Imbalance in the blood antioxidant system in growth hormone-deficient children before and after 1 year of recombinant growth hormone therapy

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Background/Aims: The aim of our study was to examine the effects of recombinant growth hormone (rGH) treatment in children with growth hormone deficiency (GHD) on the blood antioxidant system: total antioxidant capacity (TAC) of plasma measured by FRAP (ferric reducing antioxidant power or ferric reducing ability of plasma); superoxide dismutase (SOD) and catalase activities; non-protein thiol (NT) and ceruloplasmin levels.
Methods: Eleven treatment-native prepubertal children with growth hormone deficiency were included in the study. The state of the antioxidant system was examined and compared with these of a control group.
Results: Before rGH treatment, the TAC of plasma and NT level were significantly lower whilst SOD activity was significantly higher than corresponding control data. After rGH therapy only SOD activity value differed from control. Though neither of the values except for TAC of plasma exhibited any significant improvement toward the end of the 12-month treatment but nonsignificant changes were also observed. The value of TAC significantly increased by 30% during treatment.
Conclusions: The present work has demonstrated that some parameters of the blood antioxidant system are in a state of imbalance and are impaired in GHD children. A 12-month treatment with rGH resulted in partial improvement of the antioxidant system conditions.

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Introduction

Free radicals and other reactive species are thought to play an important role in many human diseases. The serious imbalance between production of reactive species and antioxidant defense in results of increasing of reactive species' production or diminished the antioxidants levels lead to potential oxidative damage (oxidative stress) and progression of various disorders (Halliwell, 2004). So the evaluation of oxidative stress level could be used as a "nonspecific marker" of body systemic disorders. Moreover the oxidative stress progression (or preservation) during standard treatment must demand the modification of therapy, for example by additional examination administration of vitamins, antioxidants or etc. We believe right use for evaluation of oxidative stress level some parameters of blood antioxidant status.

It's known the substantially increased oxidative stress parameters in GHD children compared to healthy controls and normalization of these parameters after GH treatment (Mohn, 2005). Beyond, as stated earlier, data exists suggesting a direct effect of GH on blood and erythrocytes (Christ, 1997;Ramos, 2011).

The aim of present work was to examine the effects of 1 year of rGH treatment the GHD children on the antioxidant status of the blood.

Materials and Methods

Eleven treatment-naive GH-deficient patients were included in this study (2 girls and 9 boys aged 3 to 9 years old). The mean chronological age (CA) was 6.1±2.2 years old, and the mean bone age (BA) was 2.6±0.9 years.

Three sets of parameters were investigated and were compared against control group. The parameters of blood antioxidant system were compared with those in a control group of 11 healthy prepubertal children (2 girls and 9 boys; aged 6 to 11 years old; mean CA 9.3±1.4 years). The control groups did not receive any placebo injections.

All patients underwent a standard set of clinical and laboratory tests, which included: physical and anthropometric evaluations; x-rays of both hands and wrist joints (in case of inclusion in the study); and CT or MRI imaging of the head (in case of inclusion). To verify the diagnosis, GH provocation tests were performed: 5 samples were evaluated with clonidine (0, 30, 60, 90, and 120 minutes), and 7 samples were evaluated with insulin (0, 15, 30, 45, 60, 90, and 120 minutes). A value less than 10 pg/L was considered supportive of the diagnosis. Clinical and biochemical blood analysis, as well as an evaluation of IGF-1 levels and IGF-1 binding protein-3 levels (IGFBP-3), was performed before starting treatment and

at 12 months into rGH treatment. The levels of IGF-1 were measured using a commercial enzyme-linked immunosorbent essay (ELISA) for IGF-1 RIA (producer DSL, Sinsheim, Germany); the level of IGFBP-3 was determined via a commercial ELISA DSL-10-6600 ACTIVE™ IGFBP3 ELISA kit (producer DSL, Sinsheim, Germany). Biochemical blood analysis was performed in the biochemical laboratory of the Endocrinology Research Centre, using the Hitachi 912 analyzer according to a standardized methodology. The hormone was subcutaneously administered daily, once in the evening. The dose of rGH was 0.033 mg per 1 kg of body mass per day (GH Research Society. Consensus guidelines, 2000).

The evaluations of the antioxidant system were performed on whole blood samples, which were collected in the morning before breakfast prior to rGH therapy.

Antioxidant Status Evaluation

The blood antioxidant system was examined using a Hitachi 556 (Japan) spectrometer was used. The activity of SOD was estimated via the inhibition of epinephrine self-oxidation (Sun, 1978), and the level of ceruloplasmin was estimated by measuring the enzymatic reaction with o- phenylenediamine (Brazhe, 2014). Catalase activity was measured at a temperature of 37°C, according to Aebi (Aebi, 1984). Non-protein thiols were determined as described by Sedlak et al. (Akhalaya, 2006). The total reducing power of the antioxidants in the blood plasma was determined by the ferric reducing ability of plasma (FRAP) assay, as described by Benzie and Strain (Benzie, 1996) with modifications: 350 μL of distilled water was added to the tube containing 3 mL of the reagent (working solution); and 50 μL of the sample (plasma) was added and mixed. Next, the samples were read at a wavelength of 593 nm after 10 minutes of exposure. The method for photometric determination of blood haemoglobin was based on the transformation of haemoglobin into its haemachrome form by sodium dodecyl sulphate, followed by measuring light absorption at 540 nm (Brazhe, 2014). Changes in the optical density were recorded with a Hitachi-556 spectrophotometer (Japan).

Statistics

The results were statistically processed using Statistica software, version 8.0. Because the parameters did not always correspond to a normal distribution, the statistical significance of differences was calculated via the nonparametric Mann-Whitney and Wilcoxon tests. Changes were considered significant at $p<0.05$.

Ethics Statement

The study was approved by the Ethics Committee of the Endocrinology Research Centre, Moscow, Russia Federation (reference number: 14). Written informed consent was obtained from the patients and/or their parents or legal guardians.

Results

Anthropometric and Biochemical Parameters

Children treated with rGH demonstrated increases in height, weight, height velocity, height SDS, and height velocity SDS (Table 1). To assess therapeutic safety and compliance, IGF-1 and IGFBP-3 were measured, and their levels also increased during rGH treatment.

The Antioxidant Status

To evaluate the blood antioxidant status we chose those parameters that characterize it most fully: total antioxidant capacity (TAC) of plasma measured by FRAP (ferric reducing antioxidant power or ferric reducing ability of plasma), superoxide dismutase (SOD) activity, catalase activity, non-protein thiol (NT) levels, ceruloplasmin levels. SOD and ceruloplasmin are responsible for both utilization of the superoxide anion radical and the regulation of variable valence metal levels (copper and iron); catalase and non protein thiols play their part in hydrogen peroxide utilization; increase in LP products reflects the development of oxidative stress.

Before treatment, the TAC of plasma and the amount of NT were significantly decreased than corresponding control data ($p=0.0178$ and $p=0.0193$, consequently) and SOD activity was significantly elevated than control ($p=0.0193$). After rGH therapy only SOD activity value differed from control (Figure 1). Though neither of the values except for TAC of plasma exhibited any significant improvement toward the end of the 12-month treatment (see Table 2). The value of TAC significantly increased by 30% during treatment. The NT level and SOD activity nonsignificantly increased while catalase activity and ceruloplasmin level nonsignificantly decreased during treatment. We didn't observe the significant increasing the NT level using the Wilcoxon test ($p<0.05$) during treatment in GHD children unlike the comparing the GHD and control group where the Mann-Whitney test ($p<0.05$) was used but the nonsignificant increasing this value was registered. Probably it's determined by an inadequate sampling of subject participating in the experiment.

Discussion

We have demonstrated that the before treatment the parameters of antioxidant system

are in a state of imbalance in GHD children, with decreased TAC, NT level and elevated SOD activity.

The imbalance of antioxidant parameters were reported in some works (Evans, 2000;Gonzalez-Duarte, 2012), who found that patients with adult GHD exhibited an increased degree of oxidative stress, although the investigated parameters differed from those in our study.

The decreased TAC, NT level, elevated SOD activity and insignificantly raised level of ceruloplasmin registered in GHD children during rGH treatment indicate the oxidative stress development.

After treatment the some evidence of improvements in blood antioxidant system was observed. But it's equally tangible as it was shown did not exhibit any tangible improvements during therapy. These findings confirm the data reported in Mohn et al. (Mohn, 2005), who showed an improvement in antioxidant status, evidenced by a decrease in the number of free radicals in GHD children after rGH treatment.

The tendency to harmonies the blood antioxidant system parameters during rGH therapy is obvious, but it does not extend to all parameters, and for some parameters, it remains only a tendency.

Our results demonstrate that therapy of a GH-deficient child in this treatment should not be limited to rGH administration alone but should clearly include antioxidant therapy. In addition, the administration of vitamins, a special diet (wherein the calorie content, proteins, carbohydrates, lipids, micro-element composition, etc., are controlled), an appropriate physical program, and psycho-emotional therapy should also be considered.

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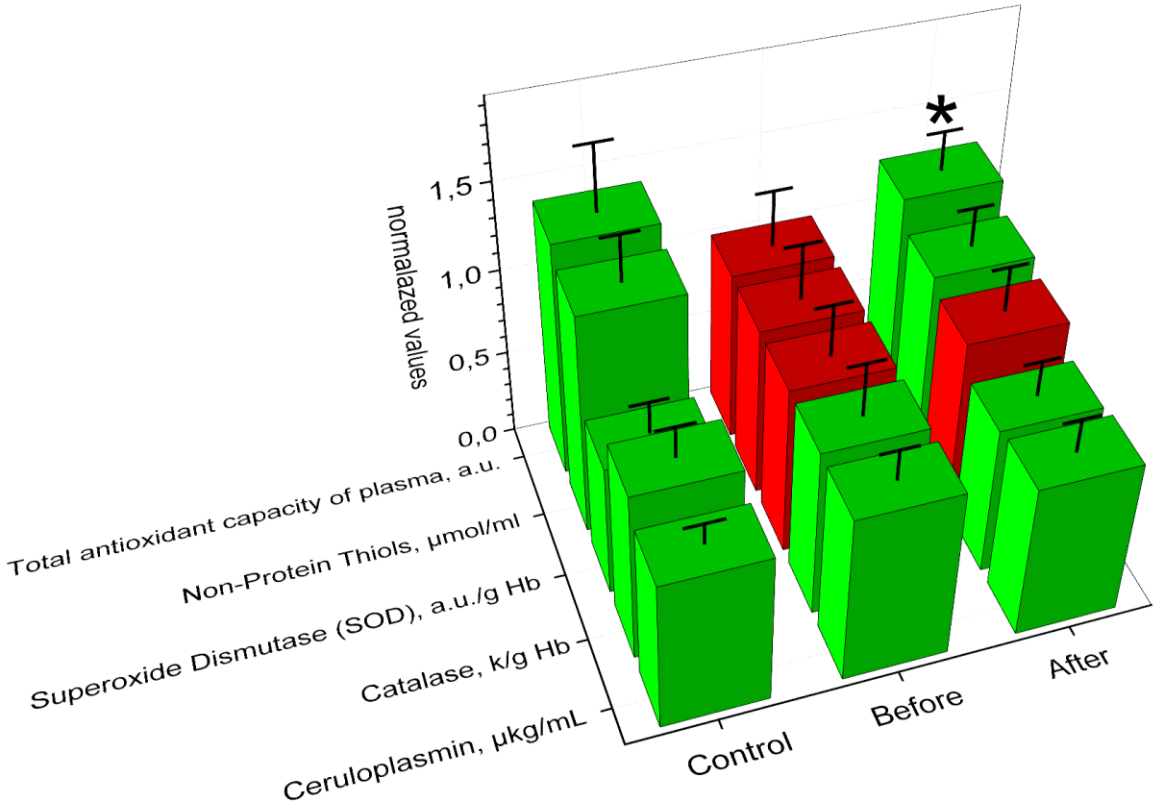

Figure 1. The normalized Antioxidant status parameters in control and GHD patients during rGH treatment.

The antioxidant parameters were normalized on those values CHD patients before the treatment. The red bars – significant difference between control and treatment groups (Mann-Whitney test, $p<0.05$). * - significant difference between parameters before and after treatment (Wilcoxon test, $p<0.05$).

Table 1. Morphofunctional parameters of erythrocytes after 12 months of growth hormone therapy

Parameters	Before GH therapy	After 12 months of GH therapy
Height, cm	96.2±10.5	108.0±10.8 p1=0.0033*
Weight, kg	15.2±3.9	17.8±5.5 p1=0.0033*
Height velocity, cm/years	3.4±1.2	12.5±3.5 p1=0.0033*
Height SDS	-3.6±0.9	-2.2±1.3 p1=0.0033*
Velocity of height SDS	-3.2±1.8	7.4±3.9 p1=0,0033*
IGF-1, nMol/L	5.7±4.1	13.5±9.3 p1=0.0044*
IGFBP-3, nMol/L	51.9±36.1	99.3±36.5 p1=0.0033*

p1 - statistical significance between parameters before and after treatment; value was evaluated using the Wilcoxon test, $p<0.05$; (significant difference between data marked asterisk *)

Table 2. Antioxidant status parameters after 12 months of treatment

Parameters	Control	Before GH therapy	After 12 months of GH therapy
Total antioxidant capacity of plasma, a.u.	0.26±0.07	0.19±0.06 p2=0.0302*	0.23±0.04 p1=0.0208* p2=0.4116
Non-Protein Thiols, μmol/ml	0.92±0.18	0.70±0.22 p2=0.0328*	0.78±0.15 p1=0.5937 p2=0.1007
Superoxide Dismutase (SOD), a.u./g Hb	14.5±3.4	18.8±5.4 p2=0.0328*	19.6±4.7 p1=0.5337 p2=0.0165*
Catalase, k/g Hb	216±38	215±64 p2=0.6695	190±43 p1=0.1823 p2=0.1783
Ceruloplasmin, μkg/mL	518±70	581±100 p2=0.0940	531±111 p1=0.0619 p2=0.9215

p1 - stastistical significance between parameters before and after treatment; value was evaluated using the Wilcoxon test, $p<0.05$ p2 - statistical significance between control and treatment groups; value was evaluated using the Mann-Whitney test, $p<0.05$; (significant difference between data marked asterisk *)