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

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(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 echocardiography or impedance cardiography respectively (29, 30). The intravenous infusion of 60 \( \mu \)g/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 \( \mu \)g/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.

Antral examination, blood pressure, electrocardiographic and echocardiographic findings were also normal.

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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.

Plasma 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-automated 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.
Mean and individual LVEF variations at baseline and after rhIGF-I administration.

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 feeling 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).
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 SV; no change was recorded in CO, 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.

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