Insulin-like growth factor I and II preserve myocardial structure in postinfarct swine

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Abstract

Background—Insulin-like growth factors (IGF) I and II improve myocardial function after coronary occlusion in different animal models.

Objectives—To investigate the mechanism of improved myocardial function after administration of IGF-I or IGF-II in acute myocardial infarction.

Methods—Female pigs (mean (SD) weight 25 (5) kg) were subjected to acute myocardial infarction by microembolisation with 75–150 µm affigel blue beads. The beads contained and slowly released 150 µg/pig of IGF-I (n = 6), IGF-II (n = 6), or pig albumin (n = 6). Echocardiography, perfusion imaging, and haemodynamic measurements were performed before infarction and during four weeks after infarction. Regional wall motion of different left ventricular segments was scored semiquantitatively on the basis of a three point scoring system, from normal = 0 to dyskinesia = 3. Serum cardiac troponin I concentration was measured before, immediately after, and three hours after the infarct. Excised hearts were analysed for actin, desmin, blood vessel density, and DNA ladder within the infarct, border, and normal myocardial areas.

Results—Myocardial function of the infarct related area improved significantly during the four weeks of follow up in both the IGF groups (p = 0.01). Myocardial perfusion, heart rate, and blood pressure were similar in all the animals during the study. Treated animals had lower serum cardiac troponin I concentration (p = 0.001), more actin in the border area (p = 0.01) and infarct area (p = 0.0001), and reduced DNA laddering in the infarct area compared with the controls (p < 0.05). IGF groups had more blood vessels in the border area (p = 0.04) and the infarct area (p = 0.003).

Conclusions—Both types of IGF improved myocardial function and the improvement was associated with preservation of myocardial structure. IGF-I was more effective than IGF-II.

Keywords: myocardial infarction; growth factors; ventricular function; troponin I
IGF-II (150 µg/pig), and six received pig albumin (150 µg/pig). Echocardiographic functional studies were performed before and after infarction and during the four week follow up period of the study.

INDUCTION OF ACUTE MYOCARDIAL INFARCTION
The animals were premedicated with 2–3.5 mg/kg azaperone intramuscularly. After intravenous administration of thiopentone (thiopental) sodium (10–12 mg/kg), general anaesthesia was achieved using halothane 1.5–2% in oxygen, inhaled through an endotracheal tube. Left coronary artery catheterisation was performed through the femoral artery using the Seldinger technique. An over-the-wire intracoronary balloon (2.0 or 2.5 mm) was advanced into the distal left anterior descending coronary artery or into one of its branches. After the balloon had been inflated to 6 atm for 30 seconds, 50 µl of affigel blue non-biodegradable beads (75–150 µm, Bio-Rad Laboratories, California, USA) suspended in 0.5 ml of saline were injected through the distal lumen of the balloon catheter to induce a microembolisation infarct of the distal left anterior descending coronary artery territory. The experimental groups received beads containing recombinant human IGF-I or IGF-II (150 µg/pig), while the control group received beads containing pig albumin (150 µg/pig). IGF-II was given in phosphate buffered saline (PBS) and IGF-I was freeze dried and dissolved in 0.1 M acetic acid, after which it was diluted in PBS to 5 mg/ml. Thus the beads were used both to induce acute myocardial infarction and to provide a gradual (slow) release delivery system of the peptides (unpublished data).

ECHOCARDIOGRAPHY
To evaluate regional and global left ventricular function, parasternal long and short axis cross sectional echocardiography (77020A Ultrasound, Hewlett Packard, Andover, Massachusetts, USA) was carried out under general anaesthesia at baseline (preinfarction), immediately after the infarct, and two hours, two weeks, and four weeks after the infarct. Short axis echocardiographic images obtained at the basal, mid-papillary muscle, and apical levels were recorded on half inch videotapes and analysed by two unbiased investigators. Left ventricular cavity area, myocardial thickening, and myocardial shortening were measured to assess left ventricular global myocardial function. Images were divided into septal, anterior, lateral, posterior, and inferior wall segments within the left ventricle, on the basis of the recommendations of the American Society of Echocardiography. Abnormalities of regional wall motion in the different left ventricular segments were scored as follows: 0, normal; 1, hypokinesia; 2, akinesia; 3, dyskinesia. In this three point scoring system, the segmental wall motion is inversely related to the score. Calculations of global left ventricular function were based on the summation score of all segments for each animal at each time period. No regional dysfunction following myocardial infarction was observed at the basal parts of the heart and no changes were observed in global left ventricular function; thus these “segments” received the score of 0 (normal motion) and are not reported in detail.

PERFUSION IMAGING
To evaluate regional and global left ventricular perfusion, technetium-99m tetrofosmin was injected intravenously (12 mCi). A 10 minute image was obtained in the anterior oblique projection 30 minutes after the injection. Using a mobile gamma camera (Apex 215M, Elscint, Haifa, Israel), imaging was performed before myocardial infarction, immediately after myocardial infarction, and two and four weeks later. At the four week time period, myocardial perfusion was also measured following adenosine administration. Adenosine was given until a 30% reduction in mean systolic blood pressure was recorded. Perfusion images were obtained at basal, mid-papillary muscle, and apical levels.

Data were stored on magnetic disks for subsequent analyses. Images at each time period and at each level were projected on a computer screen and visually divided into septal, anterior, lateral, posterior, and inferior left ventricular wall segments. An unbiased investigator made a qualitative assessment of the perfusion of each left ventricular segment according to a standard technique. In this five point scoring system, the segmental perfusion is inversely related to the score and directly related to the damage in myocardial perfusion, with normal = 0.

HAEMODYNAMIC MEASUREMENTS
Arterial blood pressure was monitored continuously through a catheter positioned in the femoral artery and recorded at baseline (preinfarction), immediately after the infarct, two hours after the infarct, and four weeks later, with measurements during adenosine administration at that time as well, using a physiological pressure transducer (60–800 Trantec, Baxter Health Corporation, Uden, Holland) connected to a polygraph system (Nihon Kohden, Tokyo, Japan). Heart rate was monitored and recorded at the same time as blood pressure.

SERUM TROPOinin
For serum cardiac troponin I evaluation, venous blood samples from each animal were collected at baseline, immediately after infarction (20–30 minutes), and three hours after infarction. The samples were centrifuged for five minutes at 1400g, and the separated serum from each animal was frozen at −80°C. A microparticle enzyme immunoassay was used to determine cardiac troponin I concentration in the serum samples. Quantitative determination was performed on the AxSYM system (Abbott Diagnostics, Abbott Park, Illinois, USA), using their troponin I reagent pack for in vitro diagnostic use.

PATHOLOGICAL PREPARATION
Four weeks after the infarction the animals were reanaesthetised and killed. The chest was opened and the heart was quickly excised and removed. Samples were collected from representative macroscopically determined infarct areas (white), peri-infarct border areas (white
with red), and normal areas (red) (fig 1). The samples from normal myocardial areas were collected from the opposite side to the infarcted left ventricular wall. The samples (0.5–1.0 g) were immediately frozen in liquid nitrogen and stored at −80°C, or cut and fixed in 10% buffered formaldehyde and embedded in paraffin for subsequent analyses. In order to verify that the material collected was appropriately defined, all stained slides were microscopically analysed by an unbiased pathologist.

**BLOOD VESSEL DENSITY**

Slides from each myocardial area in each animal (5 µm thick) were stained with factor VIII (Von Willebrand factor, VWF) antibody (1:200), using the Histostatin-SP Zymed kit (San Francisco, California, USA). Nine fields on each slide representing each myocardial area were randomly chosen for counting stained blood vessels. Counting was performed using a grid inserted into the eyepiece (1 cm² divided into 10 × 10 squares of 1 mm², at ×400 magnification). All stained vessels with lumens dissected by the vertical lines were counted. Vascular counts for each slide were calculated as the sum of the counts in nine fields. The number of blood vessels per area in each group is presented as mean (SD).

**MYOCARDIAL ACTIN AND DESMIN**

Slides were stained with monoclonal mouse IgG2a α-actin and monoclonal mouse IgG, κ α-desmin, respectively, using the Histostatin-SP Zymed kit. The quantitative evaluation of actin and desmin stained areas was performed at ×150 magnification. The slides were projected on a PC image monitor, using a Galai Scannaray/Supercue-3 computerised coloured image analyser (Galai, Migdal Haemek, Israel). Nine fields of 23.55 × 10³ µm² from each myocardial area of each animal were randomly chosen for evaluation. The values of actin and desmin were expressed as a percentage of the total stained area at each field and presented as the mean (SD) of nine fields.

**DNA LADDERING**

The frozen myocardial samples were used for biochemical analyses. To identify and quantify DNA fragmentation associated with myocyte death, ligation mediated polymerase chain reaction (PCR) of blunt DNA ends was used, as described previously.

For densitometric analysis, 20 µl of PCR products for each lane were loaded on 4% polyacrylamide gels, which was followed by electrophoresis, staining with a silver staining kit (Amersham Pharmacia Biotech, Buckinghamshire, UK), photography, and scanning with a scanner linked to a computer. Only bands between 150 and 1000 base pairs were considered to be fragmented DNA and served as a marker for cell death. The integrated optical density value for each lane was calculated using a Gel-Pro Analyze software (Media Cybernetics, Des Moines, Iowa, USA). Only lanes obtained on the same gel were evaluated and compared. All PCR and electrophoretic experiments were performed in duplicate. The control group signal served as the reference for

![Image of myocardial areas stained with Masson-trichrome](http://heart.bmj.com/content/86/6/693)

**Figure 1** Example of macroscopic (left panel) and microscopic (right panel) examination. Infarct, border, and normal myocardium stained with Masson-trichrome: viable myocardium stains red, collagen stains blue (magnification ×200). Macroscopic picture shows the left ventricle (LV), right ventricle (RV), and septum (S). White area of the left ventricle represents scar tissue from which the “infarct” tissue sample was taken. The “normal” tissue sample was taken from the contralateral wall, and the “border” tissue sample from the area bridging the scar and the normal myocardium.
Table 1. Changes in mean blood pressure following adenosine administration in IGF-I treated, IGF-II treated, and control animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Before adenosine</th>
<th>After adenosine</th>
<th>Per cent reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I</td>
<td>85 (11)</td>
<td>64 (9)</td>
<td>30 (3)</td>
</tr>
<tr>
<td>IGF-II</td>
<td>84 (9)</td>
<td>64 (9)</td>
<td>32 (6)</td>
</tr>
<tr>
<td>Control</td>
<td>78 (13)</td>
<td>56 (11)</td>
<td>30 (8)</td>
</tr>
</tbody>
</table>

*p value 0.447

Data are mean (SD).

HEART RATE

The heart rate was similar in all animals at baseline (107 (12) vs 117 (15) vs 112 (18) beats/min for IGF-I, IGF-II, and control groups, respectively (p = 0.54)). Myocardial infarction caused an increase in heart rate in all animals, with no difference among the three groups (115 (14) vs 120 (21) vs 116 (10) beats/min for IGF-I, IGF-II, and control groups, respectively (p = 0.84)). No significant differences were observed in heart rate among the three groups during the postinfarction follow up or following adenosine administration.

SERUM TROPOVIN

Serum cardiac troponin I concentration in the three groups was statistically similar before and immediately after infarction. However, a significantly higher serum cardiac troponin I concentration was detected three hours after the infarction in the control group, and this increase was abolished in IGF-I and IGF-II treated animals (1.9 (1.2) vs 1.9 (1.2) vs 8.0 (3.9) ng/ml for IGF-I, IGF-II, and control groups, respectively (p = 0.001) (fig 3)).

ACTIN AND DESMIN

Figure 4 shows immunostaining for actin (upper panel) and desmin (lower panel) within normal, border, and infarct areas (magnification ×200). In the normal myocardium there were no differences in the actin content among the three groups. However, in the border and infarct areas the actin content was higher in IGF-I and IGF-II treated animals than in the control group (36.7 (6.4)% vs 30.5 (3.8)% vs 25.4 (4.8)% (p = 0.01), and 4.5 (0.2)% vs 4.4 (0.9)% vs 2.1 (0.9)% (p = 0.0001) for IGF-I, IGF-II, and control animals in the border and infarct areas, respectively (fig 4).

STATISTICAL ANALYSIS

The two way analysis of variance with repeated measures was used for comparison between IGF-I, IGF-II, and control animals. A two sample Student t test assuming unequal variances was used to compare the IGF-I and IGF-II groups. The results are presented as mean (SD) (or mean (SE) for DNA laddering), and a probability value p ≤ 0.05 was considered significant.

Figure 2. Comparison of myocardial wall motion abnormality in IGF-I, IGF-II, and control groups during the four week follow up period. Pre-MI, baseline; post-MI, immediately after myocardial infarction; 2 hours post, two hours after infarction; 2 weeks post, two weeks after infarction; 4 weeks post, four weeks after infarction. *Significant difference from control group, p = 0.01.

Figure 3. Comparison of serum cardiac troponin I (cTnI) concentrations in IGF-I, IGF-II, and control groups at baseline (pre-MI), immediately after myocardial infarction (post-MI), and three hours after infarction (3 hours post-MI). *Significant difference from control, p = 0.001.

Results

MYOCARDIAL FUNCTION

Segmental myocardial wall motion of IGF-I and IGF-II treated animals improved significantly during the four week period after the myocardial infarct: 0.3 (0.4) vs 0.6 (0.7) vs 1.2 (0.6) (p = 0.01) for IGF-I, IGF-II, and control, respectively (fig 2).

MYOCARDIAL PERFUSION

Before the myocardial infarction, myocardial perfusion was normal in all the animals. Infarction resulted in a myocardial perfusion defect that was evident in all animals. No significant differences in myocardial perfusion within the injured area were observed among the three groups, either during the four week postinfarction period (2.38 (0.75) vs 2.88 (0.98) vs 2.25 (1.38) for IGF-I, IGF-II, and control groups, respectively (p = 0.94)), or after adenosine administration (1.88 (1.16) vs 2.00 (2.12) vs 1.90 (1.59) for IGF-I, IGF-II, and control groups, respectively (p = 0.70)).

BLOOD PRESSURE

No significant differences were seen in systolic/diastolic and mean blood pressures among the three groups of animals before infarction, immediately after infarction, or four weeks after infarction (85 (11) vs 84 (9) vs 78 (13) mm Hg for mean blood pressure in IGF-I, IGF-II, and control groups, respectively (p = 0.094)), or after adenosine administration (1.88 (1.16) vs 2.00 (2.12) vs 1.90 (1.59) for IGF-I, IGF-II, and control groups, respectively (p = 0.70)).
infarct areas, respectively) (fig 5A). Moreover, the IGF-I treated group had more actin in the border area than the IGF-II treated animals (36.7 (6.4)% vs 30.5 (3.8)%, respectively (p = 0.03)).

In the normal and border areas there was no difference in desmin content among the three groups. However, the infarct area of both IGF-I and IGF-II groups had a larger desmin content than control animals (7.0 (0.9)% vs 7.1 (1.0)% vs 4.4 (0.8)%, for IGF-I, IGF-II, and control groups, respectively (p = 0.0002) (fig 5B)).

Figure 4 Immunostaining with monoclonal mouse IgG2a α-actin (upper panel) and monoclonal mouse IgG, κ α-desmin (lower panel) of normal, border, and infarct areas. Lower staining intensity is observed within infarcted and border myocardial areas than in the normal area. A typical diffgel blue bead microsphere is also shown.

Figure 5 (A) Comparison of the actin content in the normal, border, and infarct myocardial areas of IGF-I, IGF-II, and control groups. *Significant difference from control group in the border area (p = 0.01) and the infarct area (p = 0.0001). **Significant difference between IGF-I and IGF-II groups in the border area (p = 0.03).

(B) Comparison of desmin content in the normal, border, and infarct areas of IGF-I, IGF-II, and control groups. *Significant difference from control group in the infarct area (p = 0.0002).

Figure 6 Immunostaining with factor VIII (Von Willebrand factor, VWF) antibody of normal, border, and infarct areas. Larger numbers of positively stained endothelial cells are seen within the infarcted myocardial area than in the border and normal areas.

IGF-I and IGF-II preserve myocardial structure
number of DNA strand breaks is present in the control animal. No differences were observed between IGF-I and IGF-II in any myocardial area, neither was there any difference among the three groups in the normal and border areas. However, both IGF-I treated (23.3 (8.5)%) and IGF-II treated animals (33.3 (8.3)%) showed reduced DNA laddering in the infarcted area compared with the controls (100%) (p < 0.05).

Discussion

Our study shows that the exogenous administration of IGF-I and IGF-II improves regional myocardial function of injured regions in an experimental swine model of acute myocardial infarction. Although a beneficial effect of IGF variants on myocardial function has been reported previously, this is the first study comparing their effects in the same animal model of acute myocardial infarction and exploring some of the underlying mechanisms of these effects.

IGF-I AND IGF-II EFFECTS ON MYOCARDIAL FUNCTION

Accumulating evidence has indicated that IGF variants play a specific role in the intricate cascade of events of cardiovascular function, in addition to their well established growth promoting and metabolic effects.21 The effects of exogenous administration of IGF-I and IGF-II have recently been investigated.21 In a study performed by Bisi and colleagues, subcutaneous administration of a low dose of recombinant human IGF-I significantly increased basal left ventricular ejection fraction in healthy human adults.22 Intravenous administration of recombinant human IGF-I improved cardiac performance in patients with chronic heart failure by afterload reduction and possibly by positive inotropism.23 In these studies, IGF-I did not cause any haemodynamic changes.22,23 Similarly, in the present study, IGF-I administration caused a significant improvement in resting myocardial function without haemodynamic changes. Thus several investigations including our own study have shown improved myocardial function in different settings of normal and pathological conditions following IGF-I administration.

In contrast to IGF-I, there are very limited data on IGF-II administration and its effects on the infarcted myocardium. Vogt and colleagues have shown that myocardial infarction was significantly decreased by direct intramyocardial infusion of IGF-II in a swine model of acute occlusion and reperfusion.24 In a previous study from our laboratory, Battler and colleagues showed improved left ventricular function following IGF-II administration, using the same animal model as in the present work.9 We found that IGF-II caused an increase in regional myocardial function; however, this was smaller than that achieved with IGF-I. Thus at the doses given in the present study (150 µg/pig of IGF-I and IGF-II), both IGF variants
exerted cardioprotection following acute myocardial infarction. However, additional pharmacokinetic and pharmacodynamic studies are warranted.

**Preservation of Myocardial Structure**

The concentration of serum cardiac troponin I detected in our present study was significantly lower in IGF-I and IGF-II treated animals compared with non-treated animals three hours after the infarction. Recently, the serum concentration of heart specific contractile troponin I has been found to be a highly sensitive marker for the detection of myocardial cell death within the first few hours of acute coronary syndromes. Thus a reduced serum cardiac troponin I concentration suggests that the IGFs attenuate infarct induced myocardial cell injury during the early postinfarction period.

We have also detected more actin and desmin in the infarct area and more actin in the border area of IGF treated animals four weeks after infarction. The combination of increased contractile and cytoskeletal proteins in these areas, and reduced serum cardiac troponin I concentration, may reflect myocardial preservation by the IGFs. Furthermore, both IGF variants were found to stimulate protein synthesis in cardiomyocytes in vitro, an additional mechanism that may be responsible for these findings.

**Proposed Mechanism of the IGF Effect**

In ventricular biopsies from patients with isolated aortic stenosis and patients with aortic regurgitation, IGF-I formation was positively related to the velocity of circumferential fibre shortening. In animals, IGF-I exerted a positive inotropic effect by increasing the availability of ionised calcium, and increased the shortening magnitude of myocytes isolated from the left ventricle of healthy adult mongrel dogs and dogs with heart failure. Thus the improvement in myocardial function caused by IGF administration in the present study may be at least partially explained by increasing the availability of [Ca2⁺]. In addition, in our present study the improvement in myocardial function was associated with preservation of myocardial structure in postinfarction IGF-I and IGF-II treated animals.

The mechanisms of preservation of myocardial structure after infarction may fall into three broad categories: the first is increasing myocardial oxygen supply following infarction; the second is decreasing myocardial oxygen demand following infarction; and the third is preventing the initiation of events induced by myocardial infarction that lead to irreversible cell damage. As no significant differences in myocardial perfusion, blood pressure, or heart rate were observed among the three groups during the study, any increase in myocardial oxygen supply or decrease in myocardial oxygen demand was less likely to occur in the IGF-I and IGF-II treated animals. Thus it is conceivable that IGF administration preserved myocardial structure and so improved myocardial function in the treated animals by the third mechanism.

**Limitations**

Our study examined the effects of exogenous IGFs in a patchy type of myocardial infarction. This is different from the usual human form of myocardial infarction caused by occlusion of an epicardial coronary artery. The extrapolation of the results of the current study to the “homogeneous” type of myocardial infarction should be done with caution.

Myocardial perfusion was analysed and evaluated by planar scintigraphic imaging, at a resolution of approximately 10 mm. Thus changes in small vessel circulation may go undetected. Systems with higher resolution...
should be used to examine microperfusion following myocardial infarction in this model.

We examined only a few myocardial cytoskeletal and contractile proteins in the present study. While these showed promise as possible contributors to the preserved myocardial function in IGF treated hearts, a more complete investigation should be conducted to consolidate the findings.

DNA laddering is not an adequate method for determining the type of cell death. Thus additional tests should be performed for specific definition of the mechanism of cell death in this model (that is, necrosis, apoptosis, or oncosis)\(^\text{17}\).

**CONCLUSIONS**

Intracoronary administration of IGF-I and IGF-II in the postinfarction pig model caused a significant improvement in myocardial function during the early phase of myocardial infarction. This was associated with preservation of myocardial structural and contractile proteins in the infarct and border areas. These findings may form the basis for investigating the role of IGFs in the treatment of acute myocardial infarction.

The study was performed by A-KK as part of a PhD degree fellowship at Sackler Faculty of Medicine, Tel-Aviv University. We thank Pharmacia AB, Sweden, for providing us with IGF-I and IGF-II. We thank the Joan and James Constantin Institute for Molecular Genetics of Tel-Aviv University for providing us with partial financial support.