Limitations to the Assessment of Reperfusion Injury With Radiolabeled 2-Deoxyglucose

To the Editor:

Matsumura et al. propose a new method to determine reperfusion injury in dog heart. The phosphorylation rate constant, \( k_s \), for two 2-deoxyglucose moieties (\( ^{14}C \)-2-DG and \( ^{18}F \)-2-deoxy-2-fluro-D-glucose [FDG]) was compared with histological assessment of ischemic damage. The results suggest that a large portion of the infarcted myocardium loses viability during the first hours of reperfusion. If true, this would be the first demonstration of a bimodal time course of reperfusion injury in vivo.

Two factors may have affected the interpretation of the results. First, the assumption is made that the total \( ^{14}C \) radioactivity in each sample is proportional to the phosphorylation rate. However, the contribution of unphosphorylated deoxyglucose, although declining, is never negligible, and in some circumstances, it can represent the majority of the tissue radioactivity. Correction for this is the main supposition of the deoxyglucose method. Neglecting this correction is particularly problematic because the relationship between phosphorylated and unphosphorylated deoxyglucose concentrations is almost certain to be regionally variable.

Second, and more important, is the cancellation of the lumped constant (LC) from the calculations for the 2 tissue regions. We have shown that the LC is subject to considerable variability depending on the experimental conditions. It has been postulated that the LC is high when transport is rate limiting and low when phosphorylation is rate limiting. After 90 minutes of total ischemia, the available glucose in the ischemic area is likely to be very low. Any uptake of glucose at the onset of reperfusion is likely to be limited by transport because intracellular glucose is low and hexokinase is not saturated. Thus, the LC would be high. It is conceivable that during reperfusion, transport begins to exceed phosphorylation because transport capacity is high and glycolysis becomes inhibited by the resumption of fatty acid oxidation. Now hexokinase will become rate limiting, resulting in a low value for the LC. If one applies this scenario to the results in the triphenyltetrazolium chloride (TTC)-negative regions, the initially high \( k_s \) (5 minutes), which suggests viability, may be the result of an overestimation of glucose uptake (LC high), whereas the low \( k_s \) after 3 hours may be due to an underestimation of glucose uptake (low LC) and may not be related to reperfusion injury at all.

In conclusion, we agree that a method for the detection of reperfusion injury in vivo has long been elusive. In our view, the goal remains elusive.

response

Dr. Taegtmeyer and colleagues raise issues about sequential measurements of 2-deoxyglucose uptake in reperfused myocardium. Their first concern is possible contamination by unphosphorylated tracer. The proportion of phosphorylated 2-deoxyglucose reaches steady state after 60 minutes, a time course consistent with our experimental design. If a lot of unphosphorylated tracer contributed to measured tissue activity, then a decline in activity should be observed in sequential measurements, owing to loss of unphosphorylated tracer from myocytes. We observed no significant tracer loss from normal or reperfused myocardium over 4 hours.

Another concern is use of the same lumped constant for calculation of \( k_t \) during early and late reperfusion. Some in vitro data show that addition of insulin or a change in fatty acid substrate availability results in a discrepancy between measured rates of glucose and 2-deoxyglucose uptake, but the same data show that rates of glucose and 2-deoxyglucose uptake are similar during steady-state conditions and are unaffected by changes in workload. Other data show that the lumped constant does not change in reperfused myocardium and is independent of blood flow and workload. Our \( k_t \) values are comparable to previous studies in reperfused canine myocardium.

Could changes in substrate utilization alter hexokinase activity in reperfused myocardium? First, there was unlikely to be a significant change in substrate availability during our experiments. Second, the myocardium prefers fatty acids as a substrate for oxidation, even after only 20 minutes of reperfusion. It is suggested that we may have overestimated \( k_t \) and thus viability, during early reperfusion, but we also compared \( k_t \) during early reperfusion with electron microscopy findings. The threshold \( k_t \) had a predictive accuracy of 88% for detection of viability.

It might be argued that some change in lumped constant might occur and the \( k_t \) threshold for viability might differ between early and late reperfusion. In samples that are necrotic (noref) and those that are salvaged (TTC positive), there is minimal discrepancy between the samples judged viable or necrotic.
It Takes Time to Heal a Broken Heart

To the Editor:

The excellent article “Coronary Angioscopic Findings in the Infarct-Related Vessel Within 1 Month of Acute Myocardial Infarction: Natural History and the Effect of Thrombolysis” by Eric Van Belle and coworkers 1 in the January 6/13, 1998, issue of Circulation takes on special significance today. Although managed care (MC) stands at the forefront of decision making, MC must not be allowed to cancel the significance of the biology of healing. Their article demonstrates that the vascular lesion responsible for myocardial infarction requires a longer time for healing than has been appreciated by most modern cardiologists. Although previous studies had already demonstrated that acute infarction of the cardiac muscle requires weeks, not days, to heal, 2 modern textbooks of clinical cardiology provide little emphasis for that essential process. Nuclear changes begin in infarcting muscle within hours and continue for at least 10 days. Necrosis and phagocytosis of muscle fibers persist for 2–3 weeks. Neutrophils and basophils continue to proliferate for 2 to 3 weeks, at which time macrophages come in and proliferate for >6 weeks.

Now Van Belle and colleagues have demonstrated angioscopically that “healing of the infarct-related [arterial] lesion requires more than 1 month and that an ‘unstable’ yellow plaque with adherent thrombus is common during that period . . . [and] may partly explain the unique behavior of recent infarct-related lesions, which are more prone to occlude than other lesions.”

These new angioscopic findings reinforce the need for essential attention to the time required for healing and provide explanation for the substantial number of deaths that occur after the first 2 days after coronary occlusion. 3,4 Early discharge conceals a substantial number of patients with infarct who are destined to die after intrahospital study (41% to 60%). Early discharge deprives them of the potential life-saving benefits of early detection of dysrhythmia and timely treatment by control of pump failure (including intra-aortic balloon pumping, management of recurrent infarction, and cardioversion with cardiopulmonary resuscitation); such lives could have been salvaged if the patient had been under adequate surveillance with life-saving equipment, whether in hospital or otherwise. Family survivors are often hard pressed to accept these later deaths, and managing physicians may be at a loss to explain their occurrence after the elaborate treatments provided in the hospital period. This important angioscopic study can help!

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Response

We welcome the opportunity to respond to the letter from Drs Wehrmacher and Lewis regarding our article in which we showed that the majority of infarct-related lesions had angiographic evidence of instability, a complex surface with thrombus, when studied during the first month after myocardial infarction. 1

We agree with them that “the vascular lesion responsible for myocardial infarction requires a longer time for healing that has been appreciated by most modern cardiologists.” This may explain the tendency of infarct-related arteries to reocclude and the high frequency of adverse thrombotic events observed late (>10 days) after acute myocardial infarction (MI). As they suggest, part of the solution may be to monitor patients for a longer period of time in order to treat them appropriately when such an adverse event occurs. However, from a practical standpoint, it is not possible to keep these patients in the hospital for such an adverse event occurs. However, from a practical standpoint, it is not possible to keep these patients in the hospital for the time required for the culprit lesion to heal (>1 month).

Ultimately, the ideal solution would be to prevent the occurrence of thrombotic events. Our observation of persistent “plaque instability” for weeks after acute MI provides a new potential target to achieve this goal. This could be accomplished either by (1) passivation of the plaque during the time period required for healing or (2) acceleration of the healing process itself. Plaque passivation could be achieved by drugs designed to eliminate thrombus from the unstable plaque. Although the results of the
EPIC trial demonstrated proof of the concept of plaque passivation using glycoprotein IIb/IIIa antagonists, it is likely that such a short duration of intravenous treatment will be insufficient to prevent late thrombus formation and related events after MI. The development of oral forms of glycoprotein IIb/IIIa antagonists will open new perspectives, and it will be important to test these drugs in this setting. Acceleration of the healing process could be achieved by mechanical means. Indeed, implantation of a stent, by virtue of its scaffolding properties, may mechanically seal the ulcerated segment and exclude the thrombus from the lumen, leading to "mechanical healing." The recent demonstration of a reduction in late vessel occlusion of the infarct-related lesion by stent implantation tends to validate this theory and supports the use of stents in this situation. Importantly, the results of the EPISTENT trial, by showing a benefit of abximab used in conjunction with stent implantation, lead us to expect that the combination of the pharmacological and mechanical approaches will provide additive effects.

Overall, our observation of persistent plaque instability for weeks after the occurrence of MI should encourage prospective studies to evaluate strategies designed to target the "unstable plaque" to prevent late thrombotic events. Whether a therapeutic strategy leading to a decrease of late vessel occlusion after MI improves late cardiac survival will also require investigation.

**Effects of Acute L-Arginine Administration in Coronary Atherosclerosis**

**To the Editor:**

Lerman et al in their interesting study reported that supplementation with L-arginine (the precursor of nitric oxide synthesis) for 6 months in patients without significant coronary artery stenosis reversed coronary small-vessel endothelial dysfunction and improved symptoms. Furthermore, it decreased plasma endothelin concentrations in patients. However, they did not examine the effects of L-arginine on epicardial coronary arteries and stenoses.

We have examined by quantitative angiography the vasomotor effects of intracoronary infusion of normal saline (2 μL/min) for 2 minutes followed by 50 and 150 μmol/min L-arginine and bolus administration of 250 μg of nitroglycerin on 38 nonstenotic epicardial coronary artery segments and 22 stenoses in patients with coronary artery disease and a positive treadmill exercise test result (≥0.1 mV ST-segment depression at between 5 and 7 metabolic equivalents [METS] using the modified Bruce protocol).

The diameter of the stenosis after saline, 50 μmol/min L-arginine, 150 μmol/min L-arginine, and nitroglycerin was 1.52±0.10, 1.60±0.1 (P<0.01 versus saline), 1.64±0.14 (P<0.01 versus saline), and 1.76±0.12 mm (P<0.01 versus saline, P<0.05 versus L-arginine), respectively. The diameter of nonstenotic segments was 2.93±0.10, 3.02±0.11 (P<0.01 versus saline), 3.14±0.12 (P<0.01 versus saline), and 3.18±0.10 mm (P<0.01 versus saline), respectively. The percentage change from baseline was 0.9±0.4%, 6.7±1.6%, 10.9±2.0%, 16.9±2.1% in stenosis diameter and 0.7±0.2%, 4.2±1.0%, 7.8±0.9%, and 9.9±1.5% in nonstenotic segment diameter for saline, 50 and 150 μmol/min L-arginine, and nitroglycerin, respectively. The diameter change after 150 μmol/min L-arginine was ~84% of the diameter change after nitroglycerin for nonstenotic segments and 50% for stenoses.

The results of our study are consistent with stimulation of nitric oxide synthase activity by L-arginine administration. Our study provides further evidence of preserved nitric oxide synthase activity at the site of coronary stenoses. Furthermore, because this effect was also found in nonstenotic segments of diseased coronary arteries, it is either a physiological response or a pathological response requiring only minimal coronary disease. An alternative explanation for the stimulation of nitric oxide synthase would be a physiological action on vascular smooth muscle that augments its responsiveness to nitric oxide or other endogenous vasodilator mechanisms.

The substrate-deficiency hypothesis is supported by experimental evidence in hypercholesterolemic rabbits that arginine administration restores cholinergic (nitric oxide dependent) relaxation of thoracic aorta and also by clinical studies that show correction of endothelial dysfunction by L-arginine in the coronary microcirculation of hypercholesterolemic patients. Our findings further support and extend the interesting observations of Lerman et al and suggest that L-arginine supplementation also has a beneficial effect on diseased epicardial coronary arteries.

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Response

We read with interest the response of Tentolouris et al reporting their experience with administration of intracoronary L-arginine in humans. Their group demonstrated that intracoronary administration of L-arginine resulted in a significant increase in coronary artery diameter in stenotic as well as nonstenotic segments. Their findings are consistent with the emerging data and concept that L-arginine may serve as a therapeutic tool to improve coronary as well as peripheral vascular reactivity.

The use of L-arginine as a tool to examine the nitric oxide pathway grew into a potential role for L-arginine as a therapeutic tool in cardiovascular disease. However, the mechanism by which L-arginine improved vascular reactivity may vary between acute and chronic administration of L-arginine. The acute administration of L-arginine may exert its mechanism mainly through the nitric oxide pathway by increasing substrate bioavailability. However, there is a continuous body of evidence to suggest that additionally, chronic administration of L-arginine may improve vascular reactivity. These observations were initially termed the arginine paradox because the $K_m$ of nitric oxide synthase for L-arginine is lower than the intracellular concentration. New data are currently presented to explain this paradox. One of the main mechanisms by which L-arginine may improve endothelial function may be related to its role in antagonizing the competitive inhibitor of nitric oxide synthase, asymmetric dimethylarginine (ADMA). Indeed, 2 recent studies in humans demonstrated that the administration of L-arginine may exert a beneficial effect by changing the L-arginine/ADMA ratio.1,2

Currently, we have data that L-arginine acutely or chronically improves endothelial function in patients with hypercholesterolemia, coronary endothelial dysfunction,3 heart failure, and intermittent claudication. Thus, new prospective studies should address the role of L-arginine therapy in the regression of atherosclerosis in humans.

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Limitations to the Assessment of Reperfusion Injury With Radiolabeled 2-Deoxyglucose
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