Oxidative stress after reperfusion with primary coronary angioplasty: Lack of effect of glucose-insulin-potassium infusion

Guillermo Díaz-Araya, PhD; Danniels Nettle, BSc; Pablo Castro, MD; Fernando Miranda, MD; Douglas Greig, MD; Ximena Campos, BSc; Mario Chiong, BSc; Carolina Nazzal, RN; Ramón Corbalán, MD; Sergio Lavandero, PhD

Objective: **To evaluate the oxidative stress status and the modification with glucose-insulin-potassium (GIK) therapy in patients with acute myocardial infarction undergoing primary percutaneous transluminal coronary angioplasty.**

Design: **Prospective, randomized, double-blinded, placebo-controlled study.**

Setting: **Cardiac intensive care unit at the university hospital.**

Patients: **Twenty patients were randomized to GIK solution (30% glucose in water with insulin 50 IU/L, and KCl 40 mM) vs. placebo (normal saline) at 1.5 mL/kg/hr for 24 hrs. The control group was 15 healthy volunteers with no heart disease.**

Interventions: **Eligible patients were randomized by a blinded pharmacist, patients with acute myocardial infarction were treated by primary percutaneous transluminal coronary angioplasty and randomized to GIK or placebo (saline solution). Primary angioplasty was successful in nine of ten patients (90%) and ten of ten patients (100%) in the GIK and placebo groups, respectively. Nine (100%) and six (60%) patients from GIK and placebo groups, respectively, underwent stent implantation.**

Measurements and Main Results: **We determined plasma levels of lipid peroxidation estimated by the malondialdehyde assay, superoxide dismutase, glutathione peroxidase, and catalase erythrocyte activities at admission and 0.5 and 24 hrs after angioplasty. Baseline determinations were compared with a control group (n = 15). Baseline clinical characteristics and time to treatment (4.5 ± 3.5 hrs) were similar between groups. Angioplasty success rate (Thrombolysis in Myocardial Infarction [TIMI] 3 flow with residual stenosis ≤30%) was 90% and 100% in GIK and placebo groups, respectively. Patients with acute myocardial infarction had an increase of malondialdehyde at baseline (2.9 ± 1.7 vs. 1.1 ± 0.3 μM, $p < .01$) and lower enzymatic activities of superoxide dismutase (0.5 ± 0.5 vs. 1.3 ± 0.4 U/mg hemoglobin, $p < .01$) and catalase (147 ± 73 vs. 198 ± 31 U/g hemoglobin, $p < .01$). These measurements did not change significantly after angioplasty and no differences were observed between GIK and placebo groups.**

Conclusion: **Patients with acute myocardial infarction had increased levels of oxidative stress associated with a reduction in enzymatic antioxidant reserve. Administration of GIK solution did not improve these abnormalities among patients undergoing primary angioplasty.**

Key Words: insulin; glucose; oxidative stress; free radicals; myocardial infarction; angioplasty

Ischemia-reperfusion injury may occur as damage to the myocardium following blood restoration after a critical period of coronary occlusion (1). Several studies have proposed an essential role for reactive oxygen species (ROS), such as superoxide anions, H_2O_2, hydroxyl, and peroxyl radicals, in the pathogenesis of myocardial ischemia-reperfusion injury (2, 3). Ischemia-reperfusion injury is a clinical condition associated with thrombolysis, angioplasty, and coronary bypass surgery, all commonly used to reestablish blood flow and minimize damage to the heart resulting from severe myocardial ischemia (4, 5).

Several trials have used glucose-insulin-potassium (GIK) therapy for treatment of acute myocardial infarction (AMI). This solution decreased mortality in humans (6, 7) and infarct size in rats when administered from the onset of reperfusion after an ischemic insult (8). The mechanisms whereby GIK mediates cardioprotection are still unknown. The pilot Latin America Cardiology Studies (ECLA) group study (6) with GIK resulted in a 66% relative decrease in mortality after AMI. This work is consonant with both the meta-analysis (28% to 48% relative mortality decrease) (9) and the Diabetes-Insulin-Glucose in Acute Myocardial Infarction (DIGAMI) trial that reported a decreased relative mortality risk of 29% to 58% in diabetic patients with AMI who were treated with glucose and insulin (7). Despite this positive trial information and an extensive experimental background, the present data are neither firm nor extensive enough to support routine use of GIK in patients with AMI. Trials based on the concepts of metabolic therapy are being organized. In addition, there are studies investigating collateral therapies that can protect the myocardium during ischemia and can reduce the injury post reperfusion. One of these studies investigates the reduction of cardiac oxidative stress by using antioxidant substances during the reperfu-

From the Departments of Pharmacological and Toxicological Chemistry (GD-A) and Biochemistry and Molecular Biology (DN, XC, MC, SL), Faculty of Pharmaceutical and Chemical Sciences, University of Chile, Santiago, Chile; and the Department of Cardiovascular Diseases (PC, FM, DG, CN, RC), Clinical Hospital, P. University Catholic of Chile, Santiago, Chile.

Supported, in part, by grants FONDECYT 1990491, 1010992 (PC), and PT2000-01, Faculty of Chemical and Pharmaceutical Sciences, University of Chile (GD-A).

GD-A and DN contributed equally to this work.

Address requests for reprints to: Guillermo Díaz-Araya, PhD, Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Olivos 1007, Santiago, Chile. E-mail: gadiaz@ciq.uchile.cl

sion (3, 10). Because of continuing uncertainty about the potential role of this therapeutic intervention, we carried out a prospective, double-blind, placebo-controlled trial to evaluate the impact of GIK infusion during the first hours of AMI on cardiac oxidative stress status. Patients with AMI were treated by primary percutaneous transluminal coronary angioplasty (PTCA) and randomized to GIK or placebo (saline solution). Cardiac oxidative stress was monitored by measurement of changes in the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) and on the malondialdehyde (MDA) plasma concentrations.

MATERIALS AND METHODS

Patients and Controls. The criteria for inclusion were presentation within 6 hrs of the onset of chest pain (consistent with ischemia, lasting at least 20 mins, and not relieved by sublingual nitroglycerin) with ST segment elevation >0.1 mV in two or more contiguous electrocardiographic leads, and a clinical decision to treat with primary angioplasty. Criteria for exclusion were prior infarction, cardiogenic shock, Killip class III, known hyperkalemia or glycemia >300 mg/dL, and patency of the infarct related artery (Thrombolysis in Myocardial Infarction [TIMI] 3 flow) as revealed by coronary angiography. Eligible patients were randomized by a blinded pharmacist. All the patients gave informed consent for participation and the study was approved by our institutional review board. Active treatment and control (saline solution) were allocated in a 1:1 ratio. Angioplasty was considered to be successful when grade 3 TIMI flow was obtained with a residual stenosis <30%. All patients were given aspirin, and those who had stent implantation received ticlopidine or clopidogrel. Intravenous heparin in a bolus of 10,000 IU was given before angioplasty, with subsequent dosing as needed to maintain an activated coagulation time of about 300 secs. Oxidative stress parameters of patients with AMI were measured at admission and 0.5 and 24 hrs after angioplasty. These measurements were compared with a group of 15 healthy volunteers matched by age and sex. They were asymptomatic and had an unremarkable medical history and a normal physical examination. Control subjects were excluded if they had any known coronary risk factor; if they were taking any medications, vitamin supplements, or antioxidants; or if they drank alcohol on a regular basis.

Treatment. GIK consisted of 30% glucose, 50 IU/L insulin, and 40 mM KCl at an infusion rate of 1.5 mL/kg/hr over 24 hrs. The placebo group received normal saline solution at the same infusion rate. The infusion was initiated immediately after randomization and before angiography.

Determination of MDA Concentrations and Antioxidant Enzyme Activities

Twenty milliliters of blood were obtained by venipuncture at baseline and during the reperfusion at 0.5 and 24 hrs. Each sample was centrifuged at 1250 *g* for 10 mins at 4°C. Plasmas were separated and stored at −20°C; erythrocytes were washed three times with saline solution, homogenized, and centrifuged in the same manner. Lysates, prepared by adding 0.1 mL cell pellet to 0.4 mL of water, were stored at −20°C.

MDA Assay. Lipid peroxide formation was determined by the presence of thiobarbituric acid reactive substances as described earlier (11). Commercially available MDA was used as standard. To 1.5-mL microcentrifuge tubes, the following solutions were added: 0.375 mL 0.15 M phosphoric acid, 0.125 mL 42 mM thiobarbituric acid, and 0.025 mL serum MDA standards or 0.1 M HCl (blank). The mixtures were vortex-mixed and placed in boiling water for 30 mins. Absorbances at 532 nm were measured and concentrations were expressed as micromolar.

Determination of SOD Activity. SOD was extracted from the hemolyzed blood according to McCord et al (12). The enzyme activity was assayed as described by Misra et al (13). This method was based on the fact that the rate of alkaline oxidation of epinephrine is decreased by SOD activity. Oxidation yields a chromophore that was detected at 480 nm. SOD activity was expressed as units per milligram hemoglobin (Hb).

CAT Activity. Enzymatic activity was determined as described by Beers et al (14). Briefly, 1 mL of diluted hemolyzed blood was added to a cuvette. The reaction was initiated by addition of 1 mL of 30 mM H_2O_2 and the change in absorbance at 240 nm was monitored at 25°C for 1 min. A portion of the remaining sample was used for hemoglobin protein determination. Specific activity was expressed in terms of the disappearance of micromoles of H_2O_2 per minute per gram Hb.

GSH-Px Activity. Enzymatic activity was determined as described by Paglia et al (15). The lysate was mixed with an equal volume of double-strength Drabkin's reagent. In a reaction tube, 0.100 mL of this mixture was added to 2.58 mL 50 mM phosphate buffer (pH 7.0) containing 5 mM ethylenediaminetetraacetic acid. The following reagents were then added in turn: 0.100 mL of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH, 8.4 mM), 0.010 mL glutathione reductase (30 U/mL), 0.010 mL sodium azide (1.125 M), and 0.100 mL reduced glutathione (GSH) (150 mM). The enzymatic reaction was initiated by addition of 0.100 mL of prewarmed H_2O_2 (2.2 mM). NADPH conversion to nicotinamide adenine dinucleotide phosphate (NADP) was followed by continuous recording of the change in absorbance at 340 nm, between 2 and 4 mins after initiation of the reaction. The nonenzymatic oxidation of GSH was determined by simultaneous assay of a system identical to the first, except for replacement of the hemolysate by an equal volume of water. The reaction rate of the latter system was subtracted from that of the former to determine the true enzymatic activity. Enzymatic activity is expressed as nanomoles of NADPH oxidized per minute per gram Hb.

Statistical Analysis. Results are presented as means ± SD. We utilized analysis of variance for repeated measurements for the comparison of the oxidative stress parameters on admission and 0.5 and 24 hrs after angioplasty. GIK and control group oxidative parameters were compared using Student's *t*-test. A nonpaired Student's *t*-test was used for the comparison between AMI patients (GIK or placebo) and control (healthy volunteers). A p value $<$ .05 was considered significant.

RESULTS

Clinical Parameters of Patients. The clinical characteristics of the 20 patients recruited into the study are shown in Table 1. Baseline characteristics were similar between GIK and placebo groups. They were predominantly male and mean age was 54 yrs. Seven patients had an anterior and 13 a nonanterior infarction. Ninety-five percent of patients had a single vessel disease and one patient had two vessel disease (5%). The mean time to achieving a satisfactory coronary artery patency was 2.7 ± 1.6 hrs from the onset of symptoms. Primary angioplasty was successful in nine of ten patients (90%) and ten of ten patients (100%), in the GIK and placebo groups, respectively. Nine (100%) and six (60%) patients from GIK and placebo groups, respectively, underwent stent implantation. Additional medical therapy consisted of β-blockers (90% of patients) and angiotensin converting enzyme inhibitors (50% of patients).

Plasma MDA Levels. MDA, a byproduct of the attack of ROS on unsaturated fatty acids in cell membranes, has been used as marker for lipoperoxidation. The production of MDA is initiated promptly after the generation of ROS. Figure 1 depicts this oxidative stress parameter in patients with AMI compared with controls. A significant elevation of plasma MDA levels in AMI patients, when compared with noninfarcted individuals (controls), was observed (2.9 ± 1.7 vs. 1.1 ± 0.3 μM, $p < .01$). In addition, PTCA did

Table 1. Clinical characteristics in glucose-insulin-potassium (GIK) and placebo group patients

Characteristic	GIK (n = 10)	Placebo (n = 10)	*p* Value
Age, yrs ± SD	54 ± 12	55 ± 11	NS
Male sex, n (%)	8 (80)	10 (100)	NS
Risk factors for coronary artery disease			
Hypertension, n (%)	8 (80)	7 (70)	NS
Diabetes mellitus, n (%)	0 (0)	1 (10)	NS
Hypercholesterolemia, n (%)	4 (40)	1 (10)	NS
Current smoking, n (%)	4 (40)	8 (80)	NS
Infarct location			
Anterior, n (%)	5 (50)	2 (20)	NS
Nonanterior, n (%)	5 (50)	8 (80)	NS
Heart rate			
Systolic blood pressure (mm Hg)	133 ± 18	140 ± 30	NS
Diastolic blood pressure (mm Hg)	80 ± 13	82 ± 11	NS
Killip I class, n (%)	9 (90)	7 (70)	NS
Killip II class, n (%)	1 (10)	3 (30)	NS
Baseline LVEF (%)	39 ± 12	44 ± 13	NS
Myocardial perfusion defect (% of LV)	38 ± 18	35 ± 13	NS
Coronary artery disease extension			
Single-vessel disease, n (%)	10 (100)	9 (90)	NS
Two-vessel disease, n (%)	0 (0)	1 (10)	NS
Angioplasty results			
Symptom onset to balloon time, hrs ± SD	1.9 ± 1.1	3.5 ± 2.3	NS
Stent implantation, n (%)	9 (90)	6 (60)	NS
Postangioplasty TIMI-3 flow, n (%)	9 (90)	10 (100)	NS

NS, not significant; LVEF, left ventricular ejection fraction; LV, left ventricle; TIMI, Thrombolysis in Myocardial Infarction.

not modify the plasma MDA levels after 0.5 and 24 hrs of reperfusion with saline. Infusion of GIK during the reperfusion did not decrease increased circulating MDA levels after PTCA was initiated.

Antioxidant Enzyme Activities. Figures 2 and 3 show erythrocyte SOD and CAT activities, respectively, in patients with AMI compared with controls. Erythrocyte SOD activities in the AMI group (0.5 ± 0.5 U/mg Hb) were lower than in the control group (1.3 ± 0.4 U/mg Hb, $p < .01$). Erythrocyte CAT activities in the AMI group (147 ± 73 U/mg Hb) were decreased significantly compared with the control group (198 ± 31 U/mg Hb, $p < .01$). As depicted in Figure 4, erythrocyte GSH-Px activities were not changed in patients with AMI (0.41 ± 0.17 nmol NADPH/min/g Hb) with respect to control subjects (0.33 ± 0.05 nmol NADPH/min/g Hb, not significant).

Antioxidant SOD, CAT, and GSH-Px activities at 0.5 and 24 hrs after primary PTCA were not different from those baseline levels. GIK infusion during reperfusion did not prevent the decrease in erythrocyte SOD and CAT.

DISCUSSION

Our main finding was that the GIK infusion did not ameliorate the oxidative stress status after reperfusion with primary PTCA. Increased oxidative stress status in these patients was characterized by higher MDA levels and by a decrease in erythrocyte CAT and SOD antioxidant enzyme activities. PTCA itself did not change MDA plasma levels and the activities of antioxidant enzymes.

Blood can reflect the lability of the whole body to oxidative conditions. In experiments in which oxidative stress was evaluated, erythrocytes are excellent material because of their easy availability, their simple structure, and relatively large amounts of polyunsaturated fatty acids in their membranes. The lability of erythrocyte membranes to oxidative stress *in vitro* may reflect the lability of other cell membranes to oxidative damage *in vivo* (16).

GIK therapy has a long and controversial history since the first report by Sodi-Pallares et al. (17), and clinical trials have yielded controversial results. However, the recent meta-analysis has greatly clarified the historical record (reviewed in (9)) where a significant reduction in mortality was observed. Interestingly, this beneficial effect was greater in the subset of patients treated with reperfusion therapies (ECLA pilot study).

Major metabolic changes occur during the early hours of AMI. These include increased secretion of catecholamines,

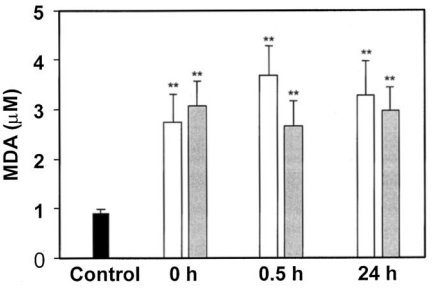

Figure 1. Effect of glucose-insulin-potassium (GIK) perfusion on plasma malondialdehyde (*MDA*) levels. Acute myocardial infarction patients were reperfused with saline (n = 10, *white bars*) or GIK solution (n = 9, *gray bars*). Infusions were started immediately before angiography and maintained until 24 hrs after angioplasty. The control group (n = 15, *black bar*) was healthy volunteers with no heart disease. $^{**}p < .01$ vs. control. No significant differences were found between GIK and placebo groups.

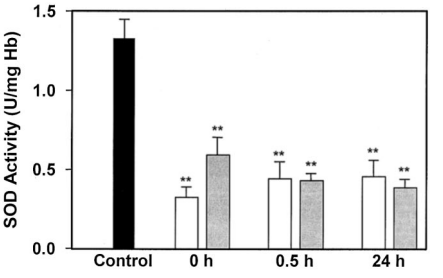

Figure 2. Effect of glucose-insulin-potassium (GIK) perfusion on superoxide dismutase (*SOD*) erythrocyte activity. Acute myocardial infarction patients were reperfused with saline (n = 10, *white bars*) or GIK solution (n = 9, *gray bars*). Infusions were started immediately before angiography and maintained until 24 hrs after angioplasty. The control group (n = 15, *black bar*) was healthy volunteers with no heart disease. $^{**}p < .01$ vs. control. No significant differences were found between GIK and placebo groups.

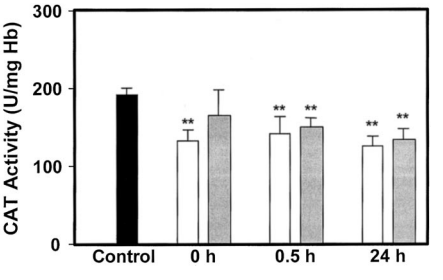

Figure 3. Effect of glucose-insulin-potassium (GIK) perfusion on erythrocyte catalase (*CAT*) activity. Acute myocardial infarction patients were reperfused with saline (n = 10, *white bars*) or GIK solution (n = 9, *gray bars*). Infusions were started immediately before angiography and maintained until 24 hrs after angioplasty. The control group (n = 15, *black bar*) was healthy volunteers with no heart disease. $^{**}p < .01$ vs. control. No significant differences were found between GIK and placebo groups.

Patients with acute myocardial infarction had increased levels of oxidative stress associated with a reduction in enzymatic antioxidant reserve.

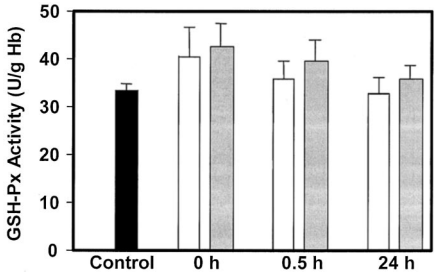

Figure 4. Effect of glucose-insulin-potassium (GIK) perfusion on erythrocyte glutathione peroxidase (*GSH-Px*) activity. Acute myocardial infarction patients were reperfused with saline (n = 10, *white bars*) or GIK solution (n = 10, *gray bars*). Infusions were started immediately before angiography and maintained until 24 hrs after angioplasty. The control group (n = 15, *black bar*) was healthy volunteers with no heart disease. No significant differences were found among control and GIK and placebo groups.

circulating free fatty acid (FFA), and glucose intolerance (18–20). Each one of these abnormalities might adversely influence the outcome of AMI, either by provoking arrhythmias or by compromising the survival of ischemic tissue. Metabolic mechanisms are probably responsible for the beneficial effects of GIK in AMI. GIK decreased both circulating levels of FFAs and myocardial FFA uptake (21). Our study clarifies for the first time that GIK does not ameliorate oxidative stress, and other unexplored mechanisms need to be investigated. In this sense, Jonassen et al. (22) showed that insulin exerts a cardioprotective effects in cardiac myocytes by an anti-apoptotic mechanism. In addition, cardiac apoptosis was abolished by preperfusing the heart with an antioxidant (23).

PTCA, GIK, and Lipid Peroxidation. Reperfusion therapy of AMI with primary PTCA has been an effective, safe, and fast method to achieve recanalization of the culprit coronary artery. This procedure is similar to the ischemia-reperfusion model in which increased levels of ROS have also been detected in myocardium tissue (24–26). Several studies have described increased production of ROS after PTCA (10, 27–29). The detection of intermediates or end products of peroxidized membrane phospholipids has been widely used to evaluate tissue damage in the reperfused myocardium. MDA determination has been used in most of these studies, probably owing to its ease of application. Our results agreed with other studies, which found high plasma MDA levels after PTCA (27, 28). In contrast, Grech et al. (30), Lafont et al. (31), and Oostenbrug et al. (32) did not find a significant change in plasma MDA levels after PTCA. Our study showed that MDA levels did not change after the PTCA procedure was initiated regardless of whether the patients were reperfused with saline or GIK solutions. Blann et al. (28) found increased plasma MDA levels after PTCA, however, the increase was moderate and the overlap between the reperfused and nonreperfused patients was considerable. The difference between all these studies could be the result of the sample times. Our recollection time of samples was 0.5 and 24 hrs after PTCA and this difference could be important for determining the lipoperoxidation damage.

PTCA, GIK, and Antioxidant Enzymes. Some of the toxic effects by ROS generated in the reperfusion may be limited by the action of antioxidant enzymes such as SOD, CAT, and GSH-Px. There is growing evidence that the extent of myocardial injury after coronary occlusion may not be related exclusively to the initial ischemia but also may result from reperfusion injury caused by oxygen free radicals (25, 26). Lower levels of free radical scavengers may be a risk factor for reperfusion injury after intervention for AMI. We found that SOD and CAT activities were reduced in patients with AMI (Figs. 2 and 3), however, GSH-Px activity was not modified (Fig. 4). These results were similar to the study of Rajakumar et al. (10), who found decreased activities of SOD and CAT. Blann et al. (28) also found a reduction in GSH-Px activity after reperfusion with PTCA. This may be related to the reduced ability of GSH-Px to scavenge ROS. In erythrocytes, the GSH-Px is generally uninfluenced by reperfusion because of the stable metabolism of the erythrocyte, but in that study they did not measure SOD and CAT activities. We found that these two enzymatic activities were not changed after primary PTCA or with the use of GIK therapy.

Because the limitation of this study was the small number of patients, we are aware that further large studies are necessary to definitively validate that GIK infusion does not ameliorate the oxidative stress status. Despite this limitation, the consistency of our findings is provocative and potentially clinically relevant.

CONCLUSIONS

Patients with AMI had increased levels of oxidative stress associated with a reduction in enzymatic antioxidant reserve. Administration of GIK solution did not improve these abnormalities among patients undergoing primary percutaneous transluminal coronary angioplasty.

ACKNOWLEDGMENT

We thank Dr. Luis Villarroel for helpful advice in the statistical analysis.

REFERENCES

1. Goldhaber JI, Weiss JN: Oxygen free radicals and cardiac reperfusion abnormalities. *Hypertension* 1992; 20:118–127
2. Griendling KK, Alexander RW: Oxidative stress and cardiovascular disease. *Circulation* 1997; 96:3264–3265
3. Jeroudi MO, Hartley CJ, Bolli R: Myocardial reperfusion injury: Role of oxygen radicals and potential therapy with antioxidants. *Am J Cardiol* 1994; 73:2B–7B
4. Hess ML, Barnhart GR, Crute S, et al: Mechanical and biochemical effects of transient myocardial ischemia. *J Surg Res* 1979; 26: 175–184
5. Bolli R, Marban E: Molecular and cellular mechanism in myocardial stunning. *Physiol Rev* 1999; 79:609–634
6. Díaz R, Paolasso EA, Piegas LS, et al: Metabolic modulation of acute myocardial infarction. The ECLA (Estudios Cardiológicos Latino-América) Collaborative Group. *Circulation* 1998; 98:2227–2234
7. 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): Effect on mortality at 1 year. *J Am Coll Cardiol* 1995; 26:57–65
8. Opie LH: Metabolism of free fatty acids, glucose and catecholamines in acute myocardial infarction and infarct size. *Am J Cardiol* 1975; 36:938–953

9. Fath-Ordoubadi F, Beatt K: Glucose-insulin-potassium therapy for treatment of acute myocardial infarction. *Circulation* 1997; 96: 1152–1156
10. Rajakumar AR, Prasad K, Mantha SV, et al: Protection of coronary angioplasty-induced oxidative stress by ISOVUE used during angioplasty. *Can J Cardiol* 1999; 15:989–999
11. Díaz-Araya G, Naranjo L, Godoy L, et al: Antioxidant effects of 1,4-dyhidropyridine and nitroso aril derivatives on brain cerebral slices. *Gen Pharmacol* 1998; 31:385–391
12. McCord J, Fridovich I: Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* 1969; 22: 6049–6055
13. Misra HP, Fridovich I: The role of superoxide anion in the autoxidation of epinephrine and a single assay for superoxide dismutase. *J Biol Chem* 1972; 247:3170–3175
14. Beers R, Sizer I: A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* 1952; 195:133–140
15. Paglia DE, Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; 70:158–169
16. Fraga CG, Tappel Al, Leibovitz BE, et al: Lability of red blood cell membranes to peroxidation; application to humans fed polyunsaturated lipids. *Lipids* 1990; 25:111–114
17. Sodi-Pallares D, Testelli MR, Fishleder BL, et al: Effects of intravenous infusion of a potassium-glucose-insulin solution on the electrocardiographic signs of myocardial infarction. *Am J Cardiol* 1962; 9:166–181
18. Valori C, Thomas M, Shinllingford J: Free noradrenaline and adrenaline excretion in relation to clinical syndromes following myocardial infarction. *Am J Cardiol* 1967; 20:605–610
19. Oliver MF, Kurien VA, Greenwood TW: Relation between serum free fatty acids and arrhythmias and death after acute myocardial infarction. *Lancet* 1968; 1:710–1714
20. Sowton E: Cardiac infarction and the glucose-tolerance test. *BMJ* 1962; 1:84–86
21. Oliver MF, Opie LH: Effects of glucose and fatty acids on myocardial ischemia and arrhythmias. *Lancet* 1994; 343:155–158
22. Jonassen AK, Brar BK, Mjos OD, et al: Insulin administered at reoxygenation exerts a cardioprotective effect in myocytes by a possible anti-apoptotic mechanism. *J Mol Cell Cardiol* 2000; 32:757–764
23. Maulik N, Yoshida T, Das DK: Oxidative stress developed during the reperfusion of ischemic myocardium induces apoptosis. *Free Radic Biol Med* 1998; 24:869–875
24. Przyklenk K, Kloner RA: Reperfusion injury by oxygen-derived free radicals? Effect of superoxide dismutase plus catalase, given at the time of reperfusion, on myocardial infarct size, contractile function, coronary microvasculature, and regional blood flow. *Circ Res* 1989; 64:86–96
25. Ferrari R, Alfieri O, Curello S, et al: Occurrence of oxidative stress during reperfusion of the human heart. *Circulation* 1990; 81: 201–211
26. Guarnieri C, Flamigni F, Caldarera CM: Role of oxygen in the cellular damage induced by re-oxygenation of hypoxic heart. *J Mol Cell Cardiol* 1980; 12:797–808
27. Guan W, Osanai T, Kamada T, et al: Time course of free radical production after primary coronary angioplasty for acute myocardial infarction and the effect of vitamin C. *Jpn Circ J* 1999; 63:924–928
28. Blann A, Midgley H, Burrows G, et al: Free radicals, antioxidants, and endothelial cell damage after percutaneous transluminal coronary angioplasty. *Coron Artery Dis* 1993; 4:905–910
29. Grech ED, Dodd NJ, Jackson MJ, et al: Evidence for free radical generation after primary percutaneous transluminal coronary angioplasty recanalization in acute myocardial infarction. *Am J Cardiol* 1996; 77: 122–127
30. Grech ED, Bellamy CM, Jackson MJ, et al: Free radical activity after primary coronary angioplasty in acute myocardial infarction. *Am Heart J* 1994; 127:1443–1449
31. Lafont A, Marwick TH, Chisolm GM, et al: Decreased free radical scavengers with reperfusion after coronary angioplasty in patients with acute myocardial infarction. *Am Heart J* 1996; 131:219–223
32. Oostenbrug GS, Mensink RP, Bar FW, et al: Lipid peroxidation-associated oxidative stress during percutaneous transluminal coronary angioplasty in humans. *Free Radic Biol Med* 1997; 22:129–136