Intracoronary administration of darbepoetin-alpha at onset of reperfusion in acute myocardial infarction: Results of the randomized Intra-Co-EpoMI trial

Administration intracoronaire de darbépoétine-alpha au moment de la reperfusion au cours de l’infarctus aigu du myocarde : résultats de l’essai Intra-Co-EpoMI

François Roubille a,*, Antoine Micheau b, Stéphane Combes c, Séverine Thibaut k, Géraud Souteyrand d, Guillaume Cayla e, Laurent Bonello f,g, Nathalie Lesavre f,g, Catherine Sportouch-Dukhan a, François Klein b, Samir Berboucha h, Stéphane Cade e, Thien-Tri Cung a, Franck Raczka a, Jean-Christophe Macia a, Richard Gervasoni a, Frédéric Cransac a, Florence Leclercq a, Stéphanie Barrière-Lemaire j, Franck Paganelli f, Pascal Mottref d, Hélène Vernhet Kovacsik b, Michel Ovize j, Christophe Piot a

a Département de cardiologie, pôle cœur-poumon, hôpital Arnaud-de-Villeneuve, CHU de Montpellier, UFR de médecine, université Montpellier 1, CNRS, UMR-5203, institut de génomique fonctionnelle, Inserm U661, 371, avenue du Doyen-Gaston-Giraud, 34295 Montpellier cedex 05, France
b Département d’imagerie cardiovasculaire, pôle cœur-poumon, hôpital Arnaud-de-Villeneuve, CHU de Montpellier, UFR de médecine, université Montpellier 1, Montpellier, France
c Service de cardiologie, clinique Pasteur, Toulouse, France
d Pôle de cardiologie, CHU G. Montpied, UFR de médecine, université Clermont-Ferrand, Clermont-Ferrand, France

Abbreviations: AAR, area at risk; CK, creatine kinase; CMR, cardiac magnetic resonance; DA, darbepoetin-alpha; EPO, erythropoietin; IS, infarct size; MI, myocardial infarction; PCI, percutaneous coronary intervention; TIMI, thrombolysis in myocardial infarction.

* Corresponding author. Département de Cardiologie, CHU A. de Villeneuve, 371, avenue du Doyen-Gaston-Giraud, 34295 Montpellier cedex 05, France. Fax: +33 4 67 33 61 86.
E-mail address: francois.roubille@gmail.com (F. Roubille).
KEYWORDS
Acute myocardial infarction; Erythropoietin; Darbepoetin; Cardioprotection; Reperfusion injury

Résumé
Contexte. — Plusieurs essais qui ont étudié l’érythropoïétine (EPO) comme un nouvel agent protecteur lors de l’infarctus du myocarde (IDM), ont échoué à traduire en clinique les études précliniques prometteuses. Ces essais pourraient avoir laissé de côté des événements cruciaux au cours des premières minutes de la reperfusion. Notre étude diffère par une administration plus précoce, intracoronarienne d’une EPO à longue durée d’action, au moment de la reperfusion.

Objectifs. — L’objectif de cet essai était d’évaluer l’intérêt de la darbépoébine-alpha (DA) intracoronarienne administrée précocement à la reperfusion pour diminuer la taille de l’IDM.

Méthodes. — Nous avons randomisé 56 patients avec IDM aigu avec sus-élévation du segment ST, pour recevoir soit un bolus intracoronarien de 150 µg de darbépoébine-alpha (groupe DA) ou du sérum physiologique (groupe témoin) au moment de la reperfusion obtenue par angioplastie primaire (PCI). L’aire à risque et la taille de l’infarctus ont été évalués par des biomarqueurs, une imagerie par résonance magnétique (IRM) et des scores angiographiques validés.

Résultats. — Il n’y avait pas de différence entre les deux groupes en termes de durée d’ischémie, de flow TIMI à l’admission et après angioplastie, d’aire à risque ou encore de collatéralité, qui sont les principaux déterminants de la taille de l’infarctus. La libération de CK n’était pas significativement différente entre les deux groupes même après ajustement sur la taille de l’aire à risque. Après trois à sept jours et à trois mois, l’aire du rehaussement à l’IRM exprimée comme un pourcentage du ventricule gauche n’était pas significativement réduite dans le groupe DS, même après ajustement sur l’aire à risque.

Summary
Background. — Several trials investigating erythropoietin as a novel cytoprotective agent in myocardial infarction (MI) failed to translate promising preclinical results into the clinical setting. These trials could have missed crucial events occurring in the first few minutes of reperfusion. Our study differs by earlier intracoronary administration of a longer-acting erythropoietin analogue at the onset of reperfusion.

Aim. — To evaluate the ability of intracoronary administration of darbepoetin-alpha (DA) at the very onset of the reperfusion, to decrease infarct size (IS).

Methods. — We randomly assigned 56 patients with acute ST-segment elevation MI to receive an intracoronary bolus of DA 150 µg (DA group) or normal saline (control group) at the onset of reflow obtained by primary percutaneous coronary intervention (PCI). IS and area at risk (AAR) were evaluated by biomarkers, cardiac magnetic resonance (CMR) and validated angiographical scores.

Results. — There was no difference between groups regarding duration of ischemia, Thrombolysis in Myocardial Infarction flow grade at admission and after PCI, AAR size and extent of the collateral circulation, which are the main determinants of IS. The release of creatine kinase was not significantly different between the two groups even when adjusted to AAR size. Between 3–7 days and at 3 months, the area of hyperenhancement on CMR expressed as a percentage of the left ventricular myocardium was not significantly reduced in the DA group even when adjusted to AAR size.

Conclusion. — Early intracoronary administration of a longer-acting erythropoietin analogue in patients with acute MI at the time of reperfusion does not significantly reduce IS.
Background

Acute myocardial infarction (MI) is a leading cause of death worldwide. In patients that survive the immediate event, infarct size (IS) is the main determinant of further prognosis [1]. Rapid reperfusion of the ischemic myocardium remains the best treatment for limiting IS and further complications [2,3]. However, recent evidence supports the idea that reperfusion itself has the potential to initiate additional lethal injury [4–6]. New strategies that directly target the reperfusion phase by adding adjunct reperfusion therapies could further reduce IS and improve clinical outcomes of acute MI [7,8].

Erythropoietin (EPO), a cytokine synthesized by the kidney in response to hypoxia, is commonly used in the treatment of the anaemia of chronic renal failure [9]. Beyond its well-known haematopoietic action, EPO inhibits apoptosis and activates prosurvival kinases, such as PI3 kinase-Akt, in adult cardiomyocytes exposed to hypoxic injury [10–12]. Several reports indicate that administration of recombinant EPO during prolonged ischemia or at the time of reperfusion reduces IS and improves cardiac function in animal models of reperfused MI [10,11,13–15]. All these experimental data suggest that EPO may be a good candidate for an adjunct to reperfusion in MI patients.

However, several recent clinical trials, designed to investigate the safety and efficacy of EPO in patients with MI, have already been published with negative or inconclusive results [16–22]. In these trials, the timing of the cardioprotective strategy could have missed crucial events that occur in the first few minutes of myocardial reperfusion. Numerous studies performed over the past two decades testing new cardioprotective strategies have already failed to translate promising laboratory research into the clinical setting. Recently, the UCL-Hatter Cardiovascular Institute 6th International Cardioprotection Workshop with the Working Group of Cellular Biology of the Heart of the European Society of Cardiology and the 2010 National Heart Lung and Blood Institute Workshop have published recommendations in order to facilitate the translation of novel cardioprotective strategies [23]. The duration of ischemia, the Thrombolysis In Myocardial Infarction (TIMI) flow grade at admission and after reopening of the culprit coronary artery, the size of the area at risk (AAR), the extent of the collateral circulation and last but not least the optimal timing of the application of the drug are key issues that need to be addressed in IS reduction trials. To protect the myocardium from reperfusion injury, the drug must be active during the first minutes of reperfusion. However, we do not know for how long the exposure to the drug should continue in order to optimize the prevention of myocardial reperfusion injury.

The objective of the present study was to determine whether the early intracoronary administration of darbepoetin-alpha (DA), a long-acting analogue of EPO, at the onset of reperfusion reduces IS in patients with ongoing acute MI.

Methods

Trial

The Intra-Co-EpoMi Trial was a prospective multicentre randomized single-blinded controlled trial. The trial was designed, the data were collected and analysed, and the manuscript was written solely by the authors. DA for the trial was purchased with institutional grant support; the manufacturer had no role in the study. The trial was performed according to the Declaration of Helsinki (revised version of Somerset West, Republic of South Africa, 1996), the European Guidelines of Good Clinical Practice (version 11, July 1990) and French laws. The study protocol was approved by the ethics committee of the institution of the principal investigator (C.P.) acting on behalf of all the institutions involved in this trial. The protocol was accepted on 01 July 2008. All subjects gave written informed consent before inclusion.

Study population

Male and female patients aged ≥ 18 years who presented within 12 hours of onset of chest pain, had ST-segment elevation ≥ 0.1 mV in at least two contiguous leads and for whom the clinical decision was made to treat with PCI were eligible for enrolment. Patients were eligible for the study whether they were undergoing primary PCI or rescue PCI. The culprit coronary artery had to be occluded at the time of admission (TIMI flow grade 0) and had to be adequately reperfused (TIMI 2–3 flow grade) after PCI.

Patients with cardiac arrest, ventricular fibrillation, cardiogenic shock, stent thrombosis or previous MI were not included. Patients with evidence of coronary collaterals (Rentrop grade > 1) supplying the region at risk were excluded from the study. Also excluded were patients with known hypersensitivity to EPO, known polyclonial, uncontrolled hypertention or thromboembolic disease, contraindication to cardiac magnetic resonance (CMR) imaging, patients already treated with EPO or cyclosporine and women who were pregnant or who were of childbearing age and were not using contraception.

Experimental protocol

After the patients gave informed consent, they were randomly assigned to either the control group or the DA group. Randomization was performed with the use of a computer-generated randomization sequence. Numbered,

Conclusions. – L’administration précoce intracoronaire d’une EPO de longue durée d’action chez de patients avec IDM aigu, au moment de la reperfusion ne réduit pas significativement la taille de l’IDM.

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sealed envelopes that contained the study group assignment were distributed to each catheterization laboratory.

All patients received clopidogrel 600 mg orally, aspirin 500 mg and unfractionated heparin with or without a glycoprotein IIb/IIIa inhibitor intravenously prior to PCI. In the treated group, the patients received an intracoronary bolus injection of DA 150 μg (Ampen® Inc., Thousand Oaks, CA, USA) at the onset of reperfusion (i.e. less than 1 minute after reopening of the culprit coronary artery). The patients in the control group received an equivalent volume of normal saline. The dose of DA was chosen based on experimental data and previous clinical studies [24]. The PCI procedure was then completed according to the physician’s judgment. Postinterventional therapy consisted of clopidogrel 75 mg/day for at least 1 year. Aspirin 75 mg/day was recommended indefinitely. Other cardiac medications were prescribed at the discretion of the treating physician.

The patient and the assessors of data analysis and clinical outcomes were blinded to the treatment allocation.

Endpoints

The primary endpoint was MI size as determined by creatine kinase (CK) release obtained at admission and repeated over the following 3 days, as described previously [7,8]. Blood samples were taken at admission, every 4 hours after opening of the coronary artery during day 1 and every 6 hours on days 2 and 3. Area under the curve (arbitrary units) of serum CK release (Beckman Kit, expressed in IU/L) was measured in each patient by computerized planimetry (Image J 1.29x) and used as a surrogate marker of infarct size. The principal secondary endpoint was MI size as assessed between 3 and 7 days and at 3 months (±1 week) postinfarction by the area of hyperenhancement on CMR expressed as a percentage of the left ventricular myocardium [7].

Other endpoints

Major adverse events that occurred during the initial hospitalization and 3 months after acute MI, including death, heart failure, acute MI, stroke, recurrent ischemia, the need for repeat revascularization, renal or hepatic insufficiency, vascular complications and bleeding, were recorded.

Angiography

Coronary angiography was performed using standard techniques. All images were reanalysed in a core laboratory separately by two senior interventional cardiologists in a blinded fashion to classify TIMI flow before and after PCI.

For each patient, the size of the AAR (i.e. the myocardium supplied by the occluded coronary artery) was assessed by two angiographical scores. The modified version of the angiographical APPROACH score is based on pathological and necropsy studies evaluating the amount of myocardium supplied by different coronary arteries [25]. The angiographical BARI score is based upon an individualized assessment of the length and calibre of coronary arteries for the assessment of the jeopardized myocardium [26]. AAR was expressed as a percentage of the left ventricular myocardium.

CMR imaging

CMR imaging was performed between 3 and 7 days and repeated at 3 months (±1 week) using a 1.5T scanner (Avanto-Siemens, Erlangen, Germany) with infusion of 0.5ml/kg of gadoterate meglumine (Gd-DOTA, Dotarem; Guerbet SA, Paris, France). T2-weighted CMR was performed by encompassing the left ventricle in cardiac short-axis, vertical long-axis and four-chamber directions with a dark-blood T2-weighted short tau inversion recovery fast spin-echo sequence. Imaging variables were repetition time, 2 heartbeats; echo time, 100 ms; turbo factor 33; field of view, 350 mm; slice thickness, 8 mm. Both cine and contrast-enhanced short-axis CMR images were prescribed every 10 mm (slice thickness, 6 mm) from base to apex. In-plane resolution was typically 1.2 × 1.8 mm. Cine CMR was performed using a steady-state free-precession sequence. Contrast CMR images were acquired on average 5–10 minutes after contrast (power injector) using a segmented inversion recovery gradient echo three-dimensional technique, constantly adjusting inversion time to null normal myocardium, and acquisition in phase sensitive inversion recovery of 10 slices on a single breath-hold. Therefore, total acquisition time was between 30 and 40 minutes for all cine and contrast images. All patients were able to tolerate lying flat in the magnet until the examination was completed. The contrast dose was 0.1 mmol/kg.

CMR analysis

All images were evaluated separately by two blinded observers (seniors radiologists) and were analysed on an offline workstation (Argus, Siemens AG, Munich, Germany).

The AAR was quantified on the T2-weighted images by delineation of myocardium with hypersignal intensity from the CMR imaging performed between 3 and 7 days postinfarction, and the IS by delineation of hyperenhancement on the contrast-enhanced CMR performed between 3 and 7 days and at 3 months (±1 week) postinfarction. The hypoenhanced surface of microvascular obstruction and hypointense T2-weighted signal within the area of increased signal intensity (haemorrhagic infarction) were included in the IS. The transmurality degree of MI was quantified in four different groups (extent of contrast enhancement/normal myocardium: 0–25%; 25–50%; 50–75%; >75% of transmurality). Areas of hyperintense T2-weighted signal and late gadolinium enhancement on contrast enhancement sequences were reported separately on a 17-segment model of the left ventricle. IS and AAR results were expressed as a percentage of the total segment area. The IS/AAR ratio was calculated as follows: number of segments with IS/number of segments with T2-weighted hypersignal.

Statistical analysis

The number of patients to be included was calculated using the following hypotheses: an infantar size of 40% (reported to the area at risk); a reduction of 15% in the treated group (standard error of mean = 7.5%), with α = 0.05, β = 0.20; and 10% without a complete follow-up or without primary endpoint evaluation. On this basis it was necessary to include 55 patients.
The data processing was performed using SAS software package version 9.1. A general descriptive analysis was done for each variable of the study. The distribution of qualitative variables between groups was compared using the chi-square test. If one of the calculated frequencies in the contingency table did not exceed 5, Fisher’s exact test was performed. Quantitative variables were compared using the Wilcoxon-Mann-Whitney test as the variables were not normally distributed.

The Kendall test was used for the correlations between the areas at risk. The area under the curve was not calculated when there were two successive missing data points. When there was only one missing dose, its value was estimated by linear regression on convex-transformed data.

**Results**

**Characteristics of the population**

Between December 2008 and November 2009, 56 patients (10 women, 46 men) 59 ± 13 years of age were randomized in four academic medical centres (Clermont-Ferrand, Nîmes, Marseille and Montpellier): 30 received DA and 26 received placebo (Fig. 1). Five patients were excluded from the study: one because reperfusion had occurred before PCI (TIMI 2 flow grade on the initial coronary angiography) and four because of Rentrop 2 and 3 collateral coronary circulation to the AAR. Table 1 summarizes the main characteristics of the randomized population. Major risk factors, angiographical findings, left ventricular ejection fraction (LVEF) and treatments were similar between the two groups except for hypertension (P = 0.02).

**Main determinants of IS**

There was no difference between the two groups with regard to duration of ischemia (i.e. time from onset of chest pain to reperfusion) (P = 0.53) (Table 1).

The two groups were also similar with respect to the size of the AAR, whatever the score used (P = 0.37 with the CMR score; P = 0.13 with the BARI score; P = 0.32 with the modified APPROACH score) (Table 2).

All the patients had a Rentrop grade ≤ 1 and TIMI 2–3 flow grade was achieved in all patients after PCI (Table 1).

**Enzymatic IS**

The peak serum CK concentration after reperfusion was not significantly different in the DA group compared with in the control group (median 2048 vs. 2290 arbitrary units, respectively; P = 0.48) (Fig. 2A). In a subgroup of 34 patients, the median area under the curve for serum CK release after reperfusion was 3,328,418 units in the DA group and 3,959,095 in the control group (Fig. 2B). This difference was not significant (P = 0.08).

The two groups remained similar when the peak serum CK level was adjusted to the size of the AAR, whatever the score used (P = 0.77 with the CMR score; P = 0.26 with the BARI score; P = 0.29 with the modified APPROACH score) (Fig. 3A). Similar results were also obtained when the median area under the curve for serum CK release after reperfusion was adjusted to the size of the AAR, whatever the score used (P = 0.62 with the CMR score; P = 0.06 with the BARI score; P = 0.05 with the modified APPROACH score) (Fig. 3B).

**IS assessed by CMR**

Between 3 and 7 days after infarction, the area of hyper-enhancement on CMR expressed as a percentage of the left ventricular myocardium was not significantly different in the DA group compared with in the control group (median 29.89% vs. 29.05%, respectively; P = 0.60) (Fig. 4A). The two groups remained similar when the area of hyper-enhancement on CMR expressed as a percentage of the left ventricular myocardium was adjusted to the size of the AAR, whatever the score used (P = 0.25 with the CMR score; P = 0.80 with the BARI score; P = 0.75 with the modified APPROACH score) (Fig. 4B).
Table 1  Baseline characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EPO group (n = 27)</th>
<th>Control group (n = 24)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.77 ± 12.61</td>
<td>55.13 ± 11.84</td>
<td>0.03</td>
</tr>
<tr>
<td>Men</td>
<td>20</td>
<td>21</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>26.70 ± 3.95</td>
<td>25.96 ± 4.17</td>
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<tr>
<td>Hypertension</td>
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<td>4</td>
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<tr>
<td>Tabagism</td>
<td>15</td>
<td>19</td>
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<tr>
<td>Familial history of coronary artery disease</td>
<td>10</td>
<td>8</td>
<td>0.78</td>
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<tr>
<td>Diabetes</td>
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<td>0.10</td>
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<td>Overweight</td>
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<td>11</td>
<td>0.66</td>
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<tr>
<td>Dyslipidaemia</td>
<td>14</td>
<td>9</td>
<td>0.30</td>
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<td>Heart rate on admission (bpm)</td>
<td>73.04 ± 12.42</td>
<td>68.08 ± 12.91</td>
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<tr>
<td>Angiographical findings</td>
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<td>Ischemia time (minutes)</td>
<td>233.73 ± 174.49</td>
<td>308.57 ± 282.53</td>
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<td>Infarct-related artery</td>
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<tr>
<td>Common trunk</td>
<td>0/27</td>
<td>0/24</td>
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<tr>
<td>Left descending artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culprit lesion</td>
<td>10/27</td>
<td>7/24</td>
<td>0.43</td>
</tr>
<tr>
<td>Successful angioplasty</td>
<td>10/10</td>
<td>6/7</td>
<td>0.35</td>
</tr>
<tr>
<td>Circumflex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culprit lesion</td>
<td>5/27</td>
<td>7/24</td>
<td>0.08</td>
</tr>
<tr>
<td>Successful angioplasty</td>
<td>5/5</td>
<td>7/7</td>
<td>0.74</td>
</tr>
<tr>
<td>Right coronary artery</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Culprit lesion</td>
<td>9/27</td>
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<tr>
<td>Successful angioplasty</td>
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<td>10/10</td>
<td>0.75</td>
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<td>Collaterality (Rentrop ≥ 2)</td>
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<td>0</td>
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<td>LVEF (%)</td>
<td>51.54 ± 11.07</td>
<td>48.88 ± 10.90</td>
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<tr>
<td>Treatment before PCI</td>
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<tr>
<td>Aspirin</td>
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<tr>
<td>Clopidogrel</td>
<td>15</td>
<td>15</td>
<td>0.85</td>
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<tr>
<td>Glycoprotein IIb/IIla inhibitor</td>
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<tr>
<td>Heparin</td>
<td>16</td>
<td>12</td>
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<tr>
<td>LMWH</td>
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</tr>
<tr>
<td>Oral anticoagulant</td>
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<td>0</td>
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<tr>
<td>Statin</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
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<tr>
<td>Beta-blocker</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>ARI</td>
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<td>1.0</td>
</tr>
<tr>
<td>Anti-inflammatory drugs</td>
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<td></td>
</tr>
<tr>
<td>Treatment at time of PCI</td>
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<td></td>
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<tr>
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<td>0.44</td>
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<tr>
<td>Anti-inflammatory drugs</td>
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</tr>
</tbody>
</table>

Data are mean ± standard deviation, number or number/total number. ACE: angiotensin-converting enzyme; ARI: angiotensin II receptor inhibitor; BMI, body mass index; EPO: erythropoietin; LMWH: low molecular weight heparin; LVEF, left ventricular ejection fraction; PCI: percutaneous coronary intervention; STEMI: ST-segment elevation myocardial infarction; TIMI: thrombolysis in myocardial infarction. 

* First evaluation between third and seventh day after STEMI.
Table 2  Area at risk among patients, assessed by three different methods.

<table>
<thead>
<tr>
<th>Scoring method</th>
<th>Total n</th>
<th>EPO group (n=27)</th>
<th>Control group (n=24)</th>
<th>P</th>
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<tr>
<td></td>
<td></td>
<td>AAR^a n</td>
<td>AAR^a n</td>
<td></td>
</tr>
<tr>
<td>CMR scan T2 hypersignal</td>
<td>38</td>
<td>39.00 ± 13.4 19</td>
<td>43.95 ± 17.3 19</td>
<td>0.38</td>
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<tr>
<td>BARI score</td>
<td>49</td>
<td>29.41 ± 6.79 26</td>
<td>26.81 ± 6.07 23</td>
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<tr>
<td>APPROACH score</td>
<td>49</td>
<td>28.58 ± 6.76 26</td>
<td>27.54 ± 8.22 23</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. AAR: area at risk; CMR: cardiac magnetic resonance; EPO: erythropoietin.
^a Expressed as a percentage of the left ventricular myocardium.

![Graph](image)

Figure 2. Primary endpoint: level of serum creatine kinase (CPK) in patients treated with darbepoetin-alpha (DA) and in patients in the control group after successful reperfusion: (A) peak and (B) area under the curve (AUC). NS: not significant.

At 3 months after infarction, the median area of hyperenhancement on CMR expressed as a percentage of the left ventricular myocardium was 26.06% in the DA group and 28.01% in the control group; this difference was not significant (P = 0.63) (Fig. 5A). The two groups remained similar when the area of hyperenhancement on CMR expressed as a percentage of the left ventricular myocardium was adjusted to the size of the AAR, whatever the score used (P = 0.21 with the CMR score; P = 0.51 with the BARI score; P = 0.64 with the modified APPROACH score) (Fig. 5B).

Adverse clinical events

During the initial hospitalization, seven serious adverse clinical events were recorded in the DA group: one death in a patient with a large anterior infarct, three cases of reversible thrombocytopenia in patients on epoetin alpha, one episode of atrial fibrillation, one acute stent thrombosis and one local haematoma. No serious adverse event was reported in the control group.

During non-hospital follow-up, one death was recorded in the DA group (unknown metastasized colon cancer diagnosed during the initial hospitalization). There were two adverse clinical events in the control group: one episode of ventricular fibrillation and one symptomatic hypotension.

Discussion

In this small-sized clinical trial, which was designed in complete accordance with the Hatter Workshop and the National Heart Lung and Blood Institute Workshop Recommendations, the early intracoronary administration of a longer-acting EPO analogue in patients with acute MI at the time of reperfusion was not associated with a smaller IS, assessed by measuring both the release of cardiac biomarkers and the area of hyperenhancement on CMR postinfarction.

DA is a long-acting EPO analogue with favourable effects on IS and postMI remodelling in rodents (mice [27–33], rats [15,30–32,34–39] or rabbits [40–42]). Baker et al. demonstrated that single intravenous DA treatment confers immediate and sustained cardioprotection in rats when administered after the onset of ischemia and at the start of reperfusion [34]. They observed reduced myocardial necrosis in a dose-dependent manner. Optimal protection was manifest at a dose of 2.5 μg/kg. In a clinically relevant porcine model of ischemia-reperfusion, intravenous administration of a larger dose of DA (30 μg/kg) 5 minutes prior to reperfusion led to increased capillary density, decreased interstitial fibrosis and significant regional functional improvement despite a lack of reduction in the IS [14]. In the first pilot clinical study, Lipsic et al. demonstrated that one bolus of DA 300 μg administered intravenously before primary PCI was safe and well tolerated [24]. However, patients enrolled in the main clinical trials that have been recently published received either epoetin alpha or epoetin beta [16–22]. These studies concluded that EPO administration in acute MI patients was safe except for the trials with the highest dose of EPO, which showed a tendency towards an increased incidence of adverse events and microvascular obstruction [16–18].

The chosen dose should obviously be discussed. In these clinical trials, doses were lower than those administered in experimental studies (3000–5000 IU/kg most often).
Very few experimental studies focused on dose-response. In rats receiving darbepoeitin 0.25–25 μg/kg intravenously, 15 minutes before ischemia, the optimal dose seemed to be 2.5 μg/kg (i.e. 175 μg in a man weighing 70 kg) [34]. In clinical trials, doses ranged from 30,000–60,000IU, roughly 500–1000 IU/kg for a patient weighing 70 kg, except one small study testing a low-dose protocol (6000IU) [21]. Only two studies adapted the dose to the individual weight [20,43], so that could introduce an additional bias in other trials. None of the studies tested several different doses. More importantly, the doses should be compared with caution, as different routes and timings of administration are probably even more important as regards pharmacokinetics and molecular targets. In our study we chose a dose by analogy with experimental models and previous clinical trials. We aimed for a high-dose to provide a bolus effect, especially as the intracoronary route was favourable for this purpose. Nevertheless, smaller or higher doses, or even repetitions of the doses, could be of interest.
Intracoronary administration of darbepoetin-α at onset of reperfusion in AMI

The timing of the cardioprotective strategy is critical in the design of clinical studies. Although available data are surprisingly sparse, there appears to be a consensus regarding the fact that the time window of protection is quite narrow because the main part of lethal reperfusion injury takes place within the first minutes of reflow after prolonged ischemia. Protective interventions must be applied either intravenously prior to the opening of the culprit coronary artery or locally at the onset of reperfusion. In this study, we chose to deliver DA early via the intracoronary route at onset of reflow (i.e. as soon as we got an angiographic TIMI flow grade of 2 or 3 in the culprit coronary artery) in order to obtain a local peak level of DA < 1 minute after adequate reperfusion. Local delivery of DA via the coronary arteries may increase local drug concentration, improve drug efficacy and reduce general side effects. As we do not know how long after reflow the exposure to the drug should continue, the administration of a long-acting drug may help to optimize the prevention of myocardial injury. It is important to note that EPO was intravenously injected from several minutes to several hours after PCI in most previous clinical trials (when reperfusion injury has already occurred) except in the study by Ludman et al., in which it was injected before PCI [16–22]. Patients in whom the infarct-related coronary artery has spontaneously recanalized (TIMI > 1) should also be systematically excluded. One patient was withdrawn from our study because reperfusion had occurred before PCI. It is of interest to note that up to 17% of patients in the EPO/AMI-1 study and 30% of patients in the REVIVAL-3 study had an initial TIMI flow grade > 1 before PCI [19,20].

The duration of ischemia, the amount of collateral circulation to the ischemic tissue and the size of the AAR are the main determinants of the IS. In our study, the duration of ischemia averaged 279 minutes in the DA group compared with 236 minutes in the control group (P not significant) and was similar to what is usually seen in clinical studies investigating cardioprotective strategies in acute MI [7,8,17–19,21,22]. Most of these studies, except for the REVIVAL-3 study, included patients who presented less than 12 hours following the onset of chest pain. The presence of coronary collateralization to the jeopardized myocardium is also a well-known confounding factor in the measure of IS. Four patients with visible coronary collaterals on angiography at admission (Rentrop grade > 1) were excluded from our study. This important issue was not taken into account in previous trials designed to investigate efficacy of EPO in MI patients [16–19,21,22]. The AAR represents also a major determinant of the final IS. Both the BARI score and the modified APPROACH score can be used clinically to accurately estimate the anatomical myocardial AAR in patients with acute MI [44]. T2-weighted CMR imaging to determine myocardial oedema constitutes an emerging surrogate of estimating the AAR [45]. However, it is of interest to note that the AAR was not measured in most of the previous clinical trials, except in the studies by Ludman et al. and Prunier et al., in which the AAR was assessed by CMR and angiography scores, respectively [16,20]. In this study, IS assessed by measuring both the release of cardiac biomarkers and the area of hyperenhancement on CMR was adjusted to the size of the AAR using three different scores (i.e. the CMR score, the BARI score and the modified APPROACH score).

Study limitations

Although we took the major determinants of IS into consideration in the experimental design of this trial in accordance with the Hatter Workshop Recommendations, the present study still has several limitations. First, it was a single-blinded trial. Second, it was a study examining a small number of patients. Third, the EPO group was significantly older and more patients had hypertension. As age, hypertension and left ventricular hypertrophy might interfere with pharmacological conditioning [46], these limitations could have hampered the ability to detect a difference. Comorbidities could impact on the myocardial sensitivity to cardioprotective strategies. Fourth, both angiographical and CMR scores have limitations for the assessment of the AAR. A recent study that aimed to determine the effect of post-conditioning on myocardial oedema in patients with ST-segment elevation MI suggested that the CMR score might underestimate the AAR in the treated group but not in the

Figure 5. A. Area of hyperenhancement on cardiac magnetic resonance (CMR) measured 3 months after infarction and expressed as a percentage of the left ventricular myocardium in patients treated with darbepoetin-alpha (DA) and in patients in the control group after successful reperfusion. B. Area of hyperenhancement on CMR adjusted to the size of the area at risk with three different scores (CMR score, BARI score and modified APPROACH score). NS: not significant.
control group, leading to the protective effect being either missed or underestimated [20]. Fifth, recruitment lasted for 1 year, introducing probable heterogeneity in clinical practices, but such difficulties appear frequently in this kind of emergency clinical trial. Finally, myocardial IS estimated from biomarkers and imaging is considered only as a surrogate for clinical outcome.

Conclusion

In summary, the early intracoronary administration of a longer-acting EPO analogue in patients with acute MI at the time of reperfusion does not significantly reduce IS.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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