Effects of recombinant human growth hormone (rhGH) replacement therapy on detailed immunologic parameters in somatotropine – deficient paediatrics patients prior and after 6 months of rhGH treatment

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Abstract

OBJECTIVE: This study aims at assessing how recombinant human growth hormone treatment of children and young people suffering from isolated growth hormone deficiency affects some selected parameters of the immune system: a percentage of lymphocytes, granulocytes, monocytes, concentrations of A, G, and M immunoglobulins, a percentage of T lymphocytes divided into subpopulations CD4 and CD8, a percentage of NK and B lymphocytes, and phagocytic activity of granulocytes and monocytes.

MATERIALS AND METHODS: The study comprised 30 children and young people aged 4.2–18 years with isolated growth hormone deficiency both prior to and 6 months after rhGH (recombinant human growth hormone) treatment with a dose of 0.093 IU/kg every 24 hr. The control group comprised 25 healthy children with normal height in the respective age bracket. Labelling was conducted by flow cytometry FACS manufactured by Becton-Dickinson using both labelled antibodies and PHAGOTEST® commercial kit (Orpegen). Concentrations of A, G and M immunoglobulins in blood serum were assessed by means of immunoturbidimetric method using COBAS (manufactured by Roche).

RESULTS: The lowest percentage of active granulocytes in PHAGOTEST® was found in a group examined prior to treatment compared to the control group. The percentage increased after 6 months of rhGH treatment to values comparable with the control group. Although mean concentrations of IgM and IgA after 6 months of treatment with rhGH significantly decreased in comparison with those determined prior to treatment, they still remained within the baseline norm.

ORIGINAL ARTICLE

No significant differences in the phagocytic activity of monocytes, IgG concentration, % of NK lymphocytes, T lymphocytes divided into CD4 and CD8 lymphocytes, B lymphocytes and CD4/CD8 lymphocytic index were found. None of the patients exhibited any clinical symptoms of immune disorders.

CONCLUSIONS: rhGH treatment of patients with isolated growth hormone deficiency can have positive influence on the phagocytic activity of scavenger cells, mainly granulocytes, which in children with isolated growth hormone deficiency seems to be lower than in their healthy peers. Growth hormone treatment of children with isolated growth hormone deficiency does not significantly affect the activity of the immune system expressed by the phagocytic activity of monocytes, the percentage of B, T and NK lymphocytes and IgG concentration in blood serum.

INTRODUCTION

A number of studies published over the last 60 years have suggested that there is a specific neuro-immunoendocrine interaction (Stokłosa 2006). Investigations suggest that the activity of immune system is modulated by a variety of endocrine factors. It is considered that administration of some hormones has inhibitory or stimulatory effects on the immune response, depending on the kind of hormone, its dose and duration of treatment. The immune and endocrine systems have a "similar" set of transmitters, which makes it possible for them "to communicate" (Stokłosa 2006).

The presence of growth hormone receptors on T lymphocytes was first discovered in 1973 (Kelley *et al.* 2007). Subsequent studies confirmed the presence of growth hormone receptors in the cells of central lymphatic organs (thymus, bone marrow), peripheral lymphatic tissue and cells circulating in the vascular bed of lymphoid system (Lebl *et al.* 2000; Manfredi *et al.* 1994; Murphy *et al.* 1992).

In the early 1980s it was shown that immunocompetent cells were both able to response to growth hormone and to synthesise hormones. In many cases these were pituitary hormones, such as ACTH, TSH, PRL or GH (Stoklosa 2006). These hormones are synthesised in significantly lower amount than those synthesised by the pituitary gland's hormones. However, taking into account the number of immune system cells they can play a certain role in hormonal homeostasis (Stokłosa 2006).

Experimental tests have shown that "dwarf" mice with lesions of anterior lobe of pituitary gland and, as a result, suffering from a deficiency of growth hormone, were diagnosed to have in their peripheral blood a lowered count of hematopoetic cell lines, hipoplasia of spleen, and a lowered count of progenitor cells for B lymphocytes in the bone marrow. The studies showed that the administration of growth hormone caused both an "improvement" of peripheral blood parameters and an increase of spleen's mass (Stokłosa 2006; Lebl *et al.* 2000; Manfredi *et al.* 1994; Murphy *et al.* 1992; Socha *et al.* 1990). Other studies conducted on animal models additionally proved that administration of growth hormone stimulates diversification of thymus into thymic cortex and medulla and leads to an increase of lymphatic organs. It also has a beneficial influence on maturation of bone marrow cells and migration of phagocytes.

The state-of-the-art knowledge on how grown hormone affects the immune system comes from studies conducted on either animal models or adult patients. While interpreting results obtained in these studies, it should be borne in mind that some studies were carried out *in vitro*. There are few reports on how grow hormone affects the immune system in children and results are often contradictory. The principal reason which makes it so difficult to unambiguously assess the influence of rhGH on the immune system is the low size of investigated groups and their significant age diversification.

Bearing in mind the above problems it only seemed reasonable to investigate first; whether in children suffering from isolated growth hormone deficiency there are deviations in some selected parameters of cell and humoral immunity before rhGH treatment; second, to try to explain whether and/or how rhGH application affects these parameters; and thirdly, which subpopulations of immunocompetent cells are mostly affected by this hormone.

MATERIALS AND METHODS

The investigations were conducted between 2004 and 2007 on children and young people suffering from isolated growth hormone deficiency treated at Clinic of Endocrinology, Diabetology, Metabolic Disorders and Cardiology of Developmental Age Pomeranian Medical University in Szczecin and Paediatric Outpatient Clinic in Autonomous Public Clinical Hospital No. 1 of Pomeranian Medical Academy in Szczecin The study was approved by the Bioethical Commission (BN-001/216/03) of the Pomeranian Medical University of Szczecin.

The investigations comprised a group of 30 children, aged 4.2–18 years of age (mean age 12.0 ± 2.8) including 25 (83%) boys and 5 (17%) girls. The control group consisted of 25 healthy children with normal height (i.e. 25–75 centile for the population of Polish children), in a respective age bracket (mean age 11.8 ± 2.4), who were looked after by the outpatients' paediatric surgery clinic staff. In the control group blood samples were taken while carrying out routine tests, i.e. before a scheduled surgery. Blood samples from children treated with growth hormone were taken prior to treatment and then at 6 months.

In the control group blood samples were taken once, in the same amount as in the investigated group. In both groups the same parameters of the immune system were assessed. In the investigated group, somatotropine pitu-

itary insufficiency was diagnosed in hospital, on the basis of physical examination, medical interview, biochemical tests, including hormonal and imaging tests.

Growth hormone (Genotropin®, manufactured by Pfizer) was administered in the evenings, 0.031 mg/kg daily. Children excluded from the trial included those with infections both before and during the hormone treatment as well as those with chronic inflammatory diseases, such as rheumatoid arthritis, multihormonal pituitary insufficiency and Turner syndrome. Other participants excluded from the study were those diagnosed with somatotropine pituitary insufficiency while at the same time suffering from other chronic diseases, such as neurofibromatosis type 1, malabsorption, phenylketonuria, etc. During treatment, follow-up evaluation was conducted at 3 and 6 months including physical examination during which height and body mass increases were measured. Thyroid activity as well as the percentage of glycosylated haemoglobin (HbA_{1c}) and skeleton maturity were assessed.

Standard techniques of immunofluorescent labelling were used to determine subpopulations of lymphocytes by means of laser flow cytometry (FACSCalibur, Becton-Dickinson).

A percentage of lymphocytes, granulocytes and monocytes in the whole population of leukocytes, a percentage of T lymphocytes (CD3) including a division into CD4+ i CD8+ subpopulations, a percentage of B lymphocytes (CD19, 20), a percentage of NK lymphocytes (CD3– 16+ 56+) were determined.

An assessment of concentrations of immunoglobulin class A, G and M in blood serum was conducted by immunoturbidimetric method using COBAS apparatus (manufactured by Roche).

Phagotest®'s commercial kit of reagents was used to determine phagocytic activity of scavenger cells. In order to label their activity heparinized whole blood (100 µl) was incubated, with a sample of labelled E. coli bacteria 20 µl (1×10^9 /ml). A suspension containing bacteria and blood were previously cooled to 0 °C, in a container with ice – an "ice bath".

The control sample was placed in an "ice bath" whereas the investigated one was put in a "water bath" at 37 °C. Both baths were closed in order to limit the amount of light to which samples could be exposed. Phagocytosis was stopped after 10 min by placing both samples in an "ice bath" and adding 100 µl of QUENCHING SOLUTION. Then the samples had been thoroughly stirred and later washed two times using WASHING SOLUTION (3 ml) to get rid of bacteria which had not been phagocytosed and only attached themselves to the surface of scavenger cells. After each washing the samples were spun down in Eppendorf Centrifuge 5810R at 4°C for 5 min (1250 rev/min) and the supernatant was discarded. An addition of 2 ml of LYSING SOLUTION led to a decomposition of erythrocytes in test tubes and as a result of subsequent, additional centrifuging (under conditions described above) the remained of erythrocytes was removed from the solution. Next, 200 µl of DNA STAINING SOLUTION was added to each test tubes to prevent the cells from aggregating. After stirring, both the test tubes were placed again in the "ice bath" for 10 min. The material thus obtained was analysed in a flow cytometry.

A minimum of 10,000 cells from each sample were subjected to analysis using Cell Quest.

Populations of lymphocytes, granulocytes and monocytes in peripheral blood were presented in a dot diagram; the y-axis showing FITS – the fluorescence level, and the x-axis showing SSC.

The results were presented as a percentage of cells, which phagocytosed labelled material, using the plot analysis. The difference between the percentage of cells "actively" phagocytosing in the investigated sample, incubated at 37 °C and that in the control sample, incubated in ace, was assumed to be the final value.

Statistical analysis

The obtained results were analysed by means of Kolmogorov-Smirnov test, Kruskal-Wallis one-way analysis of variance and Wilcoxon-Mann-Whitney test – nonparametric tests used to compare two samples. Pearson's chi-square (χ^2) test was used in order to determine statistical relations between non-linear variables. The significance level of $p<0.05$ was assumed for all the investigated and compared parameters.

RESULTS

No significant differences between the two respective groups were found during the study in the phagocytic activity of monocytes, the concentration of IgG, the percentage of NK lymphocytes, T lymphocytes divided into CD4 and CD8 lymphocytes, B lymphocytes or in the CD4/CD8 ratio. Parameters in which significant differences were observed are presented in Figures to be seen below.

Phagocytic activity of granulocytes

Phagocytic activity of granulocytes in children with isolated growth hormone deficiency prior to rhGH treat-

Tab. 1. Phagocytic activity of granulocytes assessed by means of Phagotest in children from the investigated and control groups.

Examined feature	Gr. 1 n=30 x ± SD	Gr. 2 n=30 x ± SD	Gr. contr. n=25 x ± SD
Phagotest – granulocytes [%]	69.40 ± 11.85 (51.36–90.70)	74.80 ± 11.21 (46.09–95.60)	78.48 ± 8.28 (56.70–90.40)
	p<0.05		
	p<0.01		

x – mean value, SD – standard deviation, n – number of examined patients, Gr. 1 – group examined prior to rhGH treatment, Gr. 2 – group examined after rhGH treatment had been introduced, Gr. contr. – control group

Tab. 2. Percentage of lymphocytes, monocytes and granulocytes in population of leucocytes in children from the investigated and control groups.

Examined feature	Gr. n=30 x ± SD	Gr. 2 n=30 x ± SD	Gr. contr. n=25 x ± SD
Percentage of lymphocytes	32.75 ± 9.84 (10.60–49.31)	40.58 ± 7.20 (23.56–53.61)	36.64 ± 9.92 (18.31–61.68)
	p<0.01		
Percentage of monocytes	9.64 ± 3.28 (5.72–17.89)	9.06 ± 3.29 (5.79–21.35)	12.29 ± 5.07 (5.86–26.85)
	p<0.05		
	p<0.01		
Percentage of granulocytes	57.62 ± 9.70 (38.51–77.55)	50.36 ± 6.73 (37.75–69.29)	51.06 ± 11.20 (16.45–75.83)
	p<0.01		
	p<0.05		

x – mean value, SD – standard deviation, n – number of examined patients, Gr. 1 – group examined prior to rhGH treatment, Gr. 2 – group examined after rhGH treatment had been introduced, Gr. contr. – control group

Tab. 3. Concentration of A and M immunoglobulins in blood serum of children from the investigated and control groups.

Examined feature	Gr. 1 n=30 x ± SD	Gr. 2 n=30 x ± SD	Gr. contr. n=25 x ± SD
Concentration of immunoglobuline A [mg/dl]	158.5 ± 75.8 (20–406)	138.9 ± 56.0 (20–250)	161.90 ± 48.30 (60–300)
	p<0.05		
Concentration of Immunoglobuline M [mg/dl]	110.0 ± 31.7 (46–182)	103.4 ± 33.3 (45–189)	105.8 ± 31.7 (46.0–158.0)
	p<0.01		

x – mean value, SD – standard deviation, n – number of examined patients, Gr. 1 – group examined prior to rhGH treatment, Gr. 2 – group examined after rhGH treatment had been introduced, Gr. contr. – control group

Tab. 4. Percentage of B lymphocytes (CD19, 20) in total population of lymphocytes in children from the investigated and control groups.

Examined feature	Gr. 1 n=26 x ± SD	Gr. 2 n=29 x ± SD	Gr. contr. n=25 x ± SD
Lymphcytes B (CD19.20) [%]	16.40 ± 6.57 (4.9–33.0)	17.54 ± 4.53 (8.6–28.6)	13.28 ± 5.50 (3.5–22.7)
		p<0.01	

x – mean value, SD – standard deviation, n – number of examined patients, Gr. 1 – group examined prior to rhGH treatment, Gr. 2 – group examined after rhGH treatment had been introduced, Gr. contr. – control group

ment was significantly lower compared to phagocytic activity of these cells in children from the control group ($p<0.01$) (Table 1). This activity significantly increased at 6 months of therapy ($p<0.05$) and reached a value close to that recorded in the control group.

Percentages of respective subpopulations of leukocytes

The percentage of lymphocytes in the total population of blood cells in the investigated group was on average slightly lower than that in children in the control group (Table 2) and it significantly increased ($p<0.01$) after 6 months of growth hormone therapy.

The percentage of monocytes in the total population of leucocytes in peripheral blood in the investigated group was on average slightly lower in children with isolated growth hormone deficiency both prior ($p<0.05$) and after rhGH treatment ($p<0.01$) compared to values observed in children from the control group. However, no significant differences in these cells' percentages were observed in children from the investigated groups both prior to and after rhGH treatment.

The percentage of granulocytes in the total population of leucocytes in peripheral blood in children with isolated growth hormone deficiency was significantly higher ($p<0.05$) compared to mean values of granulocytes' percentage in children in the control group and it significantly decreased ($p<0.01$) after 6-month rhGH therapy down to values observed in children from the control group.

Concentrations of A and M immunoglobulins

No significant differences in average values of A and M immunoglobulins in blood serum were found between patients with isolated growth hormone deficiency and children from the control group (Table 3).

In children with isolated growth hormone deficiency concentrations of IgA and IgM in blood serum after 6 months of rhGH treatment significantly decreased ($p<0.05$) and ($p<0.01$) respectively, as compared with concentrations of these immunoglobulins before treatment. Nevertheless, the average concentrations of A and M immunoglobulins were within the baseline.

Percentage of B lymphocytes

Due to a lack of sufficient amount of material (blood) to analyse an assessment of risk ratio of B lymphocytes (CD19, 20) only 26 children from the investigated group were analysed prior to growth hormone treatment and only 29 at 6 months of the therapy. On average, the lowest value of B lymphocytes' percentage (CD19, 20) was observed in children from the control group (Table 4). No significant differences in this parameter values were observed between the investigated group examined before the treatment and the control group. However, the percentage of B lymphocytes (CD19, 20) was significantly higher ($p<0.01$) in the investigated group at six months of the therapy than that in the control group.

DISCUSSION

The overall conclusion of research conducted so far is that growth hormone clearly affects the development, regulation and activity of the immune system.

Some researchers including Wu *et al.* (2004) and Jeschke *et al.* (2000) stressed the beneficial influence of growth hormone which can be used in supportive therapy in patients with severe burns. Their findings have shown that rhGH therapy in patients with severe burns reduced pro-inflammatory response of the organism (reduction concentrations of CRP protein, α-1 – acid glycoprotein, components C3 of complement, IL-1β and TNF-α) in the acute stage of the disease, which reduced damage of internal organs.

RhGH has also been used as medication in supportive therapy of patients suffering from death cap (*Amanita phalloides*) poisoning – its administration stimulated liver regeneration (Socha *et al.* 1990). Currently attempts are being made to apply growth hormone therapy in patients suffering from systemic diseases, such as juvenile rheumatoid arthritis, Crohn's disease or AIDS (Simon 2007). Investigations conducted so far on the role of growth hormone on the immune system of HIV positive patients have been inconclusive. Few studies suggested an increase of both thymus mass and the number of CD4 lymphocytes in patients undergoing antiretroviral therapy with growth hormone. This therapy seemed to beneficially affect metabolism through a decrease of protein catabolism and an improvement of height and body mass parameters (Hardin *et al.* 2005). Nevertheless, neither the influence rhGH exerts on immunological profile nor the optimal dose of the hormone have as yet been determined (Geffner 1997; Laurence 1995; Napolitano *et al.* 2002).

In a study conducted on a group of 16 patients with growth hormone deficiency Menfredi *et al.* (1994) found that phagocytic activity of granulocytes in those patients was lower than in a group of healthy controls. Decker *et al.* (2005) confirmed an increased "oxidative burst activity" of scavenger cells following an administration of large doses of rhGH in patients who had to undergo surgery due to abdominal aortic aneurysm. Derfalavi *et al.* (1998) found that rhGH therapy in patients with chronic renal failure improved several impaired functions of the immune system, such as phagocytosis, chemotaxis of scavenger cells and synthesis of peroxide anions performed by these cells. Conversely, Rappaport *et al.* (1991) in a study on phagocytosis did not confirm a relationship between the activity of scavenger cells and growth hormone deficiency in 8 patients either prior to or during rhGH treatment. However, the group of patients examined in this study was rather small.

Our own research provides some evidence to demonstrate how growth hormone affects some parameters of the immune system. We have shown that growth hormone can reduce phagocytic activity of granulocytes in children with growth hormone deficiency. It can also lower a percentage of monocytes and increase that of granulocytes in total population of leucocytes compared with a healthy population of children. Our studies have also shown that the phagocytic function of granulocytes significantly improved after 6 months of rhGH therapy.

Some recent studies seem to suggest that growth hormone does not affect the concentrations of A, G and M immunoglobulins in patients with growth hormone deficiency during treatment with rhGH (Manfredi *et al.* 1994; Spadoni *et al.* 1991). However, some studies present converse findings and suggest that rhGH does affect concentrations of immunoglobulins – Rekers-Mombarg *et al.* (Rekers-Mombarg *et al.* 1995) recorded a transient decrease of A immunoglobuline concentration; and Bozzola *et al.* (Bozzola *et al.* 1988) found a significant decrease of IgM in groups investigated *in vitro*.

Our own studies have also shown that concentrations of A and M immunoglobulins decreased during rhGH therapy. Therefore, it is fair to suppose that both growth hormone deficiency and a long term rhGH therapy can lead to subtle changes in humoral immune response (synthesis of immunoglobulins), which nevertheless do not translate into patients' clinical condition (Geffner 1997, Decker *et al.* 2005; Bozzola *et al.* 1988; Kiess *et al.* 1988).

Bearing in mind alterations of B lymphocytes' percentage in total population of leucocytes in patients with growth hormone deficiency treated with rhGH, it should be pointed out that both a decrease (Spadoni *et al.* 1991; Rappaport *et al.* 1986; Petersen *et al.* 1990) of these cells was recorded during therapy with growth hormone and no effect was noted on their percentage (Manfredi *et al.* 1994). Studies on how rhGH affects percentage of T lymphocytes demonstrated both a decrease of these cells' percentage (Rappaport *et al.* 1991) and no effect of rhGH on this population of leukocytes (Manfredi *et al.* 1994; Spadoni *et al.* 1991). Napolitano *et al.* (2002) and Sneppen *et al.* (2002) observed a positive correlation between a percentage and absolute number of CD4 lymphocytes circulating in a vascular bed and a supply of rhGH. The same author, while examining patients with multihormonal pituitary insufficiency observed that they had a lower percentage of NK lymphocytes in a total population of leucocytes than the control group. Additionally, no improvement was observed in these patients after 18 months of growth hormone treatment.

Kiess *et al.* (1988), in their study aiming at assessing the activity and number of NK lymphocytes' subpopulation in 20 patients with growth hormone deficiency, showed that in the investigated group both the number and the activity of NK lymphocytes were significantly lower than in the control group. However, Spandoni and Petersen's studies (Spadoni *et al.* 1991; Petersen *et al.* 1990) showed no significant differences in the popu-

lation of NK cells both prior and after growth hormone treatment. Additionally, no differences were observed compared with the control group.

Contrary to those result, this study found no significant influence of growth hormone on the percentage of T lymphocytes, on their subpopulations CD4+ and CD8+, on the size of CD4/CD8 ratio or on the percentage of B and NK lymphocytes in the total population of lymphocytes. Likewise, growth hormone seems not to affect concentrations of G immunoglobulin in blood serum.

Owing to certain discrepancies between results obtained by the above mentioned authors and those obtained in our own study while also bearing in mind the fact that growth hormone is being used ever more widely in a number of chronic diseases, in which the immune system's functions are *a priori* disturbed, it seems essential to further continue studies which will help elucidate whether or not growth hormone affects the immune system.

CONCLUSIONS

RhGH treatment in patients with isolated growth hormone deficiency can have a beneficial influence on phagocytic activity of scavenger cells, mainly granulocytes, as this activity seems to be lower in children with isolated growth hormone deficiency than that in their healthy peers.

RhGH treatment of patients with isolated growth hormone deficiency does not significantly affect the activity of the immune system expressed by phagocytic activity of monocytes, a percentage of B, T and NK lymphocytes and IgG concentration in blood serum.

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