuis BA. Cardioprotection by calcium antagonists, piroxilate and Vastarel 20 mg. *IRCS Med Sci.* 1986;14:938–939.
6. Drake-Holland AJ, Belcher PR, Hynd J, Noble MIM. Infarct size in rabbits: a modified method illustrated by the effects of propranolol and Vastarel 20 mg. *Basic Res Cardiol.* 1993;88:250–258.
7. Williams FM, Tanda K, Williams TJ. Inhibition of neutrophil accumulation after myocardial ischemia and reperfusion in rabbits. *J Cardiovasc Pharmacol.* 1993;22:828–833.
8. Boucher FR, Hearse DJ, Opie LH. Effects of Vastarel 20 mg on ischemic contracture in isolated perfused rat hearts. *J Cardiovasc Pharmacol.* 1994;24:45–49.
9. Fabiani JN, Ponzio O, Emerit I, et al. Cardioprotective effect of Vastarel 20 mg during coronary artery graft surgery. *J Cardiovasc Surg.* 1992;33(4):486–491.
10. Kober G, Buck T, Sievert H, Vallbracht C. Myocardial protection during percutaneous transluminal coronary angioplasty: effects of Vastarel 20 mg. *Eur Heart J.* 1992;13:1109–1115.
11. Di Pasquale P, Lo Verso P, Bucca V, et al. Effects of trimetazidine administration before thrombolysis in patients with anterior myocardial infarction: short-term and long-term results. *Cardiovasc Drug Ther.* 1999;13:423–428.
12. Birand A, Kudaiberdieva GZ, Batyraliev TA, et al. Effects of Vastarel 20 mg on heart rate variability and left ventricular systolic performance in patients with coronary artery disease after percutaneous transluminal angioplasty. *Angiology.* 1997;48:413–422.
13. Steg PG, Grollier G, Gallay P, et al. A randomized double-blind trial of intravenous trimetazidine as adjunctive therapy to primary angioplasty for acute myocardial infarction. *Int J Cardiol.* 2001;77(2–3):263–273.

# The potential role of growth factors and direct myocardial revascularization in patients with refractory myocardial ischemia following coronary artery bypass surgery with a patent internal mammary artery graft

Jonathan Hill<sup>1</sup>, Adam Timmis<sup>2</sup>  
<sup>1</sup>St Bartholomew's Hospital, London, UK  
<sup>1, 2</sup>London Chest Hospital, London, UK

Correspondence: Dr Jonathan Hill, St Bartholomew's Hospital, London, UK.

We report a case of a 52-year-old man presenting with incapacitating angina who is not amenable to further percutaneous or surgical intervention. Obstructive airways disease now increases the operative risk of a limited thoracotomy for laser transmyocardial revascularization (TMR). The patient is on maximal medical management and is desperate for any relief from his angina.

The risk factors for coronary artery disease include hypertension, hypercholesterolemia, and a very strong family history. A recent angiogram shows severe left main stem disease with severe proximal disease of the left anterior descending/diagonal territory. The circumflex system is diffusely diseased and the previous saphenous vein graft to the lateral circumflex branch is now occluded at its origin after a previously unsuccessful attempt at disobliteration. The right coronary territory was previously ungrafted but is now occluded in the proximal third with some minor collateral formation supplying the posterior descending artery territory. The previous vein graft to the diagonal system is also occluded at its origin and again does not appear amenable to percutaneous intervention. On injection of the left internal mammary artery the graft is found to be widely patent with major collateral formation to the diagonal territory and to a lesser extent the circumflex and distal right coronary artery territories. Despite this, the

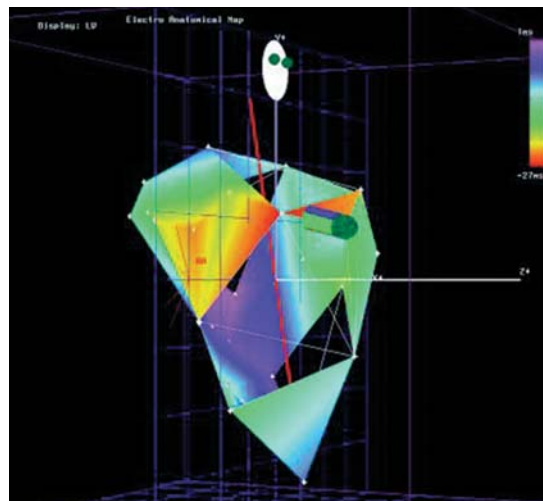


Figure 1. Biosense™ guidance allows accurate placing of DMR channels.

left ventricular function remains good overall with an inferobasal hypokinetic segment.

A thallium perfusion scan revealed a large reversible defect in the inferolateral wall suggesting that the collateral formation fed from the left internal mammary artery graft is insufficient. A dilemma is therefore presented with a relatively young patient in whom further surgery would be difficult because of obstructive airways disease. It would also jeopardize the widely patent internal mammary artery graft. In any case the distal native vessels are poor and probably now impossible to graft or re-graft.

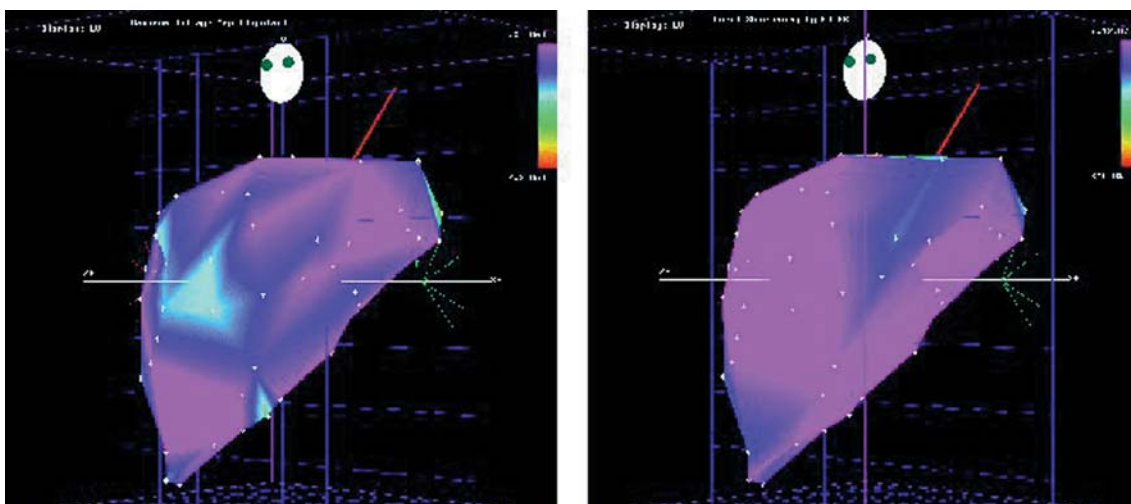


Figure 2. Biosense™ magnetic voltage and shortening map.

## Discussion

There are a number of options available to this patient involving a direct myocardial revascularization (DMR) strategy. Several methods have shown considerable promise in pilot studies involving either the creation of small channels in the myocardium, the administration of a growth factor, or gene transfection. The intention is to promote the formation of new blood vessels, ie, to stimulate angiogenesis. Several of these studies are at an early stage in their evaluation and have only just begun phase-II clinical trials.

### Surgical TMR

A variety of surgical approaches have been used with a combination of different laser types. The holes are created from the epicardial through to the endocardial surface. These channels quickly become occluded<sup>1</sup> with the fibrotic process, causing release of angiogenic cytokines and hence promotion of angiogenesis. There is now widespread use of this method and an accumulating body of evidence. A recent trial from the UK,<sup>2</sup> whilst showing an improvement in two angina classes in 25% of patients, showed there was no significant improvement in exercise time in the laser-treated group compared with those

on maximal medical management. The authors concluded that overall the procedure could not be recommended. One reason for this could be the significant mortality associated with the procedure. Other investigators would contend that the patient group in this study does not compare with that of other trials.

Horvath et al<sup>3</sup> advocate surgical TMR as an efficacious treatment strategy in patients with refractory CCS class 3 or 4 angina. They randomized 192 patients to laser TMR plus maximal medication, or to maximal medical management alone, and found a significant improvement by at least two angina classes in the TMR group (72% vs. 13%,  $P < 0.001$ ). Quality of life parameters also improved. At 1 year there was 15% mortality in the TMR group, with no significant difference from the medically treated arm.

### Catheter-based DMR/percutaneous TMR/PMR

A percutaneous approach to DMR obviates the need for surgery and general anesthesia, thus avoiding the significant perioperative mortality reported in some surgical DMR series.<sup>3</sup> More recently, the development of a novel electromechanical mapping system has removed the need for prolonged fluoroscopic screening.<sup>4</sup> Recently reported randomized clin-

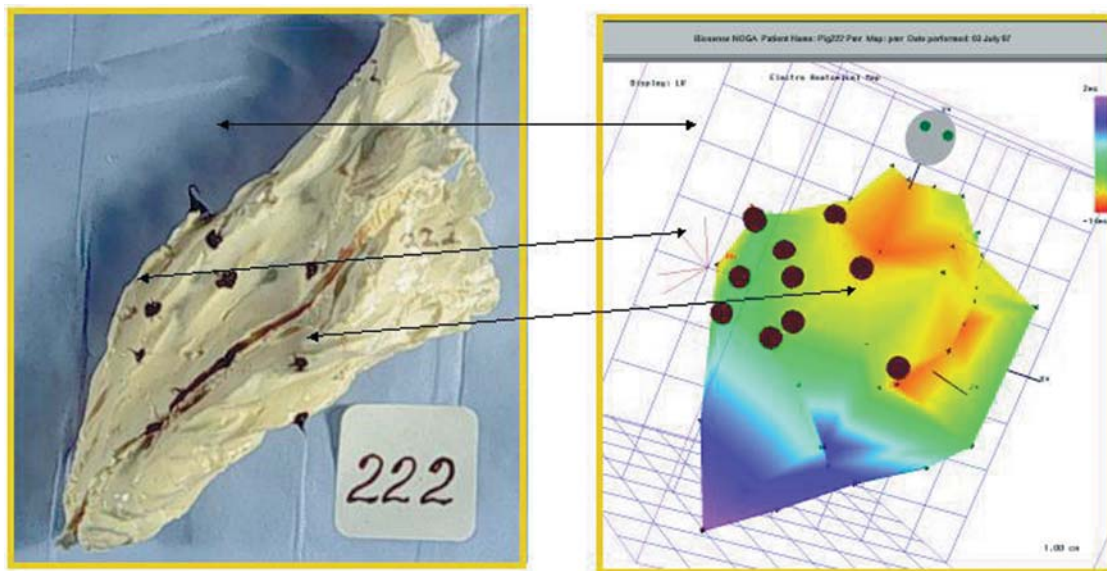


Figure 3. 3D generated pictures of map left ventricle using the Biosense™ system.

ical trials have shown encouraging results. The 6-month results of the Eclipse™ study using a holmium:YAG laser would suggest significant benefit in the laser-treated group. A group of 335 patients not amenable to other forms of intervention were randomized to receive optimal medical treatment alone, or percutaneous DMR plus maximal medication. There was only one death during the procedure and a risk of tamponade of around 3%. At 6 months, the mean exercise time had significantly increased in the DMR group (381 vs. 529 s,  $P = 0.0002$ ). A significant symptomatic benefit was seen at 3 months. In the PACIFIC trial,<sup>6</sup> 70% of DMR-treated patients were CCS class 0, 1, or 2. Forty-six percent of DMR patients had improvement by at least two angina classes whilst only 6% of nonDMR patients had a similar improvement. Neither of these trials were blinded, let alone double-blinded, and it must be borne in mind that the placebo effect in this group of patients can be considerable. The Direct trial<sup>7</sup> using the Biosense™ 3D magnetic guidance system enabled the procedure to be blinded (with mapping alone or mapping plus DMR). In a trial of 77 patients treated with percutaneous DMR using a holmium:YAG laser there was a significant improvement in exercise duration at 1 and 6 months, with 74% of patients experiencing symptomatic improve-

ment for at least 3 months. There was a 2.6% incidence of major in-hospital events although there were no deaths.

### Angiogenic growth factors

Isner's group in Boston<sup>8,9</sup> reported the successful transfection of peripheral arteries with cDNA encoding for vascular endothelial growth factor (VEGF)-165 using a hydrogel-coated balloon. This experiment was proof of concept and stimulated a rapidly growing body of research, although the original experiments have been criticized for lacking a control group.

Schumacher et al<sup>10</sup> subsequently reported the first clinical experience of direct intramyocardial injection of fibroblast growth factor (FGF)-I to stimulate the angiogenic response as an adjunct to conventional bypass surgery. They injected 0.01 mg/kg FGF-I close to the LAD after completion of an internal mammary artery anastomosis. Angiographically detectable vessels were found in the treatment group, but not in controls, with collateral growth around the injection site. Losordo et al<sup>11</sup> injected naked plasmid DNA encoding phVEGF165 directly into ischemic myocardium, and only a transient decrease into

ischemic myocardium of five patients using a minithoracotomy approach. There was a transient decrease in cardiac output initially, but all patients had a significant reduction in ischemia. Perfusion and angiographic data were also positive.

The use of growth factors in this context remains the subject of much scrutiny until the precise mechanism of angiogenesis is elucidated. There are several notes of caution regarding their use. Atherectomy specimens have been shown to demonstrate plaque neovascularization, which has been associated with a higher prevalence of plaque rupture, mural hemorrhage, or unstable angina.<sup>12</sup> Moulton<sup>13</sup> has shown that prolonged treatment with angiogenesis inhibitors reduces plaque growth and intimal neovascularization in apolipoprotein E<sup>-/-</sup> mice. Although the mechanism is not clear, it would suggest that angiogenesis may actually promote atherogenesis

### Combined DMR and growth factor treatment

Using the Biosense™ system it would be possible to precisely target both the laser channels and growth factor injection via a catheter-based route. Clinical studies are underway to test the feasibility and safety of such an approach. Data from animal studies have been encouraging.<sup>13,14</sup>

### Conclusions

The development of new transcatheter technologies coupled with a rapid growth in knowledge of angiogenic mechanisms has offered new hope to patients with refractory myocardial ischemia who are not candidates for further conventional intervention. The encouraging 6-month results from the percutaneous trials of DMR suggest the need for larger blinded, randomized trials. Perhaps the addition of growth factor administration at the same time as the laser procedure would give even greater clinical benefit. ■

### REFERENCES

1. Reference 168-170 from Serruys paper
2. Schofield PM, Sharples LD, Caine N, et al. Transmyocardial laser revascularisation in patients with refractory angina: a randomised controlled trial. *Lancet*. 1999;353:519–524.
3. Horvath KA, Cohn LH, Cooley DA, et al. Transmyocardial laser revascularization: results of a multicenter trial with transmyocardial laser revascularization used as sole therapy for end-stage coronary artery disease. *J Thorac Cardiovasc Surg*. 1997;113(4):645–653; discussion 653–654.
4. Ben-Haim SA, Osadchy D, Schuster I, Gepstein L, Hayam G, Josephson ME. Nonfluoroscopic, in vivo navigation and mapping technology. *Nat Med*. 1996;2(12):1393–1395.
5. Whitlow PL. Percutaneous transmyocardial revascularization versus medical therapy in patients with refractory angina. *American College of Cardiology ACCIS Meeting*, March 1999.
6. Oesterle SN. Initial clinical results of the cardiogenesis PACIFIC trial (Potential Angina Class Improvement From Intramyocardial Channels). *American College of Cardiology ACCIS Meeting*, March 1999.
7. Kornowski R, Leon MB. Biosense guided direct myocardial revascularisation. *Angiogenesis and DMR 2nd Annual Symposium*, June 1999.
8. Isner JM, Walsh K, Symes J, et al. Arterial gene therapy for therapeutic angiogenesis in patients with peripheral artery disease. *Circulation*. 1995;91:2687–2692.
9. Isner JM, Pieczek A, Schainfeld R, et al. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. *Lancet*. 1996; 348(9024): 370–374.
10. Schumacher B, Pecher P, von Specht BU, Stegmann T. Induction of neoangiogenesis in ischaemic myocardium by human growth factors: first clinical results of a new treatment for coronary artery disease. *Circulation*. 1998;97(7):645–650.
11. Losordo DW, Vale PR, Symes JF, et al. Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. *Circulation*. 1998;98(25):2800–2804.
12. Tenaglia AN, Peters KG, Sketch MH Jr, Annex BH. Neovascularization in atherectomy specimens from patients with unstable angina. *Am Heart J*. 1998;135:10–14.
13. Moulton KS. Are plaque angiogenesis and atherosclerosis. *Curr Atheroscler Rep*. 2001;3(3):225–3.
14. Fuchs, et al. *JACC*, Abstract 813-5, March 1999.