Release of biomarkers of myocardial damage after direct intramyocardial injection of genes and stem cells via the percutaneous transluminal route

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Received 12 October 2007; revised 9 May 2008; accepted 15 May 2008; online publish-ahead-of-print 4 June 2008

Aims
We aimed to quantify the release of biomarkers of myocardial damage in relation to direct intramyocardial injections of genes and stem cells in patients with severe coronary artery disease.

Methods and results
We studied 71 patients with ‘no-option’ coronary artery disease. Patients had, via the percutaneous transluminal route, a total of 11 ± 1 (mean ± SD) intramyocardial injections of vascular endothelial growth factor genes (n = 56) or mesenchymal stromal cells (n = 15). Injections were guided to an ischaemic area by electromechanical mapping, using the NOGA™/Myostar™ catheter system. Plasma CKMB (upper normal laboratory limit = 5 mg/L) was 2 mg/L (2–3) at baseline; increased to 6 (5–9) after 8 h (P, 0.0001) and normalized to 4 (3–5) after 24 h. A total of eight patients (17%), receiving a volume of 0.3 mL per injection, had CKMB rises exceeding three times the upper limit, whereas no patient in the group receiving 0.2 mL had a more than two-fold CKMB increase. No patient developed new ECG changes. There were no clinically ventricular arrhythmias and no death.

Conclusion
NOGA mapping followed by direct intramyocardial injections of stem cells or genes lead to measurable release of cardiac biomarkers compared with NOGA mapping alone. The increase in biomarkers was related to the injected volume.

Keywords
Stem cells therapy • Gene therapy • Myocardial enzymes • NOGA system • Intramyocardial injection

Introduction
Patients who present with advanced occlusive coronary artery disease and refractory angina and no options for medication improvements, or revascularization with percutaneous coronary intervention (PCI), or coronary artery by pass grafting (CABG), are a challenge in clinical cardiology.1,2 A number of experimental therapies aiming to induce neovascularization in ischaemic areas of the myocardium have been suggested. Therapies with direct intramyocardial injections of genes encoding vascular growth factors or stem cells from the bone marrow have been evaluated in patients with coronary artery disease. However, the results have been conflicting.3–4 Direct intramyocardial injections of bone marrow-derived stem cells and angiogenic genes in patients have been reported to be safe in small patient populations.5 Though, there have been some doubts on the safety of intramyocardial injections of skeletal myoblasts and, also, in a recent report in an animal model, ventricular arrhythmias were not frequent, in relation to intramyocardial injections of bone-marrow-derived stem cells.4–8

We aimed to study the safety of direct intramyocardial injections of stem cells or genes, via the percutaneous transluminal route, in patients with severe coronary artery disease, and in particular to quantify the release of bio-markers of myocardial damage.

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Methods

Data from all 71 patients included in our centre in controlled clinical trials with direct intramyocardial injections of genes or stem cells were included in the analyses. We compare these data with those of a control group of 25 patients who after their combined NOGA and injection procedure had a prescribed 3 months follow-up diagnostic NOGA-mapping procedure (mapping only, without injection) per protocol. These 25 patients belonged to one of the four studies analysed (EUROINJECT One Trial).3 Forty-eight patients were included in two previously published studies using intramyocardial injections of genes encoding vascular endothelial growth factor VEGF-A165.9,10 The gene therapy studies were approved by the Ethical Committee (02-078/00, 02-053/01) and Danish Medicines Agency (2612-1490, 2612-1782).9,10 Eight patients came from the terminated multi-centre NOVA™ trial, a randomized, double blind, placebo-controlled study evaluating the efficacy of BIOBYPASS® (AdG,VEGF121.10NH). The NOVA™ trial was approved by the Ethical Committee (KF 02 084/04), the Danish Medicines Agency (Eudra CT 2004-001250-91), and registered in www.clinicaltrial.gov (NCT00215696). In the NOVA trial, a total of 17 patients were included before the company terminated the trial due to a business decision. We included eight of the 17 patients in the trial at our site. It is these eight patients who were included in the present analyses. The study was still blinded when performing the present analyses.

Fifteen patients came from an ongoing trial evaluating the safety and efficacy of direct intra-myocardial injection of autologous in vitro expanded mesenchymal stromal cells. Also, this study is approved by the Ethical Committee (KF 02-268856), the Danish Medicines Agency (Eudra CT 2005-002657-45), and registered in www.clinicaltrial.gov (NCT00260338). The trial is ongoing and the analyses included all patients enrolled in the study at the time of the present analyses.

The unpublished and ongoing studies followed the same recruitment procedures of the published studies. The end point for these studies (NOVA trial unpublished and MSC trial ongoing) is to evaluate the effect of the gene or stem cells therapy on the myocardial function. During follow-up, all patients had undergone non-invasive tests (SPECT and echocardiogram) to assess the ejection fraction and the regional left ventricular motion after treatment. Secondary end point (SPECT and echocardiogram) to assess the ejection fraction and the regional left ventricular motion after treatment. Secondary end point is to evaluate the safety of the treatment. All patients received oral and written information about the studies and signed an informed consent.

Participating patients

The patients included have been selected, treated, and followed by the Cardiac Catheterization Laboratory, Department of Cardiology at Rigshospitalet. All four studies have the same inclusion and exclusion criteria. Patients included had no options for conventional revascularization therapy, and also had the following characteristics. (1) Revers-ible ischaemia at stress single photon emission computerized tomography (SPECT). (2) A myocardial thickness of 8 mm or more in the injection area measured by echocardiography. (3) At least one coronary artery or patent graft from which new collaterals/vessel could be supplied. (4) Canadian Cardiovascular Society class 2 or 3 angina.

Excluded patients had (i) ejection fraction <40%, (ii) unstable angina pectoris, (iii) acute myocardial infarction within the last 3 months, (iv) diabetes mellitus with proliferative retinopathy, or (v) been diagnosed or suspected to have cancer disease or any other serious concomitant disease.

All the patients were treated with conventional drug therapy according to guidelines.11,12

Intramyocardial injection procedures

A dedicated team of two senior cardiologists and three nurses performed all procedures using the NOGATM Unix® system (Biosense Webster A/S, Johnson and Johnson) or, from 2006 and onwards, the NOGATM XP® system (Biologic Delivery SystemsTM, Cordis Corporation) and the 8-french-sized Myostar® mapping-injection catheter. Intramyocardial injections were administered, as previously described, slowly (30–40 s) into an ischaemic area of the left ventricle with a thickness of >8 mm.9,10 The injection area was delineated by combining information from an electromechanical map, a single photon emission scinintigraphy, and a recent coronary angiography. In 25 patients, we performed a protocol defined diagnostic NOGA™ follow-up 3 months after the injection procedure to evaluate the effect of the treatment. After each procedure, an echocardiography, focusing on pericardial effusion, was performed. Patients were discharged the day after the injection/diagnostic NOGA™ procedure.

Blood samples

All enrolled patients had a blood test done before the invasive procedure. After the invasive procedure, the blood samples for plasma CKMB and TNT measurements were collected after 8 and 24 h. If the CKMB level after 24 h was above the upper normal limit (5 μg/L) then blood sampling was repeated until the normalization of the parameter. A CKMB level of more than three times the upper laboratory limits (5 μg/L) was considered a procedural myocardial infarction.13,14

Arrhythmia monitoring

All patients were ECG-monitored for 24 h in-hospital by special dedicated staff with no other assignments besides ECG monitoring. The morning after the procedure a report on the ECG monitoring and on eventual arrhythmias were presented to the cardiologist responsible for the medication and the discharge of the patient. Ventricular extra systoles were not quantified.

Statistical analysis

Continuous variables with a normal distribution are presented as mean (± standard deviation) and the variables with a non-Gaussian distribution are presented as median (first quartile–third quartile). The qualitative variables are presented as numbers and percentages. Change in CKMB and TNT following intramyocardial injections (Figure 1) was analysed in an ANOVA with the repeated measure as a within-subject factor. The Greenhouse-Geisser (Box) correction was applied to account for compound symmetry. To account for the type of procedure (Figure 2), this was subsequently included into the repeated measures ANOVA as a between-subjects factor.

Associations between (i) products injected, (ii) volume per injection, (iii) total volume injected and change in CKMB was analysed by three independent one-way ANOVA/ANCOVA.

Independent-sample Student’s t-test has been used to compare unpaired groups and paired Student’s t-test to compare two paired groups for the variables with a Gaussian distribution. The variable with a non-Gaussian distribution has been tested by the non-parametric Mann–Whitney test for two unpaired groups and Wilcoxon signed-rank test for two paired groups.

All data were analysed using SPSS statistical analysis program (SPSS version 15.0, SPSS Inc., Chicago, Il, USA) using two-sided tests. Values of P < 0.05 were considered significant.
Results

Baseline characteristics

The four trials, in which the patients were included and treated with either genes encoding VEGF or mesenchymal stromal cells, are summarized in Table 1. Clinical baseline characteristics and procedural data are shown in Table 2.

Cardiac marker release

We found a small and brief, but significant plasma CKMB (P < 0.001) and TNT (P < 0.001) increase after the combined NOGA\textsuperscript{TM} mapping and injection procedure (Figure 1). There was a significant increase of 6.8 μg/L (95% CI 4.6–9.0) and 0.12 μg/L (95% CI 0.08–0.16) for CKMB and TNT, respectively, from baseline to 8 h post-procedure. CKMB then normalized 24 h after the procedure (Figure 1A). Plasma TNT also decreased 24 h post-procedure, but it was still above normal limit (Figure 1B).

A subgroup of 25 patients had both a NOGA\textsuperscript{TM} mapping and injection procedure, and at 3 months had a follow-up diagnostic NOGA\textsuperscript{TM} mapping procedure without injection. We found a significant main effect of the type of procedure (injection or mapping alone) on increase in CKMB (P = 0.008). Also, a significant interaction between time and type of procedure was obtained (P = 0.001) (Figure 2), indicating a significantly larger increase in CKMB following injection compared with mapping alone. This was further confirmed by a post hoc analysis showing a mean difference in the increase of 9.6 μg/L (95% CI 4.1–15.1) from baseline to 8 h post-procedure.

There was no difference in enzyme release between patients who had 0.2 mL of mesenchymal stromal cell solution vs. patients who had 0.2 mL of adVEGF injections (5 (4–6) and 7 (3.8–8.5), respectively, P = 0.27). Patients, who received 0.2 mL (n = 23)
volume for each injection, had a significant lower enzyme release than patients who received 0.3 mL injection volume \( n = 48 \) (Figure 3A and 8). We found no correlation between procedure time and CKMB release \( P = 0.76, R^2 = 0.003 \). However, there was a tendency towards higher CKMB values with increasing total volume injected per procedure (Figure 3C). Only volume injected at each site was significantly associated with CKMB rise by univariate analysis.

A total of eight patients had CKMB values more than three times the upper limit of CKMB and five of them had an increase more than five times the upper limit of CKMB, \( > 25 \mu g/L \), at 8 h after the injection procedure. All these patients received 0.3 mL per injection. The mean of total volume injected and the number of injections for these patients were \( 3.2 \pm 0.16 \) and \( 10.6 \pm 0.55 \), respectively. The patients were followed in protocols for 12 months without development of any serious cardiac event.

**NOGA\textsuperscript{TM}, injection procedure and short-term follow-up**

The correlation between electromechanical mapping procedure time and team experience and between injection procedure time and team experience is depicted in Figure 4. This figure shows a significant negative correlation between procedure time and increasing team experience.

**Discussion**

The main finding of the present study is that a cardiac enzyme release can be measured 8 h after a direct intramyocardial injection procedure. This enzyme release seems related to the volume injected. The enzymes normalized the day after the procedure. Intraventricular catheter movements, in relation to a diagnostic electromechanical mapping procedure, which preceeds intramyocardial injections, do not seem to lead to enzyme release exceeding the normal upper limits. The procedure time decreases with the increasing experience of the operators. However, there was no relation between the procedure time and the changes in plasma enzymes.

Although significant, the measured cardiac enzyme release was small, for CKMB in average in the order of twice the upper normal limit, and there were no signs of clinical important side effects to this minor myocardial trauma. Guidelines of percutaneous coronary interventions consider a procedural myocardial infarction of importance to prognosis, and a CKMB increase of 3 or 5 times the upper laboratory limit as significant\textsuperscript{13,14}. In our study, 17% of patients treated with a volume of 0.3 mL per injection developed a more than three times increase of CKMB. Whereas, patients receiving 0.2 mL per injection did not have CKMB release exceeding two times the upper normal limit. Furthermore, our data seem to show that a volume of 0.3 mL per injection leads to an increase in CKMB, which is more than twice the increase after an injection of 0.2 mL (Figure 3B). Therefore, it seems that injection volumes of 0.2 mL are safer than 0.3 mL. It could be considered as a limitation in the study or a benefit for the study, that two of the studies included used 0.3 mL for each injection, and two studies used 0.2 mL for each injection. As outlined in Figure 3, this leads to heterogeneity in CKMB release between the studies. It is our hypothesis that this heterogeneity is caused by the difference in volume injected and not the study, because similar patients were included and treatment staff as well as the equipment were the same. A few patients had CKMB increases more than 3 and 5 times the upper normal limit.

**ECG-monitoring post-procedure and clinical follow-up**

There were no procedural myocardial perforations, and no post-procedural pericardial effusions. One patient developed atrial fibrillation, most likely induced by the electromechanical mapping catheter. This arrhythmia persisted for some hours in the post-procedural period, but converted spontaneously to sinus rhythm before discharge the following day. One patient with a known second degree AV block had progression to third degree AV block requiring permanent pacemaker therapy. Injections in this patient were performed in the lateral wall. Two patients needed antibiotic treatment in the immediate follow-up period. There were no ventricular tachycardia reported post-procedure. No patient required a change of medication due to ventricular arrhythmias or cardioverter/defibrillator implantation in the follow-up period. In the first 6 months after the procedures, there was no sudden cardiac death. One patient died in this period by progressive heart failure.

**Table 2** Demographic characteristics and procedural data

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n = 71</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>64 (57–69)</td>
</tr>
<tr>
<td>Sex male, n (%)</td>
<td>61 (86)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>34 (48)</td>
</tr>
<tr>
<td>Hypercholesterolaemia, n (%)</td>
<td>71 (100)</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>34 (48)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>17 (24)</td>
</tr>
<tr>
<td>Previous PCI, n (%)</td>
<td>44 (62.5)</td>
</tr>
<tr>
<td>Previous CABG, n (%)</td>
<td>70 (98.4)</td>
</tr>
<tr>
<td>EF SPECT</td>
<td>55 (50–60)</td>
</tr>
<tr>
<td>CCS Class</td>
<td>3 (3–3)</td>
</tr>
<tr>
<td>NYHA class</td>
<td>0 (0–2)</td>
</tr>
<tr>
<td>CABG number</td>
<td>1 (1–2)</td>
</tr>
<tr>
<td>PCI number</td>
<td>1 (0–2)</td>
</tr>
<tr>
<td>Max exercise time (s)</td>
<td>479 (356.3–599.8)</td>
</tr>
<tr>
<td>Angina pectoris attaches per week</td>
<td>8.3 (3.5–20)</td>
</tr>
<tr>
<td>Nitroglycerine tablets per week</td>
<td>9.1 (3.5–18.3)</td>
</tr>
<tr>
<td>NOGA-mapping time (min)</td>
<td>52 (28.3–66.8)</td>
</tr>
<tr>
<td>NOGA-mapping points</td>
<td>92.5 (75.8–103.3)</td>
</tr>
<tr>
<td>Myostar injection time (min)</td>
<td>37 (30–44.5)</td>
</tr>
<tr>
<td>Myostar injection points</td>
<td>11 (10–12)</td>
</tr>
</tbody>
</table>

ML, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; EF, ejection fraction; SPECT, single photon emission computed tomography; CCS, Canadian Cardiovascular Society functional classification of angina; NYHA, New York Heart Association classification. Quantitative variables are expressed as percentage. Qualitative variables are expressed as means \pm SD.
The patients were followed in protocols for 12 months without development of any serious cardiac event. Therefore, the CKMB increase was not related to any clinical event, but was likely a consequence and indication of the intramyocardial delivery of a treatment substance. A diagnostic NOGA-mapping procedure only without injection, as performed at follow-up in the EUROINJECT One Trial, induces a small increase in CKMB below the normal limit, indicating that this procedure is safe.

These results might serve as a reference in the planning of future studies using intramyocardial injections.

Four other relatively small clinical studies on a total of 43 patients have used intramyocardial injections of a volume of 0.3 mL or more.15–18 Results are contradictory. Losordo et al.16 injected a total of 6 mL plasmid VEGF-2 using six injections in 19 patients and found no CKMB rise above the normal limit. Whereas, Fuchs et al.15 found minor CKMB and TNT rises in half of their 10 patients receiving nine injections of 0.3 mL AdVEGF-121 each. Vale et al.17 and Briguori et al.18 did not report data on cardiac biomarkers in their injection studies using 1 and 0.5 mL per injection, respectively.

Recently, an animal experiment using rats weighing 150–200 g, reported an increase in ventricular arrhythmias after direct intramyocardial injection of mononuclear bone-marrow cells, when compared with retrograde intracoronary delivery.6 They injected a total volume of 0.2 mL intramyocardially, which probably compares with 25–50 times the intramyocardially injected volumes in the human studies mentioned in Table 3.19–23 Therefore, we are tempted to hypothesize, based on the observations presented in Figure 3, that the injected volume matters for the myocardial damage produced in this study, and that the arrhythmias found...
could be, at least partly, explained by myocardial damage secondary to the relatively large injection volume used. However, several differences in morphological and electrophysiological properties exist between rat and human myocardium and for this reason it might be difficult to compare the different reaction with the actual noxa.24

In none of the clinical studies on intramyocardial injections of VEGF and bone-marrow cells in patients presented in Table 3 there were increased tendency to arrhythmias. However, experimental and clinical studies on intramyocardial transplantation of skeletal myoblast, which repeatedly have documented engraftment in scar tissue, and associated improvement in left ventricular function might lead to malignant arrhythmic events and need of cardioverter/defibrillator treatment. These arrhythmias are late and do not seem to be related to the injection trauma.4,5 No arrhythmia was detected in the present studies.

A limitation in the analyses is that dose and study are correlated. Thus, if we adjust for study, we will also indirectly adjust for dose. However, it was an objective to test if dose was a predictor and that cannot be tested if we at the same time adjust for it. This poses the limitation that we cannot with certainty say if dose or study is the independent predictor. However, since NOGA procedure design was identical between the trials, we find it most likely that the dose is the independent predictor. Another limitation is that the clinical impact of the CKMB rise during NOGA injection procedures is unknown. However, it seems from the present data that it could be identical to the low impact of CKMB rise during percutaneous coronary interventions.

**Conclusions and implications**

Direct intramyocardial injections of genes and stem cells seem relatively safe. Injections are accompanied by a minor release of cardiac biomarkers of myocardial damage. The cardiac enzyme rise is related to the volume injected. If injection volume is limited to 0.2 mL per injection, and the number of injections from 10 to 12, the plasma CKMB level never exceeds three times the upper normal limit. Using this injection regimen, patients would not be considered having had a procedural myocardial

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**Figure 4** Correlation between diagnostic NOGA-mapping procedure time and operator’s experience (A) and between injection time and operator’s experience (B). 95% CIs indicated.

**Table 3** Studies with direct intramyocardial injection using the NOGA system

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients, n</th>
<th>Injection, n</th>
<th>Volume injection, mL</th>
<th>Injection therapy</th>
<th>Reported CKMB release after procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Losordo et al.16</td>
<td>19</td>
<td>6</td>
<td>1</td>
<td>ph VEGF-2</td>
<td>None</td>
</tr>
<tr>
<td>Fuchs et al.15</td>
<td>10</td>
<td>9</td>
<td>0.3</td>
<td>AdVEGF121</td>
<td>&lt;1.5 × upper limit</td>
</tr>
<tr>
<td>Perin et al.19</td>
<td>23</td>
<td>15</td>
<td>0.2</td>
<td>BM cells</td>
<td>NA</td>
</tr>
<tr>
<td>Tse et al.20</td>
<td>8</td>
<td>15 ± 5.4</td>
<td>0.1</td>
<td>BM cells</td>
<td>None</td>
</tr>
<tr>
<td>Fuchs et al.21</td>
<td>27</td>
<td>12</td>
<td>0.2</td>
<td>BM cells</td>
<td>NA</td>
</tr>
<tr>
<td>Vale et al.17</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>ph VEGF-2</td>
<td>None</td>
</tr>
<tr>
<td>Briguori et al.18</td>
<td>10</td>
<td>11 ± 1.8</td>
<td>0.5–1</td>
<td>BM cells</td>
<td>NA</td>
</tr>
<tr>
<td>Perin et al.22</td>
<td>21</td>
<td>15</td>
<td>0.2</td>
<td>BM cells</td>
<td>NA</td>
</tr>
<tr>
<td>Losordo et al.23</td>
<td>24</td>
<td>10</td>
<td>0.2</td>
<td>BM cells</td>
<td>NA</td>
</tr>
<tr>
<td>Kastrup et al.9</td>
<td>32</td>
<td>10.7 ± 0.7</td>
<td>0.3</td>
<td>ph VEGF-A 165</td>
<td>See Results section</td>
</tr>
<tr>
<td>Ripa et al.10</td>
<td>16</td>
<td>10.6 ± 0.5</td>
<td>0.3</td>
<td>VEGF-A165 + G-CSF</td>
<td>See Results section</td>
</tr>
</tbody>
</table>

phVEGF2, naked plasmid DNA encoding for vascular endothelial growth factor 2; AdVEGF121, replication-deficient adenovector encoding VEGF-A121; BM MNC, bone marrow mononuclear cells; G-CSF, granulocyte colony stimulating factor.
infarction, and extended hospital stay, control diagnostic angiography, and anticoagulation regimens would not be required.

Conflict of interest: J.K. is consultant for Biologics Delivery Systems, Cordis Corporation. The authors have nothing else to declare.

Funding

Biologics Delivery Systems, Cordis Corporation and GenVec Inc. was the initiator and sponsor of the NOVA trial. Private funding from The Danish Heart Foundation, The Lundbeck Foundation, Ax-e and Einar Danielsen Foundation, The Toyota Foundation, Brdr. Hartmans Foundation, Saren and Helene Hampers Foundation Research Foundation at Rigshospitalet and Danish Medical Research Council sponsored the other trials. Cordis Europe, Johnson & Johnson sponsored the injection catheters to Eureinjo One trial.

References


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