Clinical Research

Effects of Growth Hormone Administration on Plasma Levels of Proinflammatory Cytokines and Soluble Apoptosis Mediators Fas/FasLigand in Patients with Dilated Cardiomyopathy

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Introduction: Recent studies have shown that an abnormal proinflammatory cytokine expression and apoptotic process contribute to adverse left ventricular remodeling and progress of chronic heart failure (CHF). The present study investigates the effects of growth hormone (GH) administration on plasma levels of representative proinflammatory cytokines and soluble apoptosis mediators in patients with idiopathic dilated cardiomyopathy (IDC).

Methods: Plasma levels of tumor necrosis factor-α (TNF-α), its soluble receptors (sTNFRI, sTNFRII), interleukin-6 (IL-6), soluble IL-6 receptor (sIL-6R), soluble Fas (sFas) and soluble FasLigand (sFasL) were determined (ELISA method) in 10 patients with IDC (NYHA class III; EF: 24±2%) before and after a 3-month subcutaneous administration of GH 4 IU every other day (randomized crossover design). Peak oxygen uptake (VO_2 max) was also used to evaluate the functional status of IDC patients.

Results: Treatment with GH produced a significant reduction in plasma levels of TNF-α (8.2±1.2 vs 5.7±1.1 pg/ml, $p<0.05$), sTNFRI (4.0±0.4 vs 3.2±0.3 ng/ml, $p<0.005$), sTNFRII (2.6±0.3 vs 2.2±0.2 ng/ml, $p<0.05$), IL-6 (5.5±0.6 vs 4.4±0.4 pg/ml, $p=0.05$), sIL-6R (32.7±5 vs 28.2±3 ng/ml, $p<0.05$), sFas (4.4±0.8 vs 3.1±0.6 ng/ml, $p<0.05$), and sFasL (34.3±11.7 vs 18.8±7.3 pg/ml, $p<0.01$). A significant improvement was also observed in VO_2 max after the completion of 3 month treatment with GH (15.0±0.8 vs 17.2±1.0 ml/kg/min, $p<0.05$). Good correlations were found between GH-induced reduction in TNF-α levels and increase in VO_2 max ($r=-0.64$, $p<0.05$), as well as between GH-induced reduction in sFasL and increase in VO_2 max ($r=-0.56$, $p=0.08$).

Conclusions: Chronic treatment with GH reduces plasma levels of proinflammatory cytokines and soluble Fas/FasL system in patients with IDC. These immunomodulatory effects may be associated with the improvement in clinical performance and exercise capacity of IDC patients.

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Recent experimental and clinical data have shown that an abnormal stimulation of the immune system, mediated mainly by proinflammatory cytokines, plays a significant role in the progression of chronic heart failure (CHF)[1]. Increased levels of major proinflammatory cytokines, such as the tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), as well as the soluble apoptosis mediators Fas (sFas) and FasLigand (sFasL), have been found in the circulation of patients with advanced CHF[2,3]. Proinflammatory cytokines affect significant functions of the cardiovascular system, modifying the expression of nitric oxide syn-

thase isoforms, promoting the formation of oxygen free radicals and inducing the apoptosis of myocardial and endothelial cells and of the striated muscle fibers in the peripheral muscles[1,3,5]. At the same time, the sFas/ sFasL system reflects the activation of the apoptosis process in the cardiovascular system, is overexpressed in CHF and has been associated with the severity of the clinical profile and the prognosis of patients with CHF[3,6,7].

On the other hand, several experimental as well as small-scale clinical studies have shown that the administration of growth hormone (GH) improves cardiac output in experimental animals and patients with CHF, increasing cardiac contractility and reducing peripheral resistance[8,9,10]. This beneficial effect of GH was interpreted in the majority of cases as an improvement of the clinical profile and fatigue tolerance of patients with CHF[8,9,10,11]. However, the effect of GH on proinflammatory cytokines and their soluble receptor plasma levels, as well as on the apoptosis mediators sFas and sFasL plasma levels, in patients with CHF due to idiopathic dilated cardiomyopathy (IDC), has not been investigated in international literature. The purpose of this study was to investigate the effect of GH on the plasma levels of proinflammatory cytokines TNF-α and IL-6, their soluble receptors (sTNFRI, sTNFRII, sIL-6R) and the sFas/sFasL system in 10 patients with IDC, as well as how the resulting changes to these markers are related to the changes of the patients' functional status, as expressed through peak oxygen uptake (VO_2 max) in cardiorespiratory fatigue.

Methods

Study population and design

We studied 10 patients with IDC (mean age: 50±4 years) and symptoms of moderate to severe CHF (NYHA functional class III). Clinical diagnosis of IDC was based on clinical history, normal findings of coronary angiography, echocardiographic confirmation of decreased cardiac function and finally findings of endomyocardial biopsy. Mean ejection fraction (EF), as assessed using 2-D echocardiogram (Simpson method), was 24±2%. The functional status of the patients was assessed using the cardiorespiratory fatigue method, calculating peak oxygen uptake (VO_2 max).

All patients were receiving angiotensin converting enzyme (ACE) inhibitors and diuretics. Beta-blockers were administered to 3 patients, digitalis to 3 patients and anticoagulants to 2 patients. The medication remained unchanged during the various phases of the study. In addition to the above mentioned treatment, the patients received 4IU of GH subcutaneously every other day, for 3 months[11]. The randomized, crossover study compared the effect, of the 3-month administration of GH to the 3-month abstinence from any administration of GH. Patients with concomitant infections, malignancies, severe chronic renal failure (serum creatinine ≥2.5 mg/dl), thyropathy and connective tissue diseases were excluded from the study. Patients receiving anti-inflammatory or immunosuppressive drugs for a period less than 2 weeks were also excluded from the study. Finally, 10 healthy controls of similar gender and age constituted the control group. Table 1 summarizes the demographic, clinical and biochemical characteristics and the pharmaceutical treatment of patients with IDC at baseline.

Plasma cytokine and apoptosis marker levels assay

Blood samplings were performed to measure the inflammatory and apoptosis markers in patients with IDC at baseline, as well as before and after the 3-month administration of GH. Plasma samples were isolated in appropriate (vacutainer gel clotter) tubes, following centrifuge of whole blood samples at 3,000 rpm for 10 min. The plasma samples were preserved at –70°C until were performed the biochemical measurements. The plasma levels of cytokines TNF-α and IL-6, as well as of the soluble receptors sTNFRI, sTNFRII, sIL-6R and sFas, were determined using the ELISA (Enzyme-Linked ImmunoSorbent Assay) method and the available commercial kits manufactured by R&D Systems, Minneapolis, Minnesota. Furthermore, the commercially available kit manufactured by DIACLONE was used for the determination of the plasma levels of the soluble apoptosis inducer sFasL.

Statistical analysis

The results were statistically processed based on the instructions by Hills and Armitage, for studies with crossover design[12]. The comparative analysis of the values of proinflammatory cytokines, apoptosis markers and VO_2 max, during the various phases of the study was carried out using the ANOVA (ANalysis Of VAriance) method for repeated measurements,

Table 1. Demographic data, clinical characteristics and medication, as well as individual values of the various proinflammatory cytokines and apoptotic mediators of dilated cardiomyopathy patients at baseline.

ID	Age	Drugs	EF (%)	VO_2 max (ml/kg/min)	sFas (ng/ml)	sFasL (pg/ml)	TNF-a (pg/ml)	sTNF-RI (ng/ml)	sTNF-RII (ng/ml)	IL-6 (pg/ml)	sIL-6R (ng/ml)
1	56	D;I;Dig	25	18,3	6,86	2,34	6,11	4,4	3,26	2,4	39,0
2	23	D;I	28	16,3	1,12	48,60	6,76	4,8	1,41	7,9	26,0
3	48	D;I;B	35	15,1	3,15	33,80	11,47	2,6	1,81	4,6	28,0
4	59	D;I;Dig	18	12,0	8,14	55,40	5,88	3,7	1,72	7,9	28,9
5	65	D;I;B	20	12,7	4,23	101,60	3,56	5,7	1,64	6,4	55,2
6	43	D;I;A	26	19,3	3,80	9,95	12,16	4,9	4,65	5,8	39,6
7	54	D;I;B	30	15,1	4,90	22,30	15,09	5,7	4,28	4,4	20,8
8	40	D;I;Dig	16	16,8	2,88	4,27	8,23	3,4	2,37	5,2	35,4
9	52	D;I;A	23	13,6	6,62	7,54	4,92	3,9	2,49	5,2	26,7
10	61	D;I	19	13,4	1,43	21,90	11,58	2,8	3,52	6,8	21,1

EF = ejection fraction, VO_2 max = peak oxygen consumption, sFas = soluble Fas, sFasL = soluble Fas Ligand, TNF-α = tumor necrosis factor-α, sTNF-RI = soluble TNF receptor type I, sTNF-RII = soluble TNF receptor type II, IL-6 = interleukin-6, sIL-6R = soluble interleukin-6 receptor, D = diuretics, I = angiotensin converting enzyme inhibitors, Dig = digoxin, A = anti-coagulants, B = β-blockers.

and the Scheffe process. Parameter analysis was used to detect the correlations between the changes of the biochemical parameters and VO_2 max during the various phases of the study. Values of $p<0.05$ were considered statistically significant. All results were expressed in "mean value ± standard deviation" (mean±SD) format.

Results

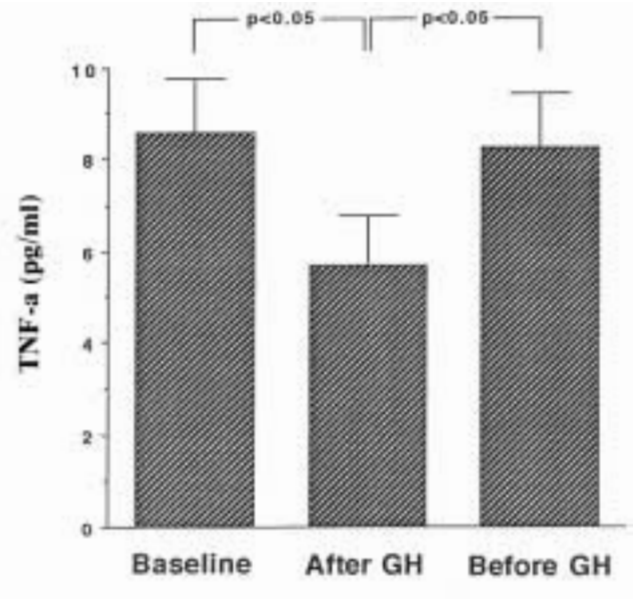
The administration of GH caused a significant reduction in the plasma levels of inflammatory agents TNF-α (8.2±1.2 vs. 5.7±1.1 pg/ml, $p<0.05$), sTNFRI (4.0±0.4 vs. 3.2±0.3 ng/ml, $p<0.005$), sTNFRII (2.6±0.3 vs. 2.2±0.2 ng/ml, $p<0.05$), IL-6 (5.5±0.6 vs. 4.4±0.4 pg/ml, $p=0.05$), and sIL-6R (32.7±5.0 vs. 28.2±3 ng/ml, $p<0.05$), as well as of the apoptosis markers sFas (4.4±0.8 vs. 3.1±0.6 ng/ml, $p<0.05$), and sFasL (34.3±11.7 vs. 18.8±7.3 pg/ml, $p<0.01$) in patients with IDC. Furthermore, GH administration was accompanied by a significant improvement of VO_2 max (15.0±0.8 vs. 17.2±1.0 ml/kg/min, $p<0.05$) in these patients. Figures 1 and 2 illustrate the effect of GH on the plasma levels of TNF-α, IL-6, sFas and sFasL factors. Good correlations were determined between the GH induced changes of VO_2 max and the plasma levels of factors TNF-α ($r=-0.64$, $p<0.05$) and sFasL ($r=-0.56$, $p=0.08$) (Figure 3).

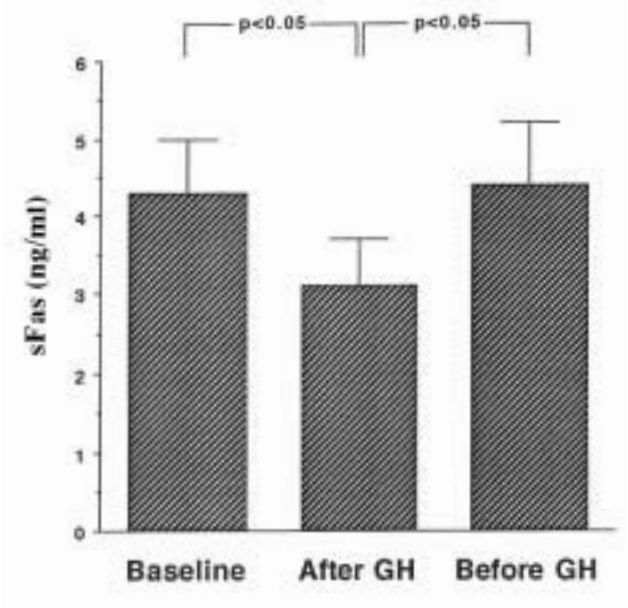
Similarly, the administration of GH also caused significant reduction (ANOVA, $p<0.05$) on proinflammatory cytokines and apoptosis marker levels as compared to baseline values. On the contrary, there were no significant differences (p:NS) among the reference values of VO_2 max (15.3±0.8 vs. 15.0± 0.8ml/kg/min), of various proinflammatory cytokines (8.6±1.2 vs. 8.2±1.2pg/ml for TNF-α, 5.7±0.5 vs. 5.5±0.6pg/ml for IL-6, Figure 1; 4.2±0.3 vs. 4.0±0.4 ng/ml for sTNFRI, 2.7±0.4 vs. 2.6±0.3ng/ml for sTNFRII, and 32.1±3.0 vs. 32.7±5.0ng/ml for sIL-6R) and of the apoptosis markers (4.3±0.7 vs. 4.4± 0.8 ng/ml for sFas and 30.8±9.8 vs. 34.3±11.7 pg/ml for sFasL, Figure 2) and of the respective values during the abstinence from GH administration phase.

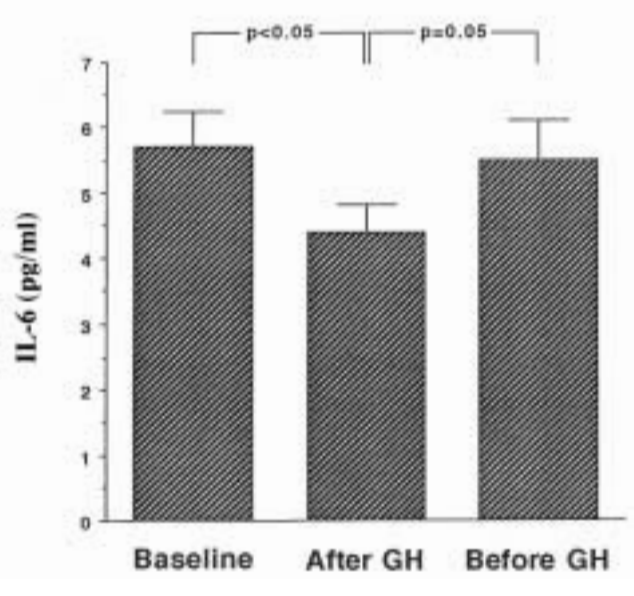
The assayed proinflammatory cytokines, their soluble receptors, as well as the apoptosis markers sFas/sFasL, were significantly increased ($p<0.001$) in patients with IDC prior to GH administration, as compared to controls (8.2±1.2 vs. 3.2±0.2pg/ml for TNF-α, 4.0±0.4 vs. 1.8±0.1ng/ml for sTNFRI, 2.6 ± 0.3 vs. 1.5±0.1ng/ml for sTNFRII, 5.5±0.6 vs. 3.6±0.2pg/ml for IL-6, 32.7± 5.0 vs. 6.2±0.3 for sIL-6R, 4.4±0.8 vs. 2.2±0.2ng/ml for sFas, and 34.3± 11.7 vs. 11.6±1.0pg/ml for sFasL). Despite the considerable reduction of the levels of the above factors following the 3-month administration of GH, these remained significantly higher ($p<0.05$) as compared to the respective values of the controls.

Discussion

Increased plasma levels of various proinflammatory cytokines (TNF-α, IL-6, IL-1β) have been det-







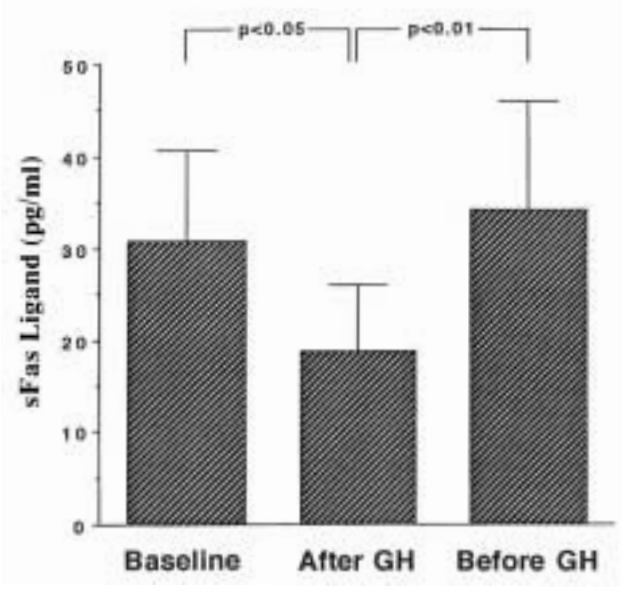


Figure 1. Effects of growth hormone (GH) administration on plasma levels of TNF-α and IL-6. Note the reduction in both cytokine levels with GH administration.

Figure 2. Effects of growth hormone (GH) administration on plasma levels of soluble apoptosis mediators sFas and sFasL. Note the significant decrease in both apoptotic parameters with GH administration.

ected in patients with severe CHF, promoting the oxidative stress and possibly participating in the progressive worsening of the syndrome[1,5,13]. The soluble receptors of proinflammatory cytokines (sTNFRI, sTNFRII, sIL-6R) are also increased in the circulation of patients with CHF and constitute significant prognostic markers reflecting the excessive activation of the immune system in the CHF syndrome[14,15]. Furthermore, the levels of the soluble apoptosis receptor Fas are increased in patients with IDC depending on the severity of the disease and provide prognostic information independent of the left ventricle geometry[16]. Finally, the soluble apoptosis inducer FasL is overexpressed in the CHF syndrome and promotes the apoptosis process in the cardiovascular system and the skeletal muscles[17]. Consequently, the sFas/sFasL system seems to play a determining role in the activation of the apoptosis process in the CHF syndrome[3,16,17].

Despite the accumulated data regarding the participation of proinflammatory cytokines and apoptosis mediators in the central (myocardial con-

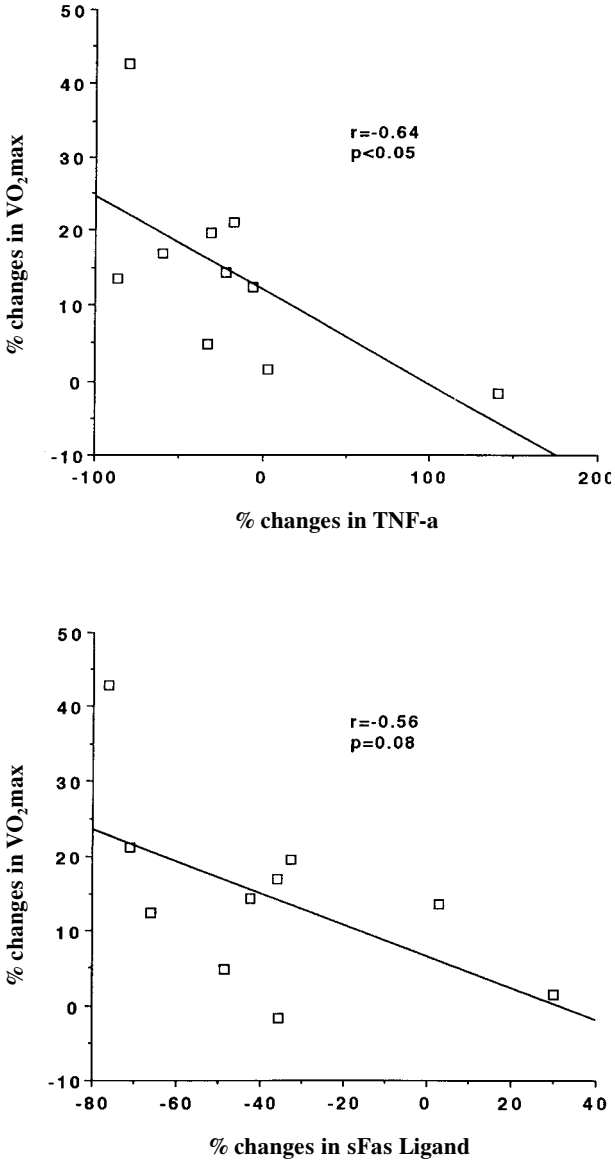


Figure 3. Correlations between growth hormone (GH)– induced changes in peak oxygen consumption (VO_2 max) and reductions in plasma levels of TNF-α and sFasL.

tractility and remodeling) and peripheral (endothelium and skeletal muscles function) disorders accompanying the CHF progress, however, there are no convincing data published in the international literature regarding the effect of traditional medicinal agents on the immune profile of patients with CHF. For example, administration of high doses of angiotensin converting enzyme inhibitors (ACEi)[18] to patients with severe CHF, or of Ca^{++} channel inhibitor amlodipine (PRAISE study)[19] to patients with IDC, was accompanied only by a reduction of the plasma levels of cytokine IL-6.

On the other hand, there is serious evidence that GH administration in experimental models of CHF improves the contractility capacity of the left ventricle and the peripheral circulation[9,10,11,20]. This beneficial effect of GH is due to vasodilation, enhancement of inotropic myocardial reserve, improvement of intracellular management of Ca^{++}, restoration of metabolism balance in myocardial cells and suppression of apoptosis activation in the cardiac tissue[10,11,20]. In addition, in smaller scale clinical studies[11,21,22,23], GH administration to patients with CHF of dilated etiology, was accompanied by the improvement of the clinical profile and the hemodynamic parameters of these patients.

This study, for the first time in international literature, describes the anti-inflammatory role of GH in the CHF syndrome, as expressed by the significant reduction of the levels of proinflammatory cytokines TNF-α and IL-6, their soluble receptors, as well as of the apoptosis mediators sFas and sFasL, i.e. factors that promote the apoptosis process in the cardiovascular system, participate in the pathophysiology of left ventricle remodeling, and possibly relate to CHF worsening. The significant correlations established between the improvement in the patients' functional status (increase of VO_2 max) and the decrease of TNF-α and sFasL levels caused by GH administration, indicate that the overexpression of inflammatory and apoptosis process mediators is directly related to the pathophysiological substrate of reduced fatigue tolerance in patients with CHF. Consequently, the recurrence of the expression of these factors constitutes a strategic therapeutic objective for the improvement of the symptoms for patients with CHF and the interruption of the progressive course of the syndrome.

Modern experimental and clinical data lead to the conclusion that a significant part of the beneficial effects of GH in the cardiovascular system is due to the stimulation of the insulin-like growth factor-1 (IGF-1) release in the systemic circulation or locally in the cardiac tissue[24]. It is known that IGF-1 reduces the plasma levels of insulin, and of VLDL and LDL cholesterols and increases the sensitivity against insulin, thus markedly modifying significant risk factors for cardiovascular diseases[25,26]. Also, the IGF-1 factor has a strong anti-oxidative and anti-apoptosis action and restores the metabolism balance in the cardiovascular system of patients with CHF[27,28]. Therefore, the anti-apoptosis and anti-inflammatory action of GH in this study may be attributed to the

GH-induced stimulation of IGF-1 production in the cardiovascular system of patients with CHF[24,27,28].

The results of this study show that administration of growth hormone constitutes a significant therapeutic intervention to the CHF syndrome, modifying the abnormal immune stimulation involved in the progress of the syndrome, possibly suppressing the activation of the biochemical pathways of apoptosis in the human cardiovascular system.

Conclusion

The administration of GH causes a significant reduction of the plasma levels of proinflammatory cytokines TNF-α and IL-6, their soluble receptors (sTNFRI, sTNFRII, sIL-6R), and the soluble apoptosis markers sFas and sFasL, in patients with CHF due to IDC. The immunomodifying and anti-inflammatory action of GH may be related to the concurrent improvement of the functional status of these patients, and constitutes a new, very promising therapeutic strategy in the CHF syndrome.

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