Radionuclide angiocardiographic evaluation of the cardiovascular effects of recombinant human IGF-I in normal adults

Gianni Bisi, Valerio Podio, Maria Rosa Valetto, Fabio Broglio, Giovanni Bertuccio, Graziano Del Rio, Muni F Boghen, Ferruccio Berti, Eugenio E Muller and Ezio Ghigo

Division of Nuclear Medicine and Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Turin, Italy, Division of Endocrinology and Metabolism, University of Modena, Modena, Italy and Department of Pharmacology, University of Milan, Milan, Italy

(Correspondence should be addressed to G Bisi, Divisione di Medicina Nucleare, Università di Torino, Ospedale Molinette, C.so Dogliotti 14, 10126 Torino, Italy)

Abstract

Objective: IGF-I possesses specific myocardial receptors and is able to promote cardiac remodelling and even inotropic effects in both humans and other animals. In fact, reduced cardiac mass and performance are present in GH deficiency and these alterations are counteracted by recombinant human (rh) GH replacement, restoring IGF-I levels. Recently, the acute administration of 60 μg/kg rhIGF-I has also been reported to be able to improve cardiac performance evaluated by echocardiography or impedance cardiography in normal subjects. The aim of our study was to verify the effects of a subcutaneous low dose of rhIGF-I (20 μg/kg) on cardiac performance in humans.

Methods: In six healthy male adults (mean ± S.E.M.: 35.7 ± 4.3 years of age), the effects of rhIGF-I on left ventricular function evaluated by radionuclide angiocardiography and on circulating IGF-I, GH, insulin, glucose and catecholamines levels were studied.

Results: Administration of rhIGF-I increased circulating IGF-I (peak at +150 min vs baseline: 330.2 ± 9.6 vs 199.7 ± 8.7 μg/l, P < 0.03) to levels which persisted similarly up to +180 min. Neither GH nor catecholamine levels were modified by rhIGF-I administration, while insulin and glucose levels showed a slight but significant decrease. Basal left ventricular ejection fraction (61.8 ± 2.0%) significantly increased at +180 min after rhIGF-I (65.3 ± 2.7%, P < 0.03). No change was recorded in mean blood pressure while a non-significant trend towards a reduction of heart rate was present by +120 min.

Conclusions: These findings indicate that even subcutaneous administration of a low dose of rhIGF-I has acute inotropic effects as evaluated by radionuclide angiography in healthy adults.

European Journal of Endocrinology

Introduction

Recent evidence indicates that the activity of the growth hormone (GH)/insulin-like growth factor (IGF-I) axis has important influences on myocardial function, which may explain cardiac abnormalities evidenced in GH hyper- and hyposecretory states in both humans and other animals (1).

IGF-I, as well as GH, possesses specific receptors at the myocardial level (1–3). IGF-I synthesis in and release from myocardial tissue have been demonstrated and they are probably involved in the auto/paracrine actions of the peptide (4–11). In addition to growth-promoting and metabolic actions, IGF-I has specific cardiovascular effects. It specifically stimulates heart, but not skeletal muscle growth, induces mRNA expression for specific contractile proteins, myocyte hypertrophy and contractility and has cardioprotective effects (11–21). In fact, reduced cardiac mass and performance are present in GH deficiency but are counteracted by a recombinant human (rh) GH replacement therapy restoring IGF-I levels (1, 22–26). More recently, in keeping with animal data (15, 16, 27, 28), acute intravenous infusion or subcutaneous rhIGF-I administration to healthy subjects has also been reported to be capable of improving cardiac performance evaluated by impedance cardiography or echocardiography respectively (29, 30). The intravenous infusion of 60 μg/kg IGF-I over 3 h induced overt systemic side-effects in addition to metabolic changes (29) while the same rhIGF-I dose injected subcutaneously did not induce side-effects or modify glucose but inhibited insulin levels as well as the GH response to physical exercise (30).

Based on the foregoing, the aim of our study was to evaluate the effects of a low rhIGF-I dose (20 μg/kg s.c.)
on cardiac performance in healthy adults using radionuclide angiocardiography. Radionuclide imaging was chosen because of its high reliability in evaluating cardiac performances (31, 32).

The effects of rhIGF-I on circulating IGF-I, GH, insulin, glucose and catecholamine levels were also studied.

**Materials and methods**

**Study design and protocol**

Six healthy male volunteers (mean ± S.E.M.; age, 35.7 ± 4.3 years; body mass index, 22.4 ± 0.5 kg/m²) gave their informed consent to the study which had been approved by the local, independent ethical committee. No subject had any history of hypertension, cardiovascular, renal, respiratory, hepatic or metabolic diseases and none was taking medication. Physical examination, blood pressure, electrocardiographic and echocardiographic findings were also normal.

On the day of the study, subjects had breakfast at 0800 h and were admitted to the study room 2 h before the beginning of the testing session (1600 h). The study room was maintained in constant conditions of temperature and light, and in the absence of noise.

An antecubital vein was cannulated for blood sampling and kept patent by slow infusion of isotonic saline. ECG was continuously monitored in lead II. Blood pressure was monitored with an automated apparatus (SpaceLabs Inc., Redmond, WA, USA). Forty-five minutes after relaxing in the recumbent position, all subjects underwent subcutaneous administration of rhIGF-I (Pharmacia & Upjohn, Stockholm, Sweden; 20.0 μg/kg at 0 min). Haemodynamic and hormonal parameters were evaluated basally and then every 30 min from +90 up to +180 min.

**Hormonal parameters: analytical methods**

Serum IGF-I levels (μg/l) were measured in duplicate by RIA (Nicholls Institute Diagnostics, San Juan Capistrano, CA, USA) after acid–ethanol extraction to avoid the interference of binding proteins. The sensitivity of the assay was 0.2 μg/l. The inter- and intra-assay coefficients of variation were 8.8–10.8% and 5.0–9.5% respectively. In our laboratory, the 3rd and 97th centile limits of normal IGF-I in adulthood are 65 and 385 μg/l respectively.

Serum GH levels (μg/l) were measured in duplicate by IRMA (hGH-CTK; Diasorin Biomedica, Saluggia, Italy). The sensitivity of the assay was 0.15 μg/l. The inter- and the intra-assay coefficients of variation were 2.9–4.5% and 2.4–4.0% respectively.

Serum insulin levels (μU/ml) were measured in duplicate by RIA (INSIK-5; Diasorin Biomedica). The sensitivity of the assay was 4.0 μU/ml. The inter- and intra-assay coefficients of variation were 5.9–6.3% and 3.5–8.7% respectively.

Serum glucose levels (mg/dl) were measured by a gluco-oxidase colorimetric method (Menarini Diagnostics, Florence, Italy).

Plasma epinephrine (E) and norepinephrine (NE) levels (ng/l) were assayed after extraction with alumina using high-performance liquid chromatography with electrochemical detection. The sensitivity of the assay was 5.0 ng/l. The inter- and the intra-assay coefficients of variation were 8.5% and 4% respectively for E, and 7% and 3% respectively for NE.

**Radionuclide imaging methods**

Equilibrium radionuclide angiocardiography was performed after in vitro labelling of red blood cells with 925 Mbq (25 mCi) of 99mTc.

Subjects were imaged supine in the best septal anterior oblique projection. Acquisition and processing were made using a 400 T GE scintillation camera, equipped with a low energy, all purpose parallel hole collimator; using 24 frames/cycle, 64×64 matrices and a ×1.6 zoom factor. Data processing was performed using a standard, highly reproducible, validated semi-automatic procedure, involving multiple regions of interest and background subtraction.

Left ventricular ejection fraction (LVEF) was calculated from the left ventricular curve. Right ventricle ejection fraction was estimated using a method of two regions of interest. Absolute left ventricular end-systolic volume and end-diastolic volume (LVEDV) were calculated using a validated non-geometric method (33).

Mean (MBP), systolic, and diastolic blood pressure as well as heart rate (HR) were measured at each time-point. Stroke volume (SV), cardiac output (CO) and systemic vascular resistance (SVR) were derived from the other measured parameters.

Eight-minute repeated acquisitions were made basally and for the first 8 min of each 30-min period from +90 to +180 min. For each acquisition, a minimum of 8 000 000 counts was acquired.

**Statistical analysis**

Results are expressed as mean ± S.E.M. Haemodynamic parameters are expressed as absolute values or as percent changes from baseline. Hormonal parameters are expressed either as absolute values or as areas under curves, calculated by trapezoidal integration. Data were analyzed using ANOVA for repeated measurement (Friedman two-way), followed by Wilcoxon signed rank test where appropriate.

**Results**

**Hormonal parameters**

Administration of rhIGF-I increased circulating IGF-I (peak at +150 min vs baseline: 330.2 ± 9.6 vs
199.7 ± 8.7 μg/l, \( P < 0.03 \) to levels which remained similar up to +180 min (Fig. 1). Following s.c. rhIGF-I administration GH levels showed no significant changes (+150 min: 0.1 ± 0.1 vs baseline, 0.3 ± 0.1 μg/l) (Fig. 1).

Catecholamine levels were not modified by IGF-I administration (E, peak at +180 min: 55.3 ± 14.2 vs 47.8 ± 9.7 ng/l; NE, peak at +180 min: 248.2 ± 20.8 vs 236.8 ± 16.4 ng/l (Fig. 1 and Table 1). On the other hand, a slight but significant decrease in both glucose (nadir at +150 min: 67.3 ± 3.7 vs baseline: 84.3 ± 1.7 mg/dl, \( P < 0.03 \)) and insulin levels (nadir at +180 min: 5.3 ± 0.2 vs baseline: 12.1 ± 1.3 μU/ml, \( P < 0.03 \)) was observed after rhIGF-I administration.

**Figure 1** Mean ± S.E.M. circulating levels of IGF-I, GH, glucose, insulin, epinephrine (E), and norepinephrine (NE) at baseline (bas) and after rhIGF-I administration in six normal male volunteers.
Haemodynamic parameter
Basal LVEF was 61.8 ± 2.0%. Following rhIGF-I administration, LVEF did not vary up to +120 min but then showed an increase which was significant at +180 min after rhIGF-I (65.3 ± 2.7%, *P < 0.03* vs baseline). Individual LVEF responses to rhIGF-I are reported in Fig. 2. No change was observed in any of the other measured haemodynamic parameters, including LVEDV. A trend towards an increase in SV and a decrease in HR was apparent, resulting in no change of CO (Fig. 2 and Table 1). No change was recorded in systolic and diastolic blood pressure.

Side-effects
Transient pain in the injection site was recorded in all subjects after rhIGF-I administration. No clear general side-effects were recorded but, late after rhIGF-I administration, all subjects complained of fatigue and of not being well.

Discussion
The results of the present study show that the acute rhIGF-I administration enhances cardiac inotropism in normal humans even after s.c. injection of a low dose.

The increase in circulating IGF-I levels that we found in the present study after the subcutaneous administration of 20 μg/kg rhIGF-I overlaps with that reported by other authors (1, 34–37) and it must be emphasized that it remained within the high normal range of basal IGF-I levels in adulthood (38). Thus, this suggests that we were investigating the effects of ‘physiological’ IGF-I doses.

In our study, rhIGF-I administration did not significantly inhibit spontaneous GH levels in spite of the well-known inhibitory action of IGF-I on somatotroph secretion (36, 39, 40). The lack of a significant inhibitory effect of rhIGF-I on somatotroph secretion in our study could be due to the fact that we did not perform frequent sampling and ultrasensitive GH assays (39).

In agreement with previous studies (29, 30, 34), insulin and glucose levels showed a reduction after rhIGF-I administration. On the other hand, after 20 μg/kg (present study) as well as after 60 μg/kg rhIGF-I s.c. (30) catecholamine levels did not show any significant variation while they have been shown to be increased by higher rhIGF-I doses leading to hypoglycaemia (34).

An inotropic effect of acute rhIGF-I administration had already been reported both in animals (15, 16, 27, 28) and in humans (29, 30). In fact, acute intravenous or subcutaneous rhIGF-I administration has been found to be capable of improving cardiac performance evaluated by impedance cardiography or echocardiography, respectively, in healthy adults (29, 30). The intravenous infusion of 60 μg/kg over 3 h induced systemic side-effects and, in addition, metabolic changes (29), while the same dose injected subcutaneously did not induce side-effects but still inhibited insulin levels and blunted the GH response to physical exercise (30).

Table 1 Effects of rhIGF-I administration on haemodynamic and hormonal parameters in six normal male volunteers.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF (%)</td>
<td>61.8 ± 2.9</td>
<td>63.5 ± 3.1</td>
<td>62.0 ± 3.1</td>
<td>64.3 ± 2.6</td>
<td>65.3 ± 2.7*</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>120.0 ± 6.8</td>
<td>115.8 ± 8.0</td>
<td>119.7 ± 8.2</td>
<td>121.7 ± 7.3</td>
<td>122.5 ± 6.8</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>74.2 ± 5.5</td>
<td>73.5 ± 6.3</td>
<td>74.4 ± 6.6</td>
<td>77.8 ± 4.2</td>
<td>79.6 ± 4.3</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>5.0 ± 0.4</td>
<td>5.0 ± 0.6</td>
<td>4.6 ± 0.5</td>
<td>4.9 ± 0.4</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>90.2 ± 4.6</td>
<td>87.8 ± 3.9</td>
<td>91.1 ± 6.9</td>
<td>86.3 ± 4.3</td>
<td>87.6 ± 5.4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>67.0 ± 2.3</td>
<td>68.0 ± 5.4</td>
<td>61.7 ± 4.0</td>
<td>63.0 ± 4.1</td>
<td>63.0 ± 4.0</td>
</tr>
<tr>
<td>SVR (dyn.s.cm⁻5)</td>
<td>1506.6 ± 159.9</td>
<td>1472.2 ± 138.8</td>
<td>1634.5 ± 120.5</td>
<td>1447.1 ± 108.0</td>
<td>1429.9 ± 113.5</td>
</tr>
<tr>
<td>E (ng/l)</td>
<td>478 ± 9.7</td>
<td>492 ± 9.3</td>
<td>553 ± 11.7</td>
<td>512 ± 10.3</td>
<td>553 ± 14.2</td>
</tr>
<tr>
<td>NE (ng/l)</td>
<td>236.8 ± 16.4</td>
<td>207.3 ± 25.5</td>
<td>241.3 ± 25.9</td>
<td>218.7 ± 34.3</td>
<td>248.2 ± 20.8</td>
</tr>
</tbody>
</table>

*P < 0.03 vs baseline.

E, epinephrine; NE, norepinephrine.
Our present findings, although from a non-randomized study, show that acute rhIGF-I administration enhances cardiac inotropism in humans even after subcutaneous injection of a dose so low as to maintain circulating IGF-I levels within the normal range.

The validity of these findings is strengthened by the use of radionuclide angiocardiography, a technique which is much more reliable than echocardiography (31, 32), for measuring cardiac performances.

The increase in LVEF after rhIGF-I could be due to a direct cardiac effect or, alternatively, to a reflection of vasodilatation. Indeed, vasodilatory effects of rhIGF-I in human forearm muscles and kidney have been reported (41–45). However, in humans in vivo after 20 µg/kg (present study) as well as after 60 µg/kg rhIGF-I (29, 30), no significant change in SVR was found. Another explanation for increased LVEF could rely on an enhanced catecholamine secretion; again, no changes were found in epinephrine and norepinephrine levels by us or by others (30).

The hypothesis that IGF-I has a direct positive inotropic effect is supported by the evidence that it has an inotropic effect in neonatal rat cardiomyocytes (27) and in the isolated rat heart (28). It is therefore reasonable to speculate that the increase in ventricular isometric force development caused by IGF-I in man could be linked either to a sensitization of cardiac neurofilaments to Ca2+ without modifying high energy phosphate metabolism (46) or the enhancement of peak cytosolic Ca2+ concentration on cardiac myocytes (47).

In contrast to another study which was performed during physical exercise (30), we found that the increase in the LVEF was coupled with a non-significant increase in CO; no change was recorded in HR, likely due to the concomitant reduction of HR. In previous studies, an increase in HR occurred after intravenous IGF-I infusion but not after subcutaneous injection (29, 30).

Interestingly, in early stage acromegaly an increased cardiac contractility has been shown while GH-deficient adults have impaired cardiac performances (1–3). These findings could be explained in part by the stimulatory effects of GH on circulating and locally produced IGF-I (1–3, 14, 45, 48, 49) since no direct effect of GH has been found on cultured cardiomyocytes (12–14). Moreover, although acute inotropic effects of rhGH administration have been reported (50) in a study protocol which overlapped with the present one, we found no effect of acute intravenous rhGH on cardiac contractility in normal adults (51).

In conclusion, the present study shows that acute subcutaneous administration of a low rhIGF-I dose leading to an increase of circulating IGF-I levels within the high normal range induced a delayed increase in cardiac inotropism in healthy human subjects.

Acknowledgements

This study was supported by MURST (Ministero Università e Ricerca Scientifica), FSMEM (Fondazione Studio Malattie Endocrino-Metaboliche), Pharmacia & Upjohn. The authors wish to thank Professor Franco Camanni and Dr Emanuela Arvat for their co-operation in the study and revision of the manuscript.

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Cardiovascular effects of hormones and growth factors


Received 26 October 1998
Accepted 14 January 1999