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One-year recombinant growth hormone therapy does not improve hemoglobin state and morphology of erythrocytes in growth hormone deficient children

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ABSTRACT

An increase in growth rates of children suffering from growth hormone deficiency (GHD) subjected to recombinant growth hormone treatment (rGHT) was shown to be accompanied by acceleration of metabolic processes that may stimulate oxygen consumption in various organs and tissues. Therefore, oxygen-transporting properties of RBC should undergo considerable changes during the rGHT. The aim of this study was to examine the effects of rGHT on erythrocyte shape and hemoglobin state in GHD children. The level of oxyhemoglobin (Oxy-Hb) in RBC was analyzed by Raman spectroscopy. The RBC count, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and other parameters were calculated. The blood of eleven treatment-naive prepubertal children with GHD (aged 3–9, median 5.7 years) was examined and compared with control group (aged 5–7; median 6.0 years) at three time points: 0, 3 and 12 months of rGHT. Before rGHT, the MI in GHD children was higher (median 0.48 vs 0.14 $p = 0.0018$) and the RBC count was lower (median 4.20 vs 4.96 10^{12} cells/L $p = 0.0022$) than in control group. After the treatment, cell count in GHD patients did not differ significantly from the control group, but Oxy-Hb level became higher (median 0.64 vs 0.41 $p = 0.0075$). During rGHT, MCV decreased (median 80.3 vs 83.2 μm^3 $p = 0.0231$). Morphological and functional characteristics of erythrocytes in GHD children were shown to differ significantly from the healthy control group. A twelve-month rGHT partially improved some of the studied parameters but Oxy-Hb level and echinocyte count remained high.

1. Introduction

An increase in growth rate of children suffering from growth hormone deficiency (GHD) subjected to recombinant growth hormone treatment (rGHT) was shown to be accompanied by changes in the blood antioxidant status [1,2] and acceleration of metabolic processes. In our previous work [2] we have demonstrated that some parameters of the blood antioxidant system were out of balance and even impaired in GHD children, whereas a 12-month rGHT resulted in a partial improvement of the antioxidant system. Erythrocytes are highly susceptible to oxidative damage, which is reflected by changes in their parameters and impairment of oxygen delivery into tissues [3]. Moreover, acceleration of metabolic processes may stimulate oxygen consumption in various organs and tissues and ultimately lead to hypoxia [4].

Red blood cells (RBC) are highly specialized cells, packed with high amounts of oxygen-transporting protein (Hb). The main function of RBC is oxygen transportation from lungs to other tissues. Therefore, oxygen-transporting properties of RBC (e.g. number of RBC, morphology or content and properties Hb in RBC) should change during the development of hypoxia.

Native Hb in RBC is known to be very sensitive to pathological factors, which was confirmed by several works performed on healthy volunteers subjected to stress or patients with various cardiovascular disorders and type 1 diabetes mellitus [5–11].

Elevation of insulin-like growth factor-1 (IGF-1), the primary mediator of growth hormone, during the rGHT has been found a long time ago. Moreover, specific IGF-1 receptors were reported to be present at the erythrocyte surface (see, e.g. [12]). In is also

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known that hematological parameters change during rGHT in GHD children [13–15]. However, no direct evidence of changes in the state of Hb in RBC has yet been demonstrated. The Raman Spectroscopy (RS) method provides direct data on the conformation of prosthetic group of Hb in native RBC, which allows to evaluate Hb state *in situ*. The aim of this work was to examine the effects of a 1-year long rGHT on the native Hb in RBC of GHD children.

2. Materials and methods

2.1. Study groups

The venous blood samples were collected from eleven treatment-naïve GHD patients (2 girls and 9 boys, 3–9 years old; median chronological age 6.1 years) and 9 healthy children (2 girls and 7 boys, 5–7 years old; median chronological age 6.0 years) in the fasting state. The control group did not receive any placebo injections.

Standard deviation scores (SDS) of height and growth velocity were assessed using the mean and standard deviation scores of the British reference population as described by Tanner [16]. All patients underwent a standard set of clinical and laboratory tests, performed in accordance to the conventional international standards [17]. Laboratory tests included a clinical blood analysis with standard blood indicators (RBC count, MCV, MCHC, MCH). To assess treatment safety and compliance, IGF-1 and IGF-1-binding protein-3 (IGFBP-3) were analyzed. An x-ray of the hands and wrists was performed in all cases and an MRI scan of the pituitary was made when necessary. For verification of the diagnosis all patients underwent two GH stimulation tests: the first one with clonidine and the second one with insulin according to standard procedures. The blood analyses were performed prior to therapy (at point 0), after 3 and 12 months of rGHT. The daily dose of rGH was 0.033 mg per kg of body weight, administered in the form of subcutaneous injections at 9–10 p.m.

2.2. Raman spectroscopy

The properties of Hb were examined by Raman spectroscopy. A DPSS laser Ventus 473 (Laser Quantum, Germany, $\lambda = 473$ nm) and a DFS-24 spectrometer (LOMO, Russia) were used to record Raman spectra; output power on the sample (1 mm^3) was 18–20 mW. Single spectrum was recorded for 1 min. The spectral resolution was 5 cm^{-1}.

Raman spectra of the whole venous blood corresponded in general to the Raman spectra of the prosthetic Hb group, the heme (Fig. 1). Raman spectra of the other blood components are negligible at this excitation wavelength. Amplitudes and locations of the bands in the Raman spectrum of the heme depended on the conformation of the molecule, the oxidation state of the iron atom, as well as on the ligands, associated with the heme [18,19]. The intensities of the ν_4 band in Raman spectra (1355–1375 cm^{-1} region) was attributed to the stretching vibration of the pyrrole half-ring (see Fig. 1). For the ligated ferrous heme molecules or the non-ligated ferric (Fe^{3+}) form of Hb the band ν_4 occurs at about 1375 cm^{-1} [20]. However, the concentrations of the non-ligated ferric form of Hb in blood do not normally exceed 1–2%, and I_{1375} value normally depends on the presence of a ligand, mainly oxygen. In ferrous heme molecules with iron in the high-spin state, which primarily corresponds to deoxygenated Hb, the maximal intensity of ν_4 is shifted to 1355 cm^{-1} (I_{1355}). Thus, $I_{1375}/(I_{1355} + I_{1375})$ ratio gives a good estimate of the oxyhemoglobin (Oxy-Hb) level [6,8,9,21,22].

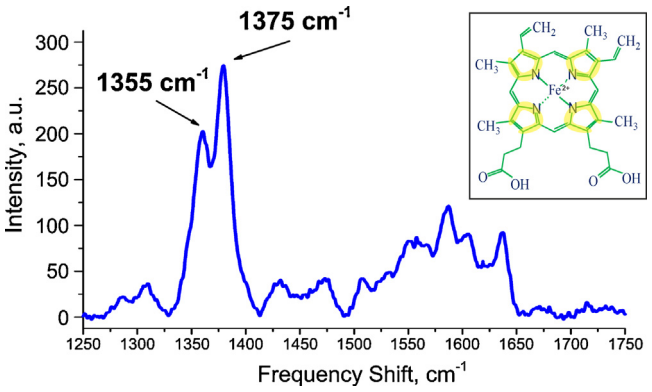

Fig. 1. A typical Raman spectrum of whole venous blood, measured using a diode laser beam, A = 473 nm, P = 18–20 mW. Inset: prosthetic group of hemoglobin, the heme. Hatched lines indicate bonds, related to the ν_4 band.

2.3. Study of erythrocyte shape

Erythrocyte shape was examined by laser interference microscopy (LIM) [23], using a MIA-1 laser interference microscope (Russian Research Institute for Optics and Physics, Russia). The technique of sample preparation was described in earlier publications [10]. To evaluate erythrocyte morphology in suspension we used the morphological index (MI) described by Gedde et al. [24]. A score was assigned to each cell depending on its shape (see Fig. 2). The overall MI was defined as an arithmetic mean of the scores of all the cells studied.

2.4. Statistics

The obtained results were statistically processed using the Graphpad Prism 6.05 demo software. We applied nonparametric tests because not all of the data demonstrated normal distribution, as revealed by the D'Agostino & Pearson omnibus normality test ($p < 0.05$). Statistical significances of differences for independent

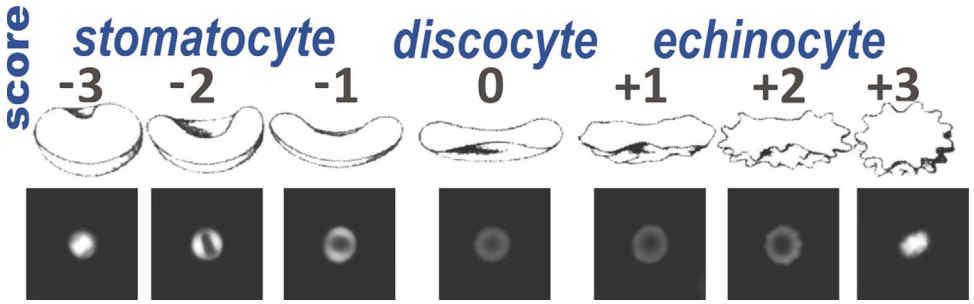

Fig. 2. Morphological indexes of erythrocytes.
Morphological indexes (MI) of erythrocytes based on LIM data. Each cell in the image was scored with a value, corresponding to its shape. Upper row is schematic cross sections of erythrocytes; lower row is the corresponding phase images of cells.

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