Modulating effect of human growth hormone on tumour necrosis factor-α and interleukin-1β

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Abstract

We measured serum tumour necrosis factor-α (TNF-α) as well as interleukin-1β (IL-1β) and GH concentrations in 15 children with isolated growth hormone deficiency (GHD), age range 5.1–13.9 years, before and 4 and 24 h after the first GH injection (0.1 IU/kg s.c.). No differences were found in basal concentrations of serum TNF-α and IL-1β between GHD children (10.01±1.55 pg/ml and 2.14±0.16 ng/ml respectively) and sex- and age-matched controls (11.57±2.16 pg/ml and 3.78±1.46 ng/ml respectively). In GHD children, serum TNF-α and IL-1β values had significantly increased (P<0.002) 4 h (26.75±5.57 pg/ml and 2.99±0.21 ng/ml respectively) and decreased again 24 h after GH administration. Likewise, serum GH levels had significantly increased 4 h (from 1.29±0.69 to 48.71±13.35 ng/ml, P<0.001) and decreased to basal values 24 h after GH administration. A significant correlation was found between basal serum concentrations of GH and those of both TNF-α (P<0.01) and IL-1β (P<0.05). However, no correlation was found between serum GH concentration and either TNF-α or IL-1β levels 4 and 24 h after GH administration. Our data suggest that GH plays a role in modulating TNF-α and IL-1β release in humans.

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Introduction

A stimulatory effect of some cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1 (IL-1) on the endocrine system has long been postulated (1–5). TNF-α is a cytokine mainly secreted by activated macrophages and is capable of modulating pituitary secretion by interacting directly with the endocrine system (4, 6–8), in particular with pituitary cells (5). Conversely, growth hormone (GH) has been suggested to modulate directly both TNF-α and IL-1β release (9). In rats, hypophysectomy depresses TNF-α synthesis by macrophages, and exogenous GH partially reverses this effect (10). However, no data about the influence of GH on TNF-α are available for humans.

IL-1, an additional monokine released by activated macrophages, has been reported to increase, decrease or have no effect on plasma GH secretion in animals (11, 12). Reciprocally, GH administration can increase IL-1β values in short children (13). Finally, a relationship between the two cytokines has been postulated, as TNF-α has been shown to elicit IL-1 release from macrophages (14, 15).

The aim of the present work was to evaluate the influence of GH on the synthesis of both TNF-α and IL-1β by monitoring cytokine concentrations in GH-deficient (GHD) patients in response to short-term GH administration.

Patients and methods

Patients

We evaluated 15 children (seven males and eight females), aged between 5.1 and 13.9 years. The diagnosis of GHD was based on the following criteria: (1) short stature (height below the 3rd percentile of Tanner’s charts); (2) decreased growth velocity (below the 10th percentile for age); (3) delayed skeletal development (bone age/chronological age <0.7); (4) blunted GH response (<10 ng/ml) to at least two pharmacological stimuli (insulin-induced hypoglycaemia, arginine, i-dopa).

Pituitary–thyroid axis function, evaluated by serum tri-iodothyronine, thyroxine, free tri-iodothyronine, free thyroxine and thyrotrophin levels, was normal in each patient. Adrenal function, estimated on the basis of basal serum cortisol concentrations at 0800 and 2000 h, was normal in all children.

No patient suffered from diabetes insipidus, chronic diseases or acquired GHD or had chromosomal abnormalities.

On the basis of clinical data and endocrinological findings, we diagnosed five cases of isolated idiopathic complete GHD (serum GH response <5 ng/ml) and ten cases of partial GHD (serum GH response between 5 and 10 ng/ml).
A group of 19 sex- and age-matched healthy subjects were studied as controls; they were taking part in a screening programme for hyperlipidaemia performed at school at 0900 h. Informed consent was obtained from the children’s parents.

Placebo was not given to both GHD and control children because the local ethical committee did not approve a double blinded or placebo controlled study.

**Methods**

Both serum TNF-α and IL-1β concentrations were evaluated at 0900 h before the first recombinant human GH injection (0.1 IU/kg s.c.) and after 4 and 24 h. Similarly, serum GH values were measured before and 4 and 24 h after GH administration.

Serum TNF-α concentrations were measured by an IRMA based on coated-tube separation and on the oligoclonal system using several monoclonal antibodies directed against distinct TNF-α epitopes (Medgenix, Fleurus, Belgium). Sensitivity was 5 pg/ml. The intra- and interassay coefficients of variation were 6 and 7% respectively. Results were expressed as pg/ml. No cross-reaction with TNF-β, IL-1, IL-2 or interferon-α, β or γ was observed.

Serum IL-1β levels were measured using an IRMA based on coated-tube separation and on the oligoclonal system in which several monoclonal antibodies directed against distinct IL-1β epitopes were used (Medgenix). Sensitivity was 5 pg/ml. The intra- and interassay coefficients of variation were 3 and 7.2% respectively. Results were expressed as ng/ml. No cross-reaction with TNF-α, IL-2 or interferon-α, β or γ was observed.

Serum GH concentrations were evaluated using a time-resolved immunofluorimetric assay (IFMA; Delfia, Pharmacia, Sweden) based on the direct sandwiching technique in which two monoclonal antibodies were directed against two separate antigenic determinants on the human GH molecule. The IFMA is highly specific (detection limit 0.039 ng/ml for the 22 kDa form of GH, and has low cross-reactivity with other GH molecular variants or pituitary hormones.

Statistical analysis was performed using ANOVA and a Student’s t-test for unpaired data. All data are expressed as means ± S.E.M.

**Results**

No significant differences in basal concentrations of serum TNF-α and IL-1β were found between children with GHD (10.01 ± 1.55 pg/ml and 2.14 ± 0.16 ng/ml respectively) and controls (11.57 ± 2.16 pg/ml and 3.78 ± 1.46 ng/ml respectively) (Figs 1 and 2).

Individual variations of serum TNF-α and IL-1β levels were observed in patients with GHD before and after the first GH injection. In GHD children, both serum TNF-α and IL-1β concentrations had significantly increased 4 h after GH administration (26.75 ± 5.57 pg/ml and 2.99 ± 0.21 ng/ml respectively) and decreased again by 24 h (14.17 ± 3.9 pg/ml and 2.31 ± 0.27 ng/ml respectively) (Figs 1 and 2).

Similarly, basal concentrations of serum GH (1.29 ± 0.69 ng/ml) increased significantly (P<0.001) 4 h after GH administration (48.71 ± 13.35 ng/ml) and decreased to basal values after 24 h (1.26 ± 0.38 ng/ml).

A significant correlation was observed between serum concentrations of GH and those of both TNF-α (r=0.695, P<0.01) and IL-1β (r=0.603, P<0.05) before GH administration. However, no correlations were
found between serum levels of GH and either TNF-α or IL-1β at 4 and 24 h after GH administration.

**Discussion**

It is well established that both TNF-α and IL-1β can modulate GH release directly, through specific hypothalamic and pituitary receptors (2, 5, 7, 12, 14). Conversely, GH has also been presumed to modulate the release of monokines (9). The increase in TNF-α levels in hypophysectomized rats after short-term administration of exogenous GH suggests that the hormone has a role in the priming of macrophages for TNF-α synthesis (10).

Unlike experimental data in animals, our results do not point to any difference in basal serum TNF-α and IL-1β concentrations between children with GHD and controls. An explanation for our finding could be only in experimental models can complete GHD be obtained by hypophysectomy.

In contrast, the finding of a significant correlation between basal concentrations of serum GH and those of both TNF-α and IL-1β in GHD children suggests that GH has a physiological role in regulating cytokine release.

However, the lack of correlation between serum GH values and serum TNF-α and/or IL-1β levels 4 and 24 h after GH administration seems to suggest that an indirect mechanism of GH is involved. A role mediated by serum GH-promoting factors such as insulin-like growth factor-I could be hypothesized (16).

The effect of GH or GH-dependent factors on peripheral macrophages is confirmed by the significant increase in both serum TNF-α and IL-1β concentrations observed 4 h after GH injection, when a serum GH peak was reached, and by their fall after 24 h, when serum GH values fell. Moreover, the close correlations between serum TNF-α and IL-1β concentrations observed before and during GH treatment confirm the immunomodulatory effect of GH on peripheral macrophages as documented in animal studies (10).

For ethical reasons our study was not double blinded or placebo controlled. However, the significant increase in both serum TNF-α and IL-1β concentrations after GH injection and their subsequent fall after 24 h do not seem to be due to spontaneous daily variations in the cytokines. In fact, it has been reported that production of both IL-1β and TNF-α from stimulated blood mononuclear cells did not change after placebo infusion started at 0900 h in healthy subjects (17). Our study was performed at the same time.

It has been observed that both IL-1β and TNF-α release is substantially diminished after 3 h of sleep, compared with that in the awake individual (18). On the other hand, a sleep-dependent circadian rhythmicity of serum TNF-α was not confirmed by other authors (19). Taking all this into consideration, the increase in serum IL-1β and TNF-α levels observed in our GHD patients probably reflects the effect of the administered GH, and not any circadian rhythm of cytokine release.

In conclusion, the role played by GH in modulating TNF-α and IL-1β release provides further argument in favour of a regulatory loop between the endocrine and immune systems.

**References**

16. Renier G, Clement I, Desfaits AC & Lambert A. Direct stimulatory effect of insulin-like growth factor-I on monocyte and

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