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; Fernanda 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

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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.

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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

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Anions, H₂O₂, 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 administrated 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 reperf-
cision (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 glyceremia >300 mg/dL, and patent 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 H2O2 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 H2O2 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 H2O2 (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 non-paired 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 displays 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
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 plasma 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 patient was 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.

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