Serum ghrelin in female patients with rheumatoid arthritis during treatment with infliximab

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Abstract Ghrelin is a gastric hormone that possesses multiple functions, including induction of growth hormone release, regulation of proinflammatory cytokines and control of food intake and energy homeostasis. A few reports on serum ghrelin level in chronic inflammatory states revealed contradictory results. The study was undertaken to determine ghrelin in patients with rheumatoid arthritis receiving infliximab, a TNF-α blocking agent. Serum ghrelin was determined in 18 female rheumatoid patients before the treatment with infliximab, 1 week after the first infusion and after 53 weeks of medication and compared with 15 age-matched healthy women. Serum ghrelin level was shown to be increased in the patients. A decrease in serum ghrelin level was found after the first infusion of infliximab and similarly decreased ghrelin level but still higher than in the control was shown in the 53rd week of medication. The obtained results suggest that ghrelin level is related to inflammation, and its serum level in patients with severe rheumatoid arthritis behaves similarly to acute-phase reactants.

Keywords Ghrelin · Infliximab · Rheumatoid arthritis

Introduction

Ghrelin was first identified in 1999 as an endogenous ligand for the growth hormone secretagogue receptor [1]. It has a strong stimulatory effect on growth hormone secretion, and the effect is at least partially mediated through the stimulation of the pituitary growth hormone secretagogue receptor [2, 3]. Ghrelin is a 28-amino acid peptide predominantly produced by the stomach, although its expression has been reported in the bowel, pancreas, pituitary gland, hypothalamus, placenta, gonads and adipose tissue [4]. The main physiological role of ghrelin is involvement in the control of food intake and energy homeostasis [5]. Serum ghrelin level was found to be increased in malnutrition and cachexia [6]. In children with congenital heart disease, a positive correlation between elevated ghrelin and TNF-α was found [7]. Ghrelin is released into blood and causes an increase in food intake and reduces fat utilization [8]. It also antagonizes leptin through the activation of the hypothalamic neuropeptide Y/Y1 receptor pathway [9]. A few other effects of ghrelin have been reported. Ghrelin has been demonstrated to stimulate the vagal efferent nerve [10]. Additionally, ghrelin exerts multiple immunoregulatory effects. Ghrelin was reported to down-regulate TNF-α and interleukin-6 in sepsis in rats [10]. Li et al. [11] have found that ghrelin inhibits proinflammatory response and nuclear factor κB activation in human endothelial cells.

Rheumatoid arthritis is a chronic inflammatory disease affecting primarily joints. TNF-α is suggested to be a key cytokine in the development and propagation of inflammation, and TNF-α blocking agents are used in the last decade as relatively successful drugs limiting inflammation in patients with rheumatoid arthritis [12]. Otero et al. [13] reported decreased levels of ghrelin in rats with adjuvant-induced arthritis and rheumatoid arthritis patients. Recently, Gonzalez-Gay et al. [14] described an increase in ghrelin level immediately after infusion of infliximab in patients with rheumatoid arthritis. On the other hand, Maruna et al. [15] suggested that ghrelin is an acute-phase reactant and its level is elevated during postoperative...
period. Ghrelin was also found to be increased in patients with vasculitis [16].

The present study was designed to determine serum ghrelin level in patients with active rheumatoid arthritis without significant reduction in body weight before and after 1 year of treatment with TNF-α blocking agent, infliximab.

Materials and methods

Eighteen female patients with active rheumatoid arthritis and 15 healthy female age-matched and body mass index-matched controls were investigated. All patients had normal body mass index (BMI); mean BMI before the treatment was 24.5 ± 1.29 kg/m² and after treatment was 24.6 ± 1.23 kg/m². BMI of the controls was 24.0 ± 1.36 kg/m². All the patients and controls were not using hormonal contraceptives thus the effect of exogenous gonadal hormones on ghrelin level was excluded. Infliximab was given in a dose 3 mg/kg b. m on 0, 2, 6 weeks and later every 8 weeks. The patients received also methotrexate in a dose of 12.5–20 mg/week. Venous blood samples were drawn between 7.00 and 8.00 AM after overnight fasting before the first infusion of infliximab, 1 week after the first infusion and 1 week after the ninth infusion that is on the 53rd week after the first infusion.

Ghrelin level was determined with radioimmunoassay using the Ghrelin RIA kit (Linco Research, St. Charles, Missouri, USA). The sensitivity of the method was 0.04 mg/ml, intraserial variability less than 5%, and interserial variability less than 7%. C-reactive protein and ESR were measured with routine procedures.

Results were expressed as mean ± standard deviation. Comparison between the patients and controls was made with the Mann–Whitney U test. The results before and after treatment in the patients were compared with ANOVA test. Only values of \( P < 0.05 \) were considered to be significant.

Results

Serum ghrelin level in the patients was higher than in healthy individuals. After the first infusion of infliximab, an evident reduction in ghrelin was found, and similar reduction was observed after a year-long management. Ghrelin levels in the patients during medication were still higher than those in the controls (Table 1). As expected, a decrease in C-reactive protein level and ESR was observed during medication. Clinical evaluation of the patients revealed significant decrease in the disease activity (measured as DAS28) (data are not shown), and all the patients were considered as “responders”.

Discussion

Our study shows that serum ghrelin level is enhanced in patients with active rheumatoid arthritis and application of infliximab resulted in some decrease in ghrelin level although after 1 year of medication the level was still higher than in the controls. A decrease in ghrelin was accompanied by the reduction of inflammatory indices and a decrease in the disease activity. This finding is different than observation of Otero et al. [13] who found decreased ghrelin level in 31 rheumatoid arthritis patients. Their group of patients was heterogenous and only 22 of them had active disease. Nine of their patients were treated with TNF-α blocking agents. It is also possible that sex of the patients had influence on difference between our results and those reported by Otero et al. [13]. Our patients were only female, and body mass of female rheumatoid patients is usually slightly higher than male patients, and ghrelin level is related to body mass. In the animal model, acute adjuvant-induced arthritis was associated with a decrease in serum ghrelin while after 15 days during recovery, the serum ghrelin level was higher than in the controls. Our findings are concomitant with studies of Kümpers et al. [16] who investigated patients with ANCA-associated vasculitis. Ghrelin level was almost twice higher in the

| Investigator group | Ghrelin [pg/ml] | C-reactive protein [mg/l] | ESR [mm/hr] |
|--------------------|----------------|--------------------------|-------------|
| A. Controls        | 1068 ± 287.5   | 2.1 ± 1.2                | 13 ± 6      |
| B. Rheumatoid arthritis before infliximab therapy | 2753.4 ± 486.8 | 44.8 ± 16.2 | 39 ± 27 |
| C. Rheumatoid arthritis 1 week after the first infliximab infusion | 1471.8 ± 361.9 | 37.3 ± 11.2 | 38 ± 17 |
| D. Rheumatoid arthritis after 53 weeks of medication | 1666.2 ± 409.1 | 16.9 ± 11.6 | 25 ± 14 |

Statistical significance of the differences

Ghrelin: A–B, A–C, A–D, B–C, B–D \( P < 0.05 \); C–D nonsignificant
C-reactive protein: A–B, A–C, A–D, B–D, C–D \( P < 0.05 \); B–C nonsignificant
ESR: A–B, A–C, A–D \( P < 0.05 \); B–C, B–D, C–D nonsignificant
patients before medication and declined during therapy. They found also positive correlation between ghrelin level and disease activity indices, including C-reactive protein level. Gonzalez-Gay et al. [14] recently studied the effect of infliximab infusion on ghrelin level. They found immediately after infusion an increase in ghrelin. It is possible that immediate increase is followed by a decrease as it has been found in our study.

It seems that serum ghrelin level in patients with rheumatoid arthritis is regulated by a number of factors; the factors are probably of metabolic nature and are related to body mass, fat mass and energy balance, immuno-inflammatory nature or others. In our study, patients had no cachexia that can be seen in some patients suffering of long-lasting inflammation. Serum leptin level alteration has been found in patients with rheumatoid arthritis [for review see: 17, 18]. The leptin/ghrelin system has been suggested to contribute to the regulation of inflammation. Ghrelin was shown to reduce leptin-induced proinflammatory response in human mononuclear and T cells [17]. A positive correlation between elevated serum ghrelin and TNF-\( \alpha \) level was reported in cachexia [16]. This finding is concomitant with our results suggesting that anti-TNF-\( \alpha \) medication is associated with a decrease in elevated ghrelin level.

In conclusion, we have found that enhanced serum level of ghrelin in patients with active rheumatoid arthritis is reduced but not normalized after 1 year of medication with infliximab. The mechanism of the alteration and role of ghrelin in rheumatoid arthritis patients deserves further study, and it is possible that some aspects of pathogenesis of the disease (e.g., vascular involvement) are related to ghrelin. Additional studies are also needed to evaluate possible application of ghrelin as a clinically applicable index of the disease activity in patients receiving biologic therapy.

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