Effect of Iron Chelation on Myocardial Infarct Size and Oxidative Stress in ST-Elevation–Myocardial Infarction

William Chan, MB, BS, PhD; Andrew J. Taylor, MB, BS, PhD; Andris H. Ellims, MB, BS; Lisa Lefkovits, MB, BS; Chiew Wong, MB, BS, PhD; Bronwyn A. Kingwell, PhD; Alaina Natoli, BSc; Kevin D. Croft, PhD; Trevor Mori, PhD; David M. Kaye, MB, BS, PhD; Anthony M. Dart, BM, BCh, DPhil; Stephen J. Duffy, MB, BS, PhD

Background—Experimental studies suggest that deferoxamine (DFO) limits the generation of reactive oxygen species by chelating redox-active iron and thereby may reduce ischemia-reperfusion injury and myocardial infarct (MI) size. We investigated whether DFO administered before reperfusion by primary percutaneous coronary intervention (PPCI) would ameliorate oxidative stress and MI size.

Methods and Results—We randomly assigned 60 patients with ST-elevation–MI to receive an intravenous bolus of DFO (500 mg) immediately before PPCI followed by a 12-hour infusion (50 mg/kg of body weight) (n = 28) or normal saline bolus and infusion (placebo group, n = 32). MI size was measured by contrast-enhanced cardiac MRI (CMRI; day 3 ± 1), creatine kinase and troponin I area-under-the-curve, and severity of wall motion abnormality on echocardiography. Clinical follow-up including repeat CMRI and echocardiography were performed at 3 months (100 ± 17 days). Oxidative stress was assessed by plasma F₂-isoprostane levels. DFO and placebo groups were well balanced with respect to baseline characteristics, symptom- and door-to-balloon times, pre-PPCI coronary patency, and infarct-related artery location. Serum iron levels were decreased with DFO treatment after PPCI compared with placebo (3.0 ± 2.5 versus 12.6 ± 5.5 μmol/L, P < 0.0001), which persisted until the end of the infusion. In DFO-treated patients, there was a significant reduction in plasma F₂-isoprostane levels immediately after PPCI (2878 ± 1461 versus 2213 ± 579 pmol/L, P = 0.04). However, there was no difference in CMRI-determined infarct size (DFO, 17.4 ± 10.8%; versus placebo, 18.6 ± 10.2%; P = 0.73), myocardial salvage index at 3 days or at 3 months, or the area-under-the-curve for creatine kinase or troponin I.

Conclusions—Adjunctive DFO treatment after the onset of ischemia and continued periprocedurally ameliorates oxidative stress without limiting infarct size.


Key Words: myocardial infarction ■ reperfusion injury ■ iron ■ oxidative stress ■ deferoxamine

Experimental studies suggest that reactive oxygen species (ROS) are the major biological mediator of ischemia-reperfusion injury. These can form during acute myocardial ischemia under low oxygen tension, but particularly during the first few minutes of reperfusion. Redox-active iron released from macrophages and intracellular myocyte stores is capable of catalyzing ROS production via Fenton chemistry to generate potent oxidants (including the highly reactive hydroxyl radical, OH•) that participate in lipid peroxidation and cellular injury.

Deferoxamine (or deferoxamine; DFO) is an extracellular iron chelator with an extremely high affinity constant (Kd = 10³¹) for Fe³⁺, which underlies its principal inhibitory effect on OH• generation, forming a stable complex with Fe³⁺ and decreasing its availability for ROS production. DFO has also been shown to reduce ROS formation by directly scavenging the OH• radical and inhibiting endothelial cell activation in response to tumor necrosis factor-α and collagen-induced whole-blood platelet aggregation. We have previously demonstrated that iron chelation with DFO in patients with coronary artery disease improved forearm endothelial-mediated, nitric oxide-dependent blood flow, an effect that might translate into improved coronary blood flow.

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From the Department of Cardiovascular Medicine, Alfred Hospital, Melbourne, Australia (W.C., A.J.T., A.HE., L.L., C.W., D.M.K., A.M.D., S.J.D.); Baker IDI Heart and Diabetes Institute, Melbourne, Australia (W.C., A.J.T., A.HE., C.W., B.A.K., A.N., D.M.K., A.M.D., S.J.D.); School of Medicine and Pharmacology, University of Western Australia, Perth, Australia (K.D.C., T.M.); and the Department of Medicine, Monash University, Melbourne, Australia (A.M.D.).
Correspondence to Stephen J. Duffy, MB, BS, PhD, Heart Centre, The Alfred Hospital, 3rd Floor, W.S. Philip Block, Commercial Rd, Melbourne 3004, Victoria, Australia. E-mail s.duffy@alfred.org.au
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Experimental studies with DFO at the time of postschematic reflow resulted in greater recovery of myocardial function and energy metabolism\(^{10}\) and decreased infarct size in canines.\(^ {16}\) In a randomized, placebo-controlled study of 45 patients undergoing cardiopulmonary bypass, DFO infused intravenously immediately after anesthesia reduced ROS production and improved left ventricular ejection fraction postoperatively and after 12 months.\(^ {17}\) No studies have examined the effectiveness of DFO in the setting of STEMI. Therefore, the primary aim of this randomized, double-blind, placebo-controlled study was to investigate whether iron chelation with DFO in patients with ST-segment elevation–myocardial infarction (STEMI) treated by primary percutaneous coronary intervention (PPCI) ameliorates oxidative stress and reduces infarct size determined by cardiac MRI (CMRI) and creatine kinase and troponin I area-under-the-curve measurements.

**WHAT IS KNOWN**

- Ischemia-reperfusion injury is a well-recognized contributor to myocardial infarct size in experimental coronary artery occlusion and in clinical ST-elevation–myocardial infarction (STEMI).
- The pathophysiology of ischemia-reperfusion injury is complex and multifactorial and includes the production of reactive oxygen species (ROS) on reperfusion and the continued postinfarct inflammatory response.
- Redox-active iron released from macrophages and myocyte intracellular stores is capable of catalyzing ROS production via Fenton chemistry to generate potent oxidants that participate in lipid peroxidation and cellular injury.

**WHAT THE STUDY ADDS**

- This study tested the hypothesis that decreasing the production of ROS by iron chelation with deferoxamine might reduce ischemia-reperfusion injury and decrease myocardial infarct size.
- In a randomized, placebo-controlled trial, iron chelation with deferoxamine after the onset of ischemia and continued periprocedurally in primary percutaneous coronary intervention for STEMI reduced serum iron concentrations and ameliorated oxidative stress but did not limit infarct size compared with placebo.
- Numerous classes of pharmacological agents, including deferoxamine, although showing promise in experimental models of myocardial infarction, have not translated positively in clinical trials.

**Methods**

**Study Participants**

Consecutive patients >18 years of age admitted with first presentation STEMI with chest pain ≥30 minutes in duration (to reduce recruitment of aborted infarcts), symptom onset <6 hours, and ECG changes of new ischemia (ST elevation at the J point ≥0.1 mV in 2 contiguous leads) were invited to participate. All patients having 1 or more of the following criteria were excluded: an intracardiac device not compatible with CMRI (eg, pacemaker); suspected or known previous MI in the same coronary artery territory as the current STEMI; rescue angioplasty; cardiogenic shock (systolic blood pressure <90 mm Hg); current iron supplementation or known iron deficient state; renal failure (estimated glomerular filtration rate ≤30 mL/min); known hypersensitivity to DFO; or severe claustrophobia.

**Trial Design**

This was a prospective, single-center, randomized, double-blind, placebo-controlled study. DFO (Novartis Pharmaceuticals, North Ryde, Australia) was purchased using a National Health and Medical Research Council (NHMRC) Program Grant and was dispensed by the Alfred Hospital Pharmacy. The study was carried out at the Alfred Hospital between August 2008 and December 2010. Patients provided written informed consent before random assignment. The study was approved by the Alfred Hospital Human Research Ethics Committee in accordance with NHMRC National Statement on Ethical Conduct in Research Involving Humans, which complies with the Declaration of Helsinki. An independent data and safety monitoring advisor reviewed all incidences of major adverse events that occurred during the study. The trial was registered with the Australian New Zealand Clinical Trials Registry on June 30, 2008 (ACTRN12608000308392). The trial design is shown in Figure 1.

An intravenous bolus dose of 500 mg of DFO reconstituted in 10 mL of water for injection (or normal saline in the placebo group) was given over 5 to 10 minutes before reperfusion by PPCI, followed by an infusion of 50 mg/kg of DFO (or placebo) over a 12-hour period (equivalent to 4.1 mg/kg per hour in an 80-kg patient). The DFO bolus dosage was chosen on the basis of work by Nitenberg et al,\(^ {15}\) who infused 500 mg of DFO over a 10-minute period in diabetic patients to restore the coronary artery endothelium-dependent vaso-dilator response to cold pressor testing and papaverine. The aim of the bolus was to achieve sufficient DFO plasma concentration and consequent iron chelation before reperfusion, as the extent of ischemia-reperfusion injury appears to be greatest immediately after reperfusion.\(^ {14}\) The infusion dose was the same as that used by Paraskevaidis et al\(^ {17}\) prepared by dissolving the prescribed amount of DFO powder, based on weight with water for injection to achieve a solution with a concentration of 10% (as per manufacturer recommendation). The solution was then further diluted in 0.9% normal saline to achieve a final volume of 100 mL to standardize the amount of fluid given in both study arms. Neither the patient, the treating interventional cardiologist, the subsequent coronary care cardiologists, nor those measuring MI size had knowledge of treatment assignment. Random assignment was performed by the Alfred Hospital Pharmacy using a computer-generated randomization sequence. Study envelopes were numbered, sealed, and stratified according to infarct-related artery distribution (anterior [left anterior descending artery, LAD] STEMI versus nonanterior STEMI).

**Routine Patient Treatment**

Coronary angiography was performed according to standard techniques. Revascularization was performed by stenting preceded by balloon predilatation. Adjunctive aspirin, clopidogrel, and intravenous unfractionated heparin were given. Periprocedural use of glycoprotein IIb/ IIIa receptor antagonists and/or thromboaspiration was at the discretion of the treating interventional cardiologist. All patients were invited to return in 3 months for clinical review and additional scanning with CMRI and echocardiography.

**ECG Analysis**

A blinded observer performed all analysis of ST-segment resolution from a preprocedural 12-lead ECG compared with the first postprocedural ECG, usually performed within 30 to 90 minutes after PPCI on the coronary care ward according to established guidelines.\(^ {18}\)
Biochemical Analysis

Serum iron parameters (iron, ferritin, soluble transferrin receptor) were measured before PCI, after PCI, at the end of DFO infusion, and at 3 months. Routine laboratory analyses including full blood examination, high-sensitivity C-reactive protein, and renal and liver function tests were measured by the Alfred Pathology Service on commercially available automated platforms. Creatine kinase and troponin I measurements were obtained before PCI and then at 6-hourly intervals over 48 hours to determine the area-under-the-curve by computerized planimetry (Image J 1.32, http://rsb.info.nih.gov/ij/). To minimize ex vivo oxidation, blood samples for F2-isoprostanes were collected in ice-cold EDTA tubes (18 mg EDTA for 10 mL of whole blood). Reduced glutathione (1 mg/mL) and butylated hydroxytoluene (200 mg/mL) were added as antioxidants before centrifugation at 3000 rpm for 15 minutes at 4°C within 80°C until analysis. F2-isoprostanes were collected in ice-cold EDTA tubes (18 mg EDTA for 10 mL of whole blood). Reduced glutathione (1 mg/mL) and butylated hydroxytoluene (200 mg/mL) were added as antioxidants before centrifugation at 3000 rpm for 15 minutes at 4°C within 30 minutes of collection. Plasma was stored at -80°C until analysis by gas chromatography-mass spectrometry using electron capture negative chemical ionization as previously described.

Angiographic Analysis

Angiographic lesion characteristics were classified according to the modified American Heart Association/American College of Cardiology classification from 2 orthogonal planes. Preprocedural and postprocedural assessments of epicardial TIMI (Thrombolysis In Myocardial Infarction) flow grade was performed by the treating interventional cardiologist blinded to patient treatment assignment. Procedural factors including symptom- and door-to-balloon inflation, glycoprotein IIb/IIIa inhibitors, thromboaspiration, and type of implanted stent were recorded.

CMRI Protocol and Analysis

All CMRI were performed on a clinical 1.5-T CMRI scanner (Signa HDx 1.5-T, GE Healthcare, Waukesha, WI). Left ventricular (LV) function was assessed by a standard steady-state free precession technique (repetition time [TR], 3.8 ms; echo time [TE], 1.6 ms; 30 phases; and slice thickness of 8 mm). LV ejection fraction was calculated by volumetric analysis from a contiguous short-axis stack (8-mm slice thickness), using the summation of disc method by at least 2 blinded cardiologists (A.J.T., A.H.E., and L.L.). For area-at-risk determination, LV short-axis slices covering the whole ventricle using a T2-weighted triple-inversion recovery breath-hold fast spin-echo pulse sequence (short tau inversion recovery; STIR) (TR 2 R-to-R intervals, TE, 80 ms; inversion time [TI], 150 ms; slice thickness, 8 mm; field of view, 40 μm; matrix, 224×224) were obtained using a body coil. Area-at-risk was quantified by manual delineation of myocardium with bright signal intensity 2 SD above the mean signal obtained in the remote, noninfarcted myocardium and multiplying the slice thickness and the myocardial density of 1.05 g/mL, and multiplying the slice thickness and the myocardial density of 1.05 g/mL and multiplying the slice thickness and the myocardial density of 1.05 g/mL to obtain the infarct mass and expressed as a percentage of LV mass. Late enhancement images covering the whole ventricle were acquired approximately 15 minutes after intravenous administration of a bolus of gadolinium-diethylene triamine penta-acetic acid (DTPA) (0.2mmol/kg, Magnevist, Schering, Germany) to identify regional necrosis/fibrosis for infarct size quantification using an inversion recovery gradient echo technique (TR, 7.1 ms; TE, 3.1 ms; TI individually determined to null the normal myocardial signal, range, 180–250 ms; slice thickness, 8 mm; matrix, 256×192; number of acquisitions=2). The area of hyperenhanced myocardium (bounded by endocardial and epicardial contours) on each short-axis slice was manually traced then multiplied by the slice thickness and the myocardial density of 1.05 g/mL to obtain the infarct mass and expressed as a percentage of LV mass (infarct size). Myocardial salvage index was calculated as the area-at-risk minus percentage infarct size divided by area-at-risk. All analyses were performed offline on dedicated workstations running AW SDC 4.4 and IDL version 6.3 with ReportCARD version 3.6 by fully blinded observers with excellent reproducibility (r=0.98) for infarct size assessment.

Echocardiographic Assessment

Blinded echocardiographic assessment of LV volume and function were performed on day 3 to 5 and at 3 months, using the Simpson biplane method (Vivid 7 systems; GE Vingmed, Horten, Norway). Regional wall motion abnormalities (wall motion score index, WMSI) were scored according to the American Society of Echocardiography 16-segment model.

Statistical Analysis

Categorical data are presented as numbers and percentages. Continuous data are presented as mean±SD unless otherwise stated. Continuous variables were compared with either paired or unpaired Student t test; categorical variables were compared between groups with Pearson χ2 test or a Fisher exact test where appropriate. Variables were checked for normality, and, where failed, logarithmic transformation of the data was performed before analysis; otherwise, nonparametric testing was performed. Mean±SD values are presented as raw data to facilitate interpretation. Repeated-measures ANOVA with post hoc testing (Bonferroni) for multiple comparisons was used to assess for treatment effects. Sample size estimation was based on creatine kinase and infarct size dataset from previously published studies. Based on a hypothesized 30% reduction in the area-under-the-curve for creatine kinase in the DFO group, we...
required 31 patients per group to achieve a statistical power of 80% and a probability of a type I error of 0.05 using a 2-sided test. The primary end point was MI size measured by both creatine kinase/troponin I area-under-the-curve and CMRI. CMRI-determined infarct size was analyzed according to treatment, location of infarct (anterior/nonanterior), pre-PCI TIMI 0 flow grade, and ischemia time (symptom-to-balloon). All reported probability values are 2-sided. Probability values <0.05 were considered statistically significant. All data analyses were performed with SPSS version 16 (SPSS Inc, Chicago, IL).

Results

Baseline and Procedural Characteristics

A total of 245 consecutive patients presented to the Alfred Hospital with ST-segment elevation and symptoms suggestive of STEMI from August 2008 to December 2010. Seventy-six patients were enrolled into the study, and 60 patients were included in the final analysis. The 16 patients initially deemed eligible but subsequently omitted from the analysis were excluded for the following reasons: 12 had no infarct-related artery and 4 patients underwent emergency coronary artery bypass grafting. One hundred sixty-nine patients were excluded for the following reasons: 45 received fibrinolysis (at other institutions and transferred for ongoing care); 32 presented with symptoms >6 hours duration; 25 were out-of-hospital cardiac arrests; 19 were not assessed because the primary investigator (W.C.) was not available; 17 patients had either stent thrombosis or previous STEMI in the same territory; 16 were deemed hemodynamically unstable or in cardiogenic shock; and 15 declined participation (see Figure 1).

The DFO and placebo groups were well balanced with respect to baseline characteristics (Table 1) including age, male sex, and cardiovascular risk factors. Infarct location was similar, with anterior (LAD) infarction accounting for 29% and 34% in the DFO and placebo groups, respectively (P=0.63). The median time from symptom onset to 1st balloon inflation was similar in both groups (211 minutes [170–274] versus 192 minutes [147–260]; P=0.25; Table 2). The number of coronary stents implanted and the mean stent length was greater in the DFO group. However, all other procedural variables including pre- and post-PCI TIMI flow grades, use of glycoprotein IIb/IIIa receptor antagonists, use of thromboaspiration, and clopidogrel preloading were similar between the 2 groups.

Measures of Serum Iron, Oxidative Stress, and Inflammation

Post-PPCI serum iron was significantly reduced in the DFO group compared with placebo (3.0±2.5 versus 12.6±5.5 μmol/L, P<0.0001; Figure 2). Serum iron remained significantly decreased in the DFO group for the duration of the 12-hour infusion (1.5±1.4 versus 13.2±5.0 μmol/L, P<0.0001). At 48 hours, serum iron in the DFO group normalized and was not different to the placebo group (10.4±4.6 versus 8.8±2.8 μmol/L, P=0.24). Treatment effect was significant by repeated measures ANOVA (P<0.0001). Serum ferritin and soluble transferrin receptor were not affected by DFO treatment (data not shown). Baseline (nonfasting) transferrin saturation for the DFO and placebo groups were 23.1±11.2% versus 25.1±9.1% (P=0.47).

Pre-PPCI plasma F2-isoprostane levels were similar between DFO and placebo groups (2878±1461 versus 2362±921 pmol/L, P=0.10). However, after PPCI there was a significant reduction in F2-isoprostane levels in the DFO but not placebo group (Δ 616±1397 versus 351±1031 pmol/L; P=0.04 for pre-PPCI versus post-PPCI intragroup comparison [Figure 3]). However, analysis of the change in plasma F2-isoprostane levels before and after PCI was not statistically significant between DFO and placebo groups (P=0.43).

Plasma C-reactive protein levels were similar between DFO and placebo groups on admission (3.7±4.9 versus 5.3±9.4 mg/L, P=0.45), at end of infusion (3.5±2.0 versus 5.3±2.9 mg/L, P=0.10), and at 48 hours (40.0±46.7 versus 41.1±67.7 mg/L, P=0.95).

Early MI Size and Salvage Assessment

More than 70% of patients in both groups (DFO=21 and placebo=23) underwent CMRI at a mean of 3±1 days after STEMI. MI size (17.4±10.8% versus 18.6±10.2%, P=0.73) and myocardial salvage index (33.8±23.1% versus 30.8±24.5%, P=0.70) were not different between the DFO or placebo groups (Figure 4). Similar results were obtained when only patients with pre-PPCI TIMI 0 flow were included (21.2±10.0% versus 18.0±9.8%, P=0.37) (Figure 4C). In

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Placebo</th>
<th>DFO</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n (%)</td>
<td>32 (53%)</td>
<td>28 (47%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Mean age, y</td>
<td>61.3±12.1</td>
<td>59.2±11.6</td>
<td>0.51</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>25 (78%)</td>
<td>24 (86%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>13 (41%)</td>
<td>13 (46%)</td>
<td>0.65</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>14 (44%)</td>
<td>12 (43%)</td>
<td>0.94</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>5 (16%)</td>
<td>3 (11%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Family history of CAD, n (%)</td>
<td>12 (38%)</td>
<td>12 (43%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Prior family history of CAD, n (%)</td>
<td>14 (31%)</td>
<td>14 (36%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8±5.6</td>
<td>28.2±5.5</td>
<td>0.36</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.9±1.0</td>
<td>4.7±0.9</td>
<td>0.54</td>
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<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.2±1.0</td>
<td>2.9±0.8</td>
<td>0.26</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.1±0.3</td>
<td>1.2±0.6</td>
<td>0.46</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>1.4±0.6</td>
<td>1.6±1.2</td>
<td>0.42</td>
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<tr>
<td>Hemoglobin, g/L</td>
<td>142±14</td>
<td>147±14</td>
<td>0.18</td>
</tr>
<tr>
<td>WCC×10⁶/L</td>
<td>12.2±3.8</td>
<td>11.0±3.1</td>
<td>0.19</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>86±28</td>
<td>88±29</td>
<td>0.77</td>
</tr>
<tr>
<td>Admission SBP, mm Hg</td>
<td>135±26</td>
<td>140±26</td>
<td>0.45</td>
</tr>
<tr>
<td>Admission DBP, mm Hg</td>
<td>80±15</td>
<td>83±17</td>
<td>0.53</td>
</tr>
<tr>
<td>Admission HR, beats/min</td>
<td>76±14</td>
<td>77±16</td>
<td>0.80</td>
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<tr>
<td>Preinfarct angina, n (%)</td>
<td>10 (31%)</td>
<td>10 (36%)</td>
<td>0.63</td>
</tr>
<tr>
<td>STEMI, n (%)</td>
<td>11 (34.4%)</td>
<td>8 (28.6%)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

DFO indicates deferoxamine; CAD, coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; WCC, white cell count; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; and STEMI, ST-elevation-myocardial infarction.
Table 2. Procedural Characteristics

<table>
<thead>
<tr>
<th>Procedural Characteristics</th>
<th>Placebo</th>
<th>DFO</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preadmission medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASA</td>
<td>2 (6%)</td>
<td>1 (4%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>0 (0%)</td>
<td>2 (7)</td>
<td>0.12</td>
</tr>
<tr>
<td>β-blocker</td>
<td>3 (9%)</td>
<td>0 (0%)</td>
<td>0.10</td>
</tr>
<tr>
<td>ACE-I/ARB</td>
<td>9 (28%)</td>
<td>7 (25%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Statin</td>
<td>5 (16%)</td>
<td>2 (7%)</td>
<td>0.31</td>
</tr>
<tr>
<td>Infarct-related artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>11 (34.4%)</td>
<td>8 (28.6%)</td>
<td>0.63</td>
</tr>
<tr>
<td>LCx</td>
<td>6 (18.8%)</td>
<td>6 (21.4%)</td>
<td>0.80</td>
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<tr>
<td>RCA</td>
<td>14 (43.8%)</td>
<td>14 (50.0%)</td>
<td>0.54</td>
</tr>
<tr>
<td>Others</td>
<td>1 (3.1%)</td>
<td>0 (0%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Symptom-to-balloon time, min</td>
<td>204.1±79.2</td>
<td>226.6±68.7</td>
<td>0.25</td>
</tr>
<tr>
<td>Door-to-balloon time, min</td>
<td>79.9±44.3</td>
<td>83.0±37.2</td>
<td>0.77</td>
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<tr>
<td>TIMI flow</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pre-PCI TIMI flow</td>
<td>0.4±0.8</td>
<td>0.6±1.0</td>
<td>0.40</td>
</tr>
<tr>
<td>Post-PCI TIMI flow</td>
<td>3.0±0.2</td>
<td>3.0±0.2</td>
<td>0.93</td>
</tr>
<tr>
<td>No. of diseased vessels</td>
<td>1.8±0.8</td>
<td>1.6±0.7</td>
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<tr>
<td>Procedural medication</td>
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<tr>
<td>GP Iib/IIa receptor antagonist</td>
<td>25 (78%)</td>
<td>19 (68%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Thromboaspiration</td>
<td>10 (31%)</td>
<td>5 (18%)</td>
<td>0.44</td>
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<tr>
<td>Clopidogrel preloading</td>
<td>17 (53%)</td>
<td>13 (46%)</td>
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<tr>
<td>Intra-aortic balloon pump (%)</td>
<td>1 (3%)</td>
<td>3 (11%)</td>
<td>0.24</td>
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<tr>
<td>Stent type BMS, n (%)</td>
<td>20 (62.5%)</td>
<td>20 (71.4%)</td>
<td>0.84</td>
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<tr>
<td>DES, n (%)</td>
<td>9 (28.1%)</td>
<td>8 (28.6%)</td>
<td></td>
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<tr>
<td>Stent No.</td>
<td>1.1±0.5</td>
<td>1.4±0.6</td>
<td>0.02</td>
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<tr>
<td>Stent diameter, mm</td>
<td>2.89±0.40</td>
<td>3.09±0.38</td>
<td>0.06</td>
</tr>
<tr>
<td>Stent length, mm</td>
<td>18.0±5.9</td>
<td>21.5±6.6</td>
<td>0.04</td>
</tr>
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DFO indicates deferoxamine; ASA, aspirin; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; LAD, left anterior descending artery; LCx, left circumflex; RCA, right coronary artery; TIMI, Thrombolysis in Myocardial Infarction; PCI, percutaneous coronary intervention; GP, glycoprotein; BMS, bare metal stent; and DES, drug-eluting stent.

Further prespecified analyses of MI size according to ischemia (symptom-to-balloon) time and infarct-related artery location, the MI size was also similar in both groups (Figure 5).

Creatine kinase (15 086±12 749 versus 14 816±10 270 arbitrary units for area-under-the-curve) and troponin I (16 326±22 181 versus 14 535±15 950 arbitrary units) release after PPCI was similar between DFO and placebo groups (P=0.93 and 0.72, respectively; Figure 6). Peak creatine kinase and troponin I between DFO and placebo groups were 2304±1869 versus 2635±2234 IU/L (P=0.54) and 86.1±105.73 versus 74.28±71.12 μg/L (P=0.61), respectively. The mean ST-segment resolution between DFO and placebo groups was 67% versus 64%, respectively (P=0.67). Analysis of WMSI by echocardiography also showed no difference between the 2 groups with respect to the degree of wall motion abnormality (DFO, 1.46±0.34 versus placebo, 1.60±0.28; P=0.10).

MI Size and Salvage Assessment at 3 Months
At 3 months of follow-up, 16 (57%) DFO and 20 (62%) placebo patients returned for their CMRI (mean, 100±17 days). Neither infarct size nor MSI were different between DFO and placebo groups (Figure 7). Echocardiographic WMSI assessment also showed no significant difference between the 2 groups at 3 months (1.42±0.32 versus 1.41±0.32, P=0.85). In those who did not undergo CMRI assessment at 3 months but who completed echocardiographic assessment, there was also no difference in LV ejection fraction between DFO and placebo groups (50.3±8.1% versus 48.9±9.2%, P=0.76) nor WMSI (1.52±0.31 versus 1.39±0.39, P=0.51).

Clinical Follow-Up and Study Safety
We did not observe any serious adverse events related to DFO bolus administration or throughout the 12-hour infusion after PPCI necessitating cessation of the study medication. At 3 months, 1 patient in the DFO group died compared with 2 patients in the placebo group. Four patients in the DFO group were readmitted after discharge (2 with heart failure, 1 with nonischemic chest pain, and 1 with melena), whereas 2 patients in the placebo arm were readmitted with atypical chest pains not due to recurrent ischemia. The 3-month cumulative incidence of heart failure was similar between DFO and placebo groups: 5 (18%) versus 7 (23%) (P=0.70).

LV ejection fraction by CMRI in both DFO and placebo groups improved from baseline to 3 months (DFO, 49.1±10.1% to 56.0±11.6%, P=0.01; and placebo, 48.9±5.4% to 52.4±7.2%, P=0.01). Serum iron levels (18.6±4.6 versus 17.2±5.2 μmol/L, P=0.38) and C-reactive protein levels (1.6±1.2 versus 3.3±6.7 mg/L, P=0.32) were comparable between the 2 groups at 3 months.

Discussion
In this randomized, double-blind, placebo-controlled study using iron chelation with deferoxamine to target ischemia-reperfusion injury in patients with STEMI treated by PPCI, there was no significant difference observed in the primary end point of MI size whether determined by CMRI, cardiac...
biomarker release, ST-segment resolution, or echocardiographic WMSI. However, deferoxamine effectively decreased serum iron levels and oxidative stress as measured by plasma F2-isoprostanes after PPCI. These data show that despite achieving a reduction in oxidative stress with active treatment that MI size was not favorably affected.

Experimental studies suggest that ROS are the major biological mediator of ischemia-reperfusion injury. ROS can form during acute myocardial ischemia under low oxygen tension, reaching peak levels during the first few minutes after reperfusion. Iron, which is the most abundant transition metal in mammalian cells, exists predominantly in the bound form (with ferritin). However, the redox-active form can readily participate in the Fenton reaction to generate ROS to induce lipid peroxidation and cellular injury. Thus, redox-active iron released from macrophages and intracellular myocyte ferritin stores are potential targets for iron chelation by DFO, which has long been known to ameliorate lipid peroxidation due to its extremely high affinity constant for Fe3+. Additionally, DFO may also directly scavenge the OH- radical and improve endothelial function, which might improve coronary blood flow. Animal studies using DFO at the time of postischemic reflow resulted in greater recovery of myocardial function and energy metabolism. Importantly, iron in the effluent of treated rabbit hearts was in the form of Fe3+-DFO chelates, suggesting that DFO reached adequate levels in the myocardium. Studies in canines with DFO administration before the onset of ischemia also resulted in decreased infarct size. No clinical studies, to our knowledge, have tested the feasibility, safety, or effectiveness of DFO therapy in patients with STEMI treated by PPCI and other contemporary pharmacological therapies.

We administered a bolus dose of DFO with the aim of achieving sufficient plasma concentration at the time of reperfusion to maximally chelate iron and inhibit the burst of ROS production after reperfusion. The bolus dosage was chosen based on work by Nitenberg et al., who showed that 500 mg DFO led to improved coronary artery vasodilator...
responses in diabetic patients. Given the relatively short half-life of DFO (5–7 minutes\textsuperscript{25}) and possible ongoing ROS production during the acute inflammatory and prooxidant phase after STEMI associated with neutrophil infiltration,\textsuperscript{26} it was critical that DFO levels be maintained to continue myocardial iron chelation. Thus, we continued DFO therapy as a 12-hour infusion after PPCI to target ongoing iron-mediated ROS production and achieved consistently low levels of serum iron.

DFO treatment was well tolerated and safe, with no observed toxicity or adverse events attributable to either the bolus or infusion in the periprocedural period and up to 3 months of clinical follow-up. After the DFO bolus, serum iron levels were significantly reduced compared with the placebo group, with some patients achieving levels of

**Figure 5.** Day-3 myocardial infarction (MI) size according to ischemia time and MI location. MI size in the prespecified subgroups of ischemia time (symptom-to-balloon time) <180 or >180 minutes (A and B) and anterior or nonanterior MI (C and D). DFO indicates deferoxamine.

**Figure 6.** Creatine kinase (CK) and cardiac troponin I (cTnI) area-under-the-curve (AUC). Biomarkers were measured every 6 hours for 48 hours. **A**, CK AUC; **B** cTnI AUC. **Closed circles** indicate placebo; **closed squares**, deferoxamine (DFO). Data are presented as mean±SEM.

**Figure 7.** Myocardial infarction (MI) size and salvage at 3 months. MI size according to treatment for all patients who had cardiac MRI (A) and myocardial salvage index (B). LV indicates left ventricular; DFO, deferoxamine.
<1.0 μmol/L. Serum iron levels also remained significantly depressed for the duration of DFO infusion. Plasma F$_2$-isoprostane levels were chosen as the measure of oxidative stress because available evidence suggest they are the most sensitive and specific in vivo markers of lipid peroxidation in humans. In the DFO-treated group, plasma F$_2$-isoprostane levels were reduced significantly after the DFO bolus compared with the placebo group, suggesting greater amelioration of oxidative stress by DFO. However, we did not measure F$_2$-isoprostane levels at the end of the DFO infusion (owing to the complex sample preparation required out of hours), when one might have expected a further reduction in oxidative stress in the active treatment group. Notably, the degree of post-STEMI inflammation, as assessed by C-reactive protein plasma levels, was not different between the 2 groups before PCI, after PCI, at 48 hours or at 3 months.

Despite the observed reduction in oxidative stress after DFO bolus treatment, the primary end point of MI size was not reduced in the DFO group when measured by CMRI, cardiac biomarker release, or WMSI by echocardiography at 3 days and at 3 months. Indices of LV systolic function and volumes and LV mass were also not significantly different between the 2 groups at 3 days and at 3 months, though LV ejection fraction improved in both groups at 3 months. Prespecified analysis of MI size according to treatment, pre-PCI TIMI 0 flow, infarct-related artery location, and ischemia time also yielded similar MI sizes between the 2 groups, suggesting the DFO treatment had no effect on MI size in any subgroups. We also assessed the effectiveness of DFO in myocardial salvage by measuring the myocardial salvage index, which is increasingly being used to underscore the success of various reperfusion strategies without the need to employ large numbers of patients and has been suggested as a better surrogate end point than infarct size per se. However, myocardial salvage index measured at 3 days and at 3 months was also not different between the 2 groups.

There are several possibilities that may account for the lack of benefit with DFO treatment in our study. First, as there are no prior clinical studies of DFO therapy in the setting of STEMI, iron chelation may simply not be effective in this context in humans. Alternatively, iron-catalyzed ROS generation may not be a major contributor to ischemia-reperfusion injury in STEMI in humans. Previous cardiovascular studies with DFO in humans have been exclusively in setting of cardiopulmonary bypass, a potent stimulant of oxidative stress. However, cardiopulmonary bypass is a vastly different clinical scenario to STEMI. Second, the timing of therapy in relation to the onset of ischemia appears critical. In contrast to the study by Paraskevaidis et al and in animal studies, DFO administration in our study was after the onset of ischemia rather than before ischemia. The previously observed improvements in postischemic LV function and MI size when treatment was administered before ischemia may not consistently translate to a beneficial effect when given after ischemia or just before reperfusion, as evidenced from studies with other agents as well. Third, our patient population was different from cohorts treated in other recent positive studies. For example, we did not have an upper age limit and included a few patients (n=16) with pre-PCI TIMI 1, 2, and 3 flow (which may have represented aborted infarcts). We also did not exclude patients based on the presence of collaterals or prior chest pain (which may have induced ischemic preconditioning). Thus, our study population may have been a more heterogeneous cohort than in these other recent studies and may have reduced our chances of detecting a possible benefit with DFO therapy. Fourth, as DFO is poorly cell permeable and predominantly acts as an extracellular iron chelator, this can potentially limit the success of intracellular iron chelation and the reduction of intracellular ROS generation and cytotoxicity. Finally, it is possible that despite the use of a DFO bolus, DFO may not have reached the ischemic myocardium in sufficient concentration to reduce oxidative stress and limit ischemia-reperfusion injury immediately on reperfusion; a scenario similar to that observed with other agents.

Limitations

The principal limitation of this study is the relatively small sample size of 60 patients, which may be underpowered to detect the hypothesized 30% reduction in creatine kinase area-under-the-curve between the 2 treatment groups. However, we based our study size on trials using similar methodology and finding positive results. Also, post hoc power calculation using CMRI determined infarct size difference of 20±10% suggested that we would require a sample size of 45 per group to achieve a statistical power of 80% with a probability of a type I error of 0.05 to detect a 30% reduction in MI size with DFO. However, given the apparent lack of benefit observed with respect to MI size and myocardial salvage index across the treatment groups, infarct-related artery location, pre-PCI TIMI 0 flow, and ischemia time, both at 3 days and at 3 months, it is unlikely that a larger sample size would detect clinically significant differences with treatment. Another limitation is that only 60% of patients successfully completed their 3-month CMRI assessment. However, in those who did not undergo CMRI assessment at 3 months but who completed echocardiographic assessment, there was also no difference in LV ejection fraction or wall motion score index between DFO and placebo groups. We based our DFO dosing on prior clinical studies but these were performed in the non-STEMI setting. It is possible that our DFO dose may not have been optimal to achieve sufficient myocardial levels at the time of reperfusion as much higher doses of (up to 100 mg/kg) are used in patients with β-thalassaemia major with cardiac disease. Despite these concerns, we observed a reduction in serum iron and decreased plasma markers of oxidative stress with the DFO regimen used.

Conclusion

In summary, DFO treatment commenced after the onset of ischemia but before reflow in patients with STEMI treated by PPCI failed to limit infarct size and improve myocardial salvage despite prompt amelioration of oxidative stress.

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Disclosures

None.

References

Effect of Iron Chelation on Myocardial Infarct Size and Oxidative Stress in ST-Elevation–Myocardial Infarction


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