CLINICAL STUDY

Improvement of ACTH response to insulin tolerance test in female patients with rheumatoid arthritis due to tumor necrosis factor inhibition

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Abstract

Objective: The hypothalamic–pituitary–adrenal (HPA) axis evaluation has been conflicting in rheumatoid arthritis (RA) patients. Our aim was to evaluate the HPA axis response to the insulin tolerance test (ITT) in premenopausal female patients with RA before and after anti-tumor necrosis factor therapy (anti-TNF therapy).

Design: A comparative cross-sectional analysis.

Subjects and methods: Ten females with RA and without previous anti-TNF therapy were included. Five healthy females were included as controls. An ITT was performed before first dose of anti-TNF therapy and then after week 12. Anti-TNF therapy was applied every 14 days for 12 weeks. Cortisol and ACTH levels were measured at 0, 30, 45, and 65 min. Prolactin was measured at 0, 30, 45, 90, 120, and 150 min.

Results: The ACTH basal plasma levels at weeks 0 and 12 did not show statistical differences, at 1.26 (0.41–2.12) vs 1.54 (0.60–2.49) respectively ($P=0.68$). The controls demonstrated a higher ACTH response than in the RA patients at week 0 before the anti-TNF therapy (349.12 area under curve (AUC)), ($P=0.004$) and a similar ACTH response to ITT to those of RA patients at week 12 after the use of the anti-TNF therapy (1087.42 AUC). Serum cortisol levels did not show significant changes when the ITT was performed before and after the anti-TNF therapy.

Conclusions: Our findings support a role for the TNF on the pituitary gland in premenopausal female patients with RA. An adequate control of RA in early stages of the disease diminishing TNF levels improves ACTH response to stress situations.

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Introduction

Rheumatoid arthritis (RA) is a systemic, inflammatory chronic disease. It is characterized by the accumulation of T cells, plasmatic cells, macrophages, and fibroblasts in the rheumatoid synovial membrane. Prevalence of RA has been demonstrated to be close to 0.5–2% (1), and is an important cause of dysfunctional incapacity as well as greater medical costs for people in productive ages (2).

The maintenance of life depends on the capacity of the body to sustain homeostasis; one gland involved in this process is the pituitary gland. It produces thyrotropin, prolactin (PRL), adrenocorticotropin (ACTH), growth hormone, and gonadotropins (follicle-stimulating hormone and luteinizing hormone), and it, in turn, is regulated by the hypothalamic–pituitary–adrenal (HPA) axis (3). In RA patients, the HPA axis shows diverse alterations and we described only some of them: i) it has been demonstrated that patients with RA have low levels of spontaneous and stimulated cortisol secretion, particularly in relation to inflammation (4–6); ii) the

basal serum levels of ACTH in RA patients are lower or similar in comparison with control groups (7, 8); iii) Harbuz showed, in seven RA active patients and six healthy subjects (all previously sensitized with an oral dose of dexamethasone), that serum cortisol levels in response to the corticotropin-releasing hormone (CRH) stimulation test were blunted in the control subjects and in four RA patients; however, three RA patients mounted an immediate and sustained cortisol response after receiving CRH. This suggested an impaired glucocorticoid feedback (9). However, the specific role of the HPA axis in the RA pathogenesis is still an open question. High pituitary tumor necrosis factor (TNF)- α expression has been shown in response to lipopolysaccharide in mice models (10), and TNF specific receptors in the hypophysis cellular lines from murine models have also been found (11, 12). In RA patients, the anti-TNF therapy demonstrated a diminished inflammatory joint event and less radiological progression (13–15). Our aim was to evaluate changes in the serum levels of ACTH, cortisol, and PRL in response to an insulin tolerance test

(ITT) in ten female patients with RA, both before and after of the use of anti-TNF therapy.

Subjects and methods

Subjects

Ten premenopausal female patients with RA were included in accordance with the American College of Rheumatology 1987 revised criteria (16). Five healthy premenopausal female volunteer subjects were included aged between 25 and 35 years as controls. Evolution of the disease was on average 48 months (range 18.6–89.39). The tuberculin test was negative for all patients. All patients were naïve to steroids. They all exhibited a normal chest X-ray plate. During the entire study period, no patient used anti-depressive drugs or any other drug that could alter the HPA axis. All patients had at least four swollen joints. Clinical information regarding the ten patients was collected by an independent rheumatologist. Each patient completed a validated Spanish version (17) of the health assessment questionnaire (HAQ), the disease activity score-28 (DAS-28) and their body mass index was measured. The serum samples were stored at -70°C until being analyzed.

Hormonal measurements

ACTH (normal values 2.2–13.2 pmol/l) was measured by a solid phase two-sided immunoradiometric assay (ELSA-ACTH kit, CIS bio international, Gif-sur-Yvette Cedex, France) with an intra-assay coefficient of variation (CV) of 3.7 and inter-assay CV of 3.8; cortisol (normal values 137.95–689.75 nmol/l) was measured by an electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics) in a Roche Elecsys model 2010 with intra- and inter-assay CV of 1.6 and 2.8 respectively. PRL (normal values 3–18 $\mu\text{g/l}$) was measured by an ECLIA (Roche Diagnostics) in a Roche Elecsys model 2010 with intra- and inter-assay CV of 3.3 and 4.6 respectively.

Anti-TNF therapy

The patients did not use any class of anti-TNF therapy prior to the study. Adalimumab therapy (a specific humanized TNF- α inhibitor) was used for 12 weeks at doses of 40 mg s.c., every other week. Adalimumab was supplied by Abbott Laboratories; the pharmaceutical company did not have any participation in the project design or development.

Protocol

We performed an ITT to evaluate the HPA axis in RA patients both before and after the use of anti-TNF therapy. The ITT was made by 0.15 IU/kg body weight crystalline insulin infusion; the insulin was given in a

bolus via a cannula previously inserted in a forearm vein. The cannula was kept clear throughout by the use of heparin (1 ml/bolus of 10 units of heparin, after each sample was taken). We administrated 75 g glucose orally to the patients if they showed prolonged hypoglycemia on a glucostix test or hypoglycemic symptoms. The patients included did not reach a state of hypoglycemia, defined as blood glucose levels below 2.2 mmol/l. However, the hormone response to the low-dose ITT as well as hypoglycemic symptoms in all patients, including five healthy female volunteers taken as controls, could validate our results in relation to low-dose ITT with serum glucose levels at least 3.5 mmol/l. Serum cortisol and plasma ACTH were measured at 0, 30, 45, and 65 min, and measurements of the serum PRL levels were made at 0, 45, 90, 120, and 150 min. After the test, we gave a meal with high carbohydrate content. The serum glucose values were determined during ITT by Glucostix test (Bayer Diagnostics) and after with an automated technique (Vitros 950, Ortho-Clinical Diagnostic, Johnson & Johnson Co., Rochester, NY, USA). The measurements were performed at intervals until 65 min after insulin administration as previous articles have shown that the cortisol peak is reached at 60 min, remaining without significant elevations after this time. The same tendency for ACTH has been observed (18). The study, as well as an amendment, was approved by the local Ethics Committee in accordance with the principles of the declaration of Helsinki. (An amendment was added to the study regarding healthy female volunteer controls that were included after the beginning of the study.) All patients, including the healthy volunteers, signed a consent form.

Statistical analysis

The values are given as the mean $\pm$ s.d., or as the median with 95% confidence intervals for the hormone measurements, except when otherwise indicated. Comparisons of variables between two groups (before and after) were made by the Wilcoxon matched pairs test. We used a Repeated Measures ANOVA test for finding inter-group serum hormone levels differences before and after the anti-TNF therapy. The Statistical program (Statsoft, Tulsa, OK, USA Version 6.0) was used for the general analysis and the Origin software Version 6.0 (Northampton, MA, USA) was used for finding the area under curve (AUC) for the hormones.

Results

No adverse events were observed during anti-TNF therapy. ITT induced hypoglycemic symptoms such as somnolence, discrete cold sweat, or feelings of hunger in all patients; three patients required oral glucose for controlling severe hypoglycemic symptoms such as anxiety, detachment, or profuse cold sweat 45 min

Table 1 Baseline characteristics of the rheumatoid arthritis patients before and after the anti-tumor necrosis factor therapy.

	Before	After	P value
Age (years)	35.8±10.17	ND	
BMI (Kg/m ²)	24.5 (19.1–27.8)	ND	
HAQ	0.80 (0.76–1.64)	0.32 (0.06–1.26)	0.37
CRP (mg/dl)	0.54 (1.15–5.48)	0.29 (0.55–2.37)	0.37
Prolactin (µg/l)	12.45 (4.8–15.1)	11.75 (9.9–16)	0.22
Cortisol (nmol/l)	284.2 (169.86–398.54)	208 (165.68–250.3)	0.22
ACTH (pmol/l)	1.26 (0.41–2.12)	1.54 (0.60–2.49)	0.68

BMI, body mass index; HAQ, health assessment questionnaire; CRP, C reactive protein.

after the beginning of the test. Adalimumab caused clinical improvement in almost all patients. The DAS-28 showed an improvement from 4.85 (2.19–6.55) to 2.39 (1.89–4.72) reaching statistical significance at $P=0.04$. The erythrocyte sedimentation rate did not show changes from 17 (0.31–48.7) to 11 mm/h (0.26–29); $P=0.07$. Table 1 shows the values for the other variables. The serum glucose levels in response to ITT achieved hypoglycemic levels between 3.5, 3.6, and 3.9 mmol/l, for both the controls and the RA patients before and after anti-TNF therapy (Fig. 1).

ACTH response

The ACTH curve comparing basal and week 12 responses to ITT shows discrete differences at 30 min. However, at 45 min, we found a clear difference between the groups: 2.90 pmol/l (0.46–5.35) vs 33.32 (14.98–51.66) which showed significance ($P=0.004$). At 65 min, we also found differences in the groups: 2.55 pmol/l (0.49–4.61) vs 20.09 (9.94–30.24); $P=0.004$ (Fig. 2). The AUC for ACTH serum levels are shown for all groups in Table 2. Correlations between ACTH and cortisol at 65 min in the controls and the patient group undergoing anti-TNF therapy were significant: $r=0.90$; $P<0.05$ and $r=0.68$; $P<0.05$ respectively. We did not find any correlation between ACTH and cortisol in the patients with RA before anti-TNF therapy.

Cortisol response

The basal cortisol serum levels had a discrete tendency to be lower after the anti-TNF therapy but this did not have statistical significance (Table 1). The cortisol curve, in response to ITT, was similar at week 0 and 12 in both groups and higher in the control group (Fig. 3). The AUC did not show differences in either group of the RA patients before or after TNF therapy (Table 2).

PRL response

The PRL response to ITT was adequate for the RA group before anti-TNF therapy, reaching the maximum response at 45 min of 233.3 µg/l (183.06–340.59) and for the control group reaching the maximum

response at 90 min of 187.7 µg/l (90–288.8). However, the PRL response to the ITT of the RA patients after the anti-TNF therapy was higher, being 384.5 µg/l (235.31–482.33), at week 12 than that of the same patients at week 0 and that of the control group (Fig. 4). The AUC showed differences between both RA groups suggesting higher serum levels of PRL after the anti-TNF therapy in response to ITT (Table 2).

Discussion

The HPA axis has been evaluated in multiple forms in former studies of RA patients; however the results have been conflicting in the majority of them. The steroid treatment, time evolution, gender, and drugs that could interfere with the HPA axis have been some causes for these non-congruent results. TNF is a key cytokine in the inflammatory processes of RA patients; the improvement seen in RA patients with anti-TNF therapy is the proof of this. We could observe a tendency to improve the disease activity measured by DAS-28 index. However, the erythrocyte sedimentation rate and HAQ did not reach statistical significance, probably due to the short time of the anti-TNF therapy. We found normal low serum levels for ACTH and cortisol in the RA patients at baseline. The ACTH serum levels before the anti-TNF therapy

Figure 1 Glucose response to insulin tolerance test in controls and RA patients before and after anti-TNF therapy. Values are expressed as mean ± s.d.

Figure 2 ACTH response to insulin tolerance test in controls and RA patients before and after anti-TNF therapy. Values are expressed as mean $\pm$ s.d.

Figure 3 Cortisol response to insulin tolerance test in controls and RA patients before and after anti-TNF therapy. Values are expressed as mean $\pm$ s.d.

did not show a response to the ITT at the time, but when the test was applied 3 months later, with anti-TNF therapy, we observed a clear rise in the ACTH serum levels at the time with a peak at 45 min, comparable to the healthy controls. These results agree with those of Straub, who observed that long-term anti-TNF therapy improves the ACTH levels in RA patients. His results indicated that the anti-TNF therapy sensitizes to the hypothalamus and pituitary gland (19). However, our study demonstrated that the TNF effect on pituitary level could interfere with normal ACTH secretion; the better ACTH response to ITT after the use of anti-TNF therapy showed this phenomenon. On the other hand, the failure to stimulate the adrenal cortex in stress situations such as RA can be secondary to the adrenal cortex atrophy that had been observed in these patients and the subsequent lower cortisol levels found in the former studies of ITT (20). We did not find any correlation between ACTH and serum cortisol levels in patients with RA before anti-TNF therapy. This is in accordance with former studies that did not find a correlation with either hormone in patients with RA (21, 22). However, at week 12, the serum cortisol levels displayed by the RA patients showed a discrete response to ITT, even when in low levels, and a correlation at 65 min with the serum ACTH levels. The control group displayed a similar response as well. This finding could indicate a better ACTH release with

adrenal gland stimulation but not one sufficient to reach normal cortisol serum levels. Barney and colleagues found in bovine adrenal cells that TNF has an inhibitory effect on ACTH-stimulated cortisol released (23), therefore, we could be improving this balance in the adrenal cells. However, the anti-TNF- α therapy not only demonstrates TNF neutralization but also diminishes the interleukin(IL)-6 and IL-1 levels to some degree. These cytokines have an effect on the adrenal gland; unfortunately our model cannot display a direct effect of these cytokines on adrenal cells. We can emphasize here that the TNF plays an important role in the HPA axis as a key factor via the ACTH inhibition at the pituitary level. However, we cannot conclude that the HPA axis has a complete improvement in response to anti-TNF therapy in RA patients because we did not observe an adequate adrenal response to ITT at week 12. Another observation is that serum PRL levels had a higher response to ITT after the TNF inhibition. A low PRL response to the induced hypoglycemia has been reported previously; improvement in this response by the treatment with

Table 2 The area under curve for the hormone measurements in the rheumatoid arthritis patients before and after anti-tumor necrosis factor therapy.

	AUC basal	AUC after	Peak (min)	P
ACTH (pmol/l)	349.12	1087.42	45	0.004
Cortisol (nmol/l)	29 067.44	20 795.13	65	0.059
PRL (μ g/l)	28 037.62	36 756	45	0.001

AUC, area under curve. Statistical analysis was made by the Wilcoxon signed rank test.

Figure 4 Prolactin response to insulin tolerance test in controls and RA patients before and after anti-TNF therapy. Values are expressed as mean $\pm$ s.d.

disease-modifying anti-rheumatic drugs was shown by Eijsbouts and Rovenski in RA patients (24, 25), but the specific role for this hormone in the RA pathogenesis is still uncertain. Our findings support a role for TNF on the pituitary gland in female patients with RA. Therefore, we conclude that an adequate control of the disease diminishing TNF serum levels improves the ACTH response to stress situations in premenopausal female patients with RA; this could avoid some degree of adrenal cortex atrophy secondary to lack of adrenal cortex stimulation by ACTH in the early stages of the disease.

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