Anti-Inflammatory and Profibrinolytic Effect of Insulin in Acute ST-Segment–Elevation Myocardial Infarction

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Background—The clinical benefits of insulin previously observed in acute ST-segment–elevation myocardial infarction (STEMI) may be partially explained by an anti-inflammatory effect. We assessed this potential effect of insulin in STEMI patients treated with fibrinolytics.

Methods and Results—Thirty-two patients receiving reteplase were randomly assigned infusions of either insulin at 2.5 U/h, dextrose, and potassium (GIK) or normal saline and potassium (C) for 48 hours. Plasma concentrations of high-sensitivity C-reactive protein (CRP), serum amyloid A (SAA), plasminogen activator inhibitor-1 (PAI-1), creatine kinase (CK), and CK-MB were measured at baseline and sequentially for 48 hours. Total p47phox protein in mononuclear cells was measured in a subgroup of 13 subjects. Baseline CRP and SAA were significantly increased (2- to 4-fold) at 24 and 48 hours in each group (P<0.01). However, in the insulin group, there was a significant (P<0.05) attenuation of the absolute rise in concentration of CRP and SAA from baseline. The absolute increase of CRP and SAA was reduced by 40% (CRP) and 50% (SAA) at 24 hours and at 48 hours compared with the control group. The absolute increase in PAI-1 from baseline and the percentage increase in p47phox over 48 hours were significantly (P<0.05) lower in the insulin-treated group. CK-MB peaked earlier and tended to be lower in insulin-treated subjects, especially in patients with inferior MI.

Conclusions—Insulin has an anti-inflammatory and profibrinolytic effect in patients with acute MI. These effects may contribute to the clinical benefits of insulin in STEMI. (Circulation. 2004;109:849-854.)

Key Words: myocardial infarction ■ inflammation ■ insulin ■ atherosclerosis

Insulin infusion during acute myocardial infarction (MI) improves clinical outcomes.1–3 Administration of insulin at the time of reperfusion decreases infarct size by 45% in animal models.4 The exact mechanism of the beneficial effect of insulin is unclear. It has been suggested that the suppression of plasma free fatty acid concentration may play a role.5

Inflammation has been implicated in the pathogenesis of atherosclerosis, plaque rupture, and thrombosis.6,7 Our recent work has shown that insulin has a powerful anti-inflammatory effect on endothelial cells in vitro and on circulating mononuclear cells (MNCs) in vivo when infused at a dose of 2.5 U/h in obese nondiabetic subjects.8,9 We hypothesized that an infusion of 2.5 U/h of insulin in patients with acute ST-segment–elevation MI (STEMI) will exert an anti-inflammatory effect, which may partially account for its effectiveness in clinical trials. This study is the first to characterize the effects of low-dose insulin infusion on the temporal evolution of inflammatory indices/mediators in patients treated with intravenous thrombolitics for acute STEMI.

Methods

Study Population
Subjects >18 years old presenting to the emergency department with acute STEMI within 6 hours of chest pain and eligible for intravenous thrombolysis were recruited. Glucocorticoid treatment, systemic infection, hypotension, congestive heart failure, creatinine >2.0 mg/dL, insulin-treated diabetes, hemoglobin <11 g/dL, and participation in another clinical research study within 30 days of enrollment were the major exclusion criteria.

The study was approved by the State University of New York at Buffalo Institutional Review Board, and all patients gave written informed consent.

Procedures

Recruitment and Randomization
Patients were alternately assigned to control and insulin groups.
**Treatment**

All patients received reteplase (10-U IV boluses, 30 minutes apart), aspirin (160 to 325 mg PO), unfractionated heparin as per 1999 ACC/AHA guidelines,10 and metoprolol (5 mg IV every 5 minutes×3) unless contraindicated. Other concomitant medications were administered at the discretion of the treating physician.

With the fibrinolytic, patients received either a glucose-insulin-potassium (GIK) infusion (10% dextrose with 40 mmol KCl at 60 mL/h and human regular insulin [Novolin R, Novo Nordisk Pharmaceuticals] at 2.5 U/h (insulin arm) (I) or 1 L normal saline with 40 mmol of KCl (control arm) (C) at 60 mL/h for 48 hours on an open-label basis. GIK infusions were titrated to maintain glucose between 80 and 200 mg/dL and potassium between 4 and 5 mmol/L.

**Data Collection**

**Plasma End Points**

Blood (20 mL) was collected in EDTA and SST tubes at baseline and at 2, 4, 6, 24, and 48 hours. High-sensitivity C-reactive protein (hsCRP), serum amyloid A (SAA), plasminogen activator inhibitor-1 (PAI-1), glucose, and insulin were measured in plasma with ELISA kits. MNC separation was performed immediately for measurement of total p47<sup>phox</sup> subunit of NADPH oxidase protein content in 13 patients.

**Cardiac Data**

Creatine kinase (CK) and CK-MB were measured at baseline and at 2, 4, 6, 8, 16, 24, and 48 hours. An ECG was obtained at baseline and at 90 minutes after thrombolysis. Percent ST-segment resolution was measured at 90 minutes.

**Analytical Methods**

CRP (mg/L) was measured by an ELISA kit (Diagnostic Systems Laboratories, Inc), lowest detection limit 0.35 ng/mL; interassay coefficient of variation (CV), 3% to 7%; intra-assay CV, <5%. SAA (mg/L) was measured by an ELISA kit (Biosource International), lowest detection limit 5 ng/mL; interassay CV, 4% to 10%; intra-assay CV, 4.9%. The methodologies for PAI-1 (ng/mL), insulin (μU/mL), glucose (mg/dL), and p47<sup>phox</sup> subunit (NADPH) oxidase protein measurements have been described previously.9,11 CK and CK-MB were measured with a commercial assay (Beckman-Coulter Inc).

**Study End Points**

For the primary end points comparing plasma CRP, SAA, and PAI-1 between and within the control and insulin groups, each data point was calculated as the absolute difference from baseline (0 hour) concentration. This was necessary to account for the inherent variability in baseline inflammatory indices caused by the heterogeneity of the population and clinical presentation. A similar approach was used in a recent study examining the effect of abciximab on circulating inflammatory markers.12 Secondary end points were plasma CK and CK-MB concentrations in the first 48 hours, ST-segment resolution at 90 minutes, and percentage change from baseline in total p47<sup>phox</sup> subunit of NADPH oxidase protein in MNCs between the control and insulin groups.

**Statistical Analysis**

Data are presented as mean±SEM for continuous variables and as absolute frequencies for categorical variables. Continuous variables were analyzed by Student’s t test for paired and unpaired data, 1-way repeated-measures ANOVA, and 2-way ANOVA when appropriate. If significant by ANOVA, differences were analyzed by use of unpaired 2-way comparisons (Student’s t test) with Bonferroni correction. Categorical variables and proportions were analyzed by Z test. Nonparametric data were transformed to logarithmic values before analysis. Pearson’s product-moment correlation was used to find correlations. Power of the study was 0.8 for detection of a 2.2-mg/L difference in absolute increase of CRP from baseline at 48 hours between the insulin and control groups. Statistical significance was set as P<0.05, analyzed using SigmaStat software (Jandel Scientific).

**Results**

Thirty-six patients were recruited into the study; 32 patients met the inclusion criteria (Table 1). Two patients in the control and 4 in the insulin group received the assigned infusions for <48 hours.

All patients received intravenous reteplase, unfractionated heparin, and aspirin within 30 minutes of arrival in the emergency department. The time from chest pain onset to initiation of reteplase was significantly (P<0.01) longer in the insulin group (137±21 [I] versus 65±12 minutes [C]). Thirty-one of 32 patients received β-blockers, 19 (57% C, 61% I) received ACE inhibitors, 27 (85% C, 83% I) received HMG-CoA reductase inhibitors, 14 received clopidogrel (42% C, 44% I), and 15 (42% C, 50% I) received eptifibatide in the first 48 hours.

**Clinical**

The incidences of rescue angioplasty (7% C, 11% I), chest pain in the 48-hour postinfarction period (35% C, 18% I), arrhythmias (22% C, 27% I), and congestive heart failure (22% C, 11% I) were similar between the 2 groups. Thirty-one of 32 patients had an angiogram, 24 (64% C, 83% I) had a stent placement, 5 (21% C, 11% I) had a CABG, and 3 (15% C, 6% I) did not require any intervention.

**Glucose**

Baseline plasma glucose was 146±19 mg/dL in controls and 132±8 mg/dL in the insulin group (P=NS). Mean glucose over 48 hours was 131 mg/dL for controls and 124 mg/dL for the insulin group. Sixteen episodes of mild asymptomatic
hypoglycemia (glucose <80, >60 mg/dL) occurred in 9 patients.

**Insulin**

Insulin infusion was started at 23±3 minutes after the first bolus of reteplase. Insulin (87±12 U) (range, 15 to 226 U) was infused over 48 hours, or 0.029±0.003 U·kg⁻¹·h⁻¹. In controls, baseline insulin concentrations were 14±3 μU/mL and ranged between 16 and 25 μU/mL over the next 48 hours (P=NS). In the insulin-treated group, insulin concentrations were 12±1 μU/mL at baseline, peaked at 24 hours, and fluctuated between 41 and 63 μU/mL during the infusion. There was a significant difference in the insulin concentrations between the groups and between the baseline and 2, 4, 6, 24, and 48 hours in the insulin group (P<0.05).

**High-Sensitivity CRP**

CRP concentrations increased significantly (P<0.01) in both groups (Table 2). The time course of the absolute change in CRP from baseline concentrations (at time 0 hour) is shown in Figure 1. The absolute increases in CRP concentrations were significantly (P<0.05) less for the insulin versus the control group at 24 hours (14.5±5 mg/L [I] versus 31±6 mg/L [C]) and at 48 hours (24±6 mg/L [I] versus 46±8 mg/L [C]) (Figure 2). Subgroup analysis in subjects receiving glycoprotein IIb/IIIa inhibitors, statins, and ACE inhibitors did not show a significant difference on CRP (2-way ANOVA).

**SAA**

SAA concentrations increased significantly (P<0.01) in both groups (Table 2). The time course of the absolute change in SAA from baseline concentrations (at time 0 hour) is shown in Figure 2. The absolute increases in SAA concentrations were significantly (P<0.01) less for the insulin versus the control group at 24 hours (14.5±5 mg/L [I] versus 31±6 mg/L [C]) and at 48 hours (24±6 mg/L [I] versus 46±8 mg/L [C]) (Figure 2). Subgroup analysis in subjects receiving glycoprotein IIb/IIIa inhibitors, statins, and ACE inhibitors did not show an independent effect on SAA (2-way ANOVA).

**PAI-1**

PAI-1 increased significantly (P<0.05) at 2 hours only in controls (Table 2). The absolute increase in PAI-1 from baseline concentrations (at time 0 hour) over time was significantly lower in the insulin group than in controls (P<0.05) (Figure 3).

**P47**

Baseline (0-hour) P47 subunit of NADPH oxidase in MNC homogenate was normalized to 100%. It increased nonsig-
nificantly in the control arm, whereas it decreased to 49±14% of baseline at 2 hours after thrombolysis (P<0.05) in the insulin arm. P47 at 2 hours was significantly (P<0.05) lower in the insulin group than in controls. P47 was significantly lower in the insulin group over 48 hours (P<0.05) (Figure 4).

**Creatine Kinase**

CK peaked to 1526±363 U/L at 8 hours in controls and to 1436±356 U/L at 8 hours in the insulin group. CK-MB peaked to 252±73 U/L at 16 hours in controls and to 240±65 U/L at 8 hours in insulin group. The CK-MB time integral was 5635±1581 U • L⁻¹ • h⁻¹ in controls and 4404±1238 U • L⁻¹ • h⁻¹ in the insulin group (P=NS).

In the inferior wall infarcts, CK peaked to 1505±384 U/L at 16 hours in controls and to 720±152 U/L at 16 hours in the insulin group. Peak CK tended to be lower in the insulin group (P=0.08). CK-MB in controls peaked to 308±111 U/L at 16 hours, whereas it peaked to 116±27 U/L at 8 hours in the insulin group. The CK time integral was 40 631±12 022 U • L⁻¹ • h⁻¹ in controls and 18 472±4812 U • L⁻¹ • h⁻¹ in the insulin group (P=0.11). The CK-MB time integral was 7196±2552 U • L⁻¹ • h⁻¹ in controls and 2467±541 U • L⁻¹ • h⁻¹ in the insulin group (P=0.09). Logarithmic transformation of nonparametric CK and CK-MB over 48 hours was significantly lower in the insulin group (P<0.01) (Figures 5 and 6).

**ST-Segment Resolution**

ST-segment resolution of <50% was seen in 38% of controls and 18% of insulin-treated subjects (P=NS), whereas ST-segment resolution of >50% was seen in 62% of controls and 82% of insulin-treated subjects (P=NS) subjects.

**Correlation**

Area under the curve (AUC) CK-MB was positively correlated to AUC CRP, r=0.58 (P=0.03) in controls, but this association was nonsignificant in the insulin group, r=0.36 (P=0.15). In both groups, AUC CRP was significantly correlated to AUC PAI-1, r=0.71 (C), 0.63 (I) (P<0.001) and AUC SAA, r=0.87 (C), r=0.87 (I) (P<0.0001).
Discussion

Our data confirm that after acute MI, plasma CRP and SAA concentrations increase, reflecting an enhanced degree of systemic inflammation. More importantly, our data are the first to demonstrate that insulin significantly reduces (40% to 50%) the magnitude of this increase of both CRP and SAA despite the anti-inflammatory effect of standard therapy in the background.

A reduction in the rise of CRP has been shown to indicate thrombolytic efficacy and a patent infarct-related coronary artery.13,14 A high CRP after MI predicts infarct expansion, cardiac rupture, and mortality.14–16 Reduction of cardiac rupture by β-blockers is associated with a decrease in the rise in CRP after MI.17 Abciximab, a glycoprotein IIb/IIIa inhibitor known to reduce CRP after percutaneous coronary revascularization, improves the incidence of ST-segment resolution with thrombolytics to 59% as opposed to a rate of 40% when thrombolytics are given alone.18 Injection of CRP in rats with induced MI significantly increases myocardial infarct size.19 Thus, we hypothesize that a reduction in the increase of CRP may explain some of the beneficial effects of insulin that have been seen in previous acute MI trials.1–3

Infarcted tissue can also raise CRP; the best correlation of CRP with infarct size is seen in the absence of thrombolytics, and a weakening of this relationship occurs with successful reperfusion.14 In our study, CRP was significantly related to CK-MB only in the control group. The lack of this correlation in the insulin group may be explained by the anti-inflammatory effect of insulin. Further investigations are needed to ascertain whether this effect is a result of the action of insulin on the circulating MNCs, as we showed previously,9 or an effect on cytokine production by inflammatory cells in the infarcted myocardium. It is also possible that a reduction in myocardial infarct size by insulin is responsible for the reduction in CRP in this group. It is interesting that glycoprotein IIb/IIIa inhibitors, statins, and ACE inhibitors had no significant effect on the inflammatory parameters in our study.

Elevations of both CRP and SAA are associated with adverse outcomes in acute coronary syndromes,20 and changes in one marker are associated with similar changes in the other. The reduction in the magnitude of rise of SAA by insulin was similar to that of CRP in our study. These observations strengthen our hypothesis regarding the anti-inflammatory effect of insulin in STEMI.

The increase in PAI-1 concentration known to occur after fibrinolysis was suppressed by insulin.21 Failure of reperfusion and reocclusion are the major disadvantages of fibrinolysis.22 An increase in PAI-1 has been shown to predict 30-day mortality in acute STEMI.23 PAI-1 is implicated in the failure of thrombolysis in animal models, and a reduction in the rise of PAI-1 correlates with thrombolytic efficacy.24 Thus, the rapid suppressive effect of insulin on PAI-1 may facilitate fibrinolysis. The rapidity of the effect of insulin on PAI-1 is consistent with our previous observations and is also suggestive of the anti-inflammatory effect of insulin.11

Consistent with our previous observations, the p47phox subunit of NADPH oxidase, the enzyme that generates superoxide radical and mediates oxidative stress, was suppressed in insulin-treated patients. This is relevant because the reperfusion injury after ischemia is mediated by oxidative stress, which also triggers the activation of redox-sensitive proinflammatory transcription factors nuclear factor-κB, early growth response-1, and activator protein-1.25

The increase in both CK and CK-MB over 48 hours was lower in insulin-treated patients with inferior MI, the largest homogeneous group within this patient set (P<0.01). Because CK and CK-MB reflect myocardial damage, it is possible that insulin reduced the myocardial infarct size. Because peak CK-MB was earlier in the insulin group, it is possible that insulin may have led to earlier reperfusion. The absence of a correlation of the AUC of CRP with the AUC of CK-MB in the insulin group, a finding usually seen in subjects with effective reperfusion, also supports this hypothesis.14

Of insulin-treated subjects, 82% had >50% ST-segment resolution, as opposed to 62% in the conventional group (P=NS), despite receiving thrombolysis 72 minutes later, an effect comparable to the addition of abciximab to thrombolytics in the TIMI 14 trial.18 Because ST-segment resolution is indicative of better epicardial and microvascular perfusion, poor recovery of which correlates with adverse outcome, it is possible that insulin may have improved recanalization and microvascular perfusion.26 Insulin is known to exert a vasodilatory and platelet antiaggregatory effect via a nitric oxide cGMP pathway.27–29 These hypotheses need to be tested in future studies.

An experimental rat model of ischemic myocardial injury has shown that infusing insulin at reperfusion reduces infarct size by 45%, an effect attributed to the antiapoptotic effect of insulin.4 Insulin also inhibits the development of atherosclerosis in apolipoprotein E–knockout mice, which develop atherosclerosis spontaneously.30 These observations are consistent with the myocardial protective effects suggested by our study.

Insulin infusions in critically ill surgical patients reduce glucose, mortality, and morbidity in parallel with a reduction in plasma CRP.31 In our study, there was no significant hyperglycemia, and glucose levels were similar in the 2 groups; thus, the anti-inflammatory effect is probably attributable to insulin itself.

One limitation of this study is the open-label administration of study infusion, because insulin had to be titrated to maintain glucose, which could have led to selection bias. However, the later presentation and higher baseline CRP may have actually led to an underestimation of the benefits of insulin in this setting. The study had insufficient power for assessing ST-segment resolution and CK-MB, therefore these observations should be interpreted cautiously. Mechanisms discussed are hypotheses that were not investigated in this study.

In conclusion, in patients with acute STEMI, the inflammatory state accelerates sharply after fibrinolitics, and a prothrombotic and an antifibrinolytic milieu is set up despite the standard anti-inflammatory and antithrombotic therapy. Insulin infusion at low doses initiated with fibrinolitics markedly reduces the magnitude of increase in inflammation and rapidly suppresses the increase in antifibrinolytic factors.
We hypothesize that these effects, along with its known vasodilatory and platelet antiaggregatory action, could improve epicardial and microvascular reperfusion, thus protecting the myocardium. The effects we describe are the first description of an additional novel mechanism that may explain the beneficial effect of insulin seen in previous clinical trials. Our study also provides the rationale for using insulin at the time of reperfusion and describes an effective dose and duration of infusion that needs to be tested in future STEMI trials with clinically relevant end points.

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References
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