CONCISE REPORT

Cortisol elimination from plasma in premenopausal women with rheumatoid arthritis

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Objective: To test the hypothesis that cortisol elimination from plasma can contribute to relatively low cortisol in premenopausal women with rheumatoid arthritis (RA).

Methods: Twelve premenopausal female patients with RA (39.8 (1.8) years) and nine healthy control women matched for age and body mass index (42 (3.3) years) were enrolled in the study. None of the patients had previously been receiving treatment with glucocorticoids. After dexamethasone suppression (2 mg by mouth) the evening before the study, 20 mg of hydrocortisone was given. Blood and saliva samples were drawn six hours after injection of hydrocortisone. Plasma and salivary cortisol were measured.

Results: Dexamethasone administration suppressed plasma cortisol concentrations to an almost undetectable level in all subjects, except one with RA. In this subject, a raised concentration of plasma cortisol was verified by repeated analysis despite the fact that cortisol concentration in the saliva sample measured simultaneously was not raised. No significant difference in the disappearance curve of cortisol in plasma or in salivary cortisol levels was found between the patients with RA and the healthy controls.

Conclusions: The profile of disappearance of total cortisol from plasma, and salivary cortisol levels during the elimination phase after its intravenous administration, are unchanged in premenopausal women with RA. Alterations in cortisol clearance are not likely to have a role in cortisol availability in patients with RA.

Activation of the hypothalamic-pituitary-adrenal (HPA) axis by proinflammatory cytokines such as interleukin 1 (IL1), IL6, or tumour necrosis factor during the inflammatory process has been regarded as an important mechanism, which counterregulates the immune inflammatory response itself. Cortisol, as one of the key hormonal products of the activated HPA axis, exhibits potent immunomodulatory actions. Therefore, deficiencies in the HPA axis function may predispose subjects to chronic inflammatory diseases such as rheumatoid arthritis (RA).¹

Depending on the methodology used, certain abnormalities of HPA axis responses to stimulation have been reported in premenopausal female patients with RA.^{2,3} It has been suggested that plasma levels of cortisol are inadequately low in relation to the inflammatory stress, which is indicated by an increased erythrocyte sedimentation rate and increased concentrations of C-reactive protein or proinflammatory cytokines in the plasma of premenopausal⁴ patients with RA and also untreated patients with early RA.⁵ Some other mechanisms of HPA axis activation, such as severe pain of those patients, have to be also taken into account.⁶

Evaluation of HPA axis perturbations in RA has been mainly based on an assessment of hormone concentrations in the

plasma. In general, an actual concentration of a hormone in plasma depends not only on its secretion but also on the concurrent elimination. Cortisol is metabolised irreversibly by 5- α - and 5- β -reductases to 5- α -tetrahydrocortisol and 5- β -tetrahydrocortisol or converted to biologically inactive cortisone by 11- β -hydroxysteroid dehydrogenases type 2. Cortisone can be reactivated by 11- β -hydroxysteroid dehydrogenases type 1 to cortisol or reduced to tetrahydrocortisone by 5- β -reductase.⁷

Our hypothesis was that cortisol elimination from plasma in patients with RA is increased. This maybe caused by the proinflammatory status or, possibly, a hereditary defect. Increased cortisol elimination would lead to relatively lower cortisol availability in patients with RA.

To test this hypothesis, cortisol clearance after its intravenous administration was evaluated in dexamethasone pretreated female patients with premenopausal onset of RA.

SUBJECTS AND METHODS

Twelve female patients with RA (age 39.8 (1.8) years), according to the criteria of the American Rheumatism Association, recruited from the National Institute for Rheumatic Diseases in Piestany and from the 1st Clinic of Internal Medicine, Medical Faculty of Comenius University in Bratislava, Slovakia, were studied. Nine healthy women matched for age (42 (3.3) years) and body mass index served as controls. To preserve the homogeneity of the study group we decided to study premenopausal female patients only. None of the patients had previously been receiving treatment with glucocorticoids. All patients had been using non-steroidal anti-inflammatory drugs or second line drugs, or both. The mean duration of the disease was 8 (1.7) years. None of the patients had active disease at the time of the study. All subjects gave informed written consent, and the study was approved by the ethical committee of the National Institute for Rheumatic Diseases.

At 9 pm the evening before the study 2 mg of dexamethasone by mouth (Dexamethazon Leciva tbl., Leciva a.s., Praha, Czech Republic) was given to all subjects. Next morning an indwelling catheter was inserted into a cubital vein. Basal blood and saliva samples were obtained. Then over a period of three minutes 20 mg of cortisol (Hydrocortison ICN, ICN Czech Republic a.s., Rožtoky, Czech Republic) in 10 ml of isotonic NaCl solution was injected into the cubital vein of the opposite arm to prevent catheter contamination. Blood samples were drawn at 10, 20, 30, 60, 90, 120, 240, and 360 minutes after the injection into the tubes containing EDTA, immediately cooled, and centrifuged at 2500 rpm for 15 minutes. Saliva samples were taken and placed in clean polyethylene tubes at the same time intervals. Both plasma and saliva samples were stored at -20°C until analysed.

Abbreviations: AUC, area under the curve; CBG, cortisol binding globulin; HPA, hypothalamic-pituitary-adrenal; IL, interleukin; RA, rheumatoid arthritis

Figure 1 Disappearance curve of plasma cortisol (A), cortisol concentration in saliva (B), and percentage of salivary and plasma cortisol (C) after intravenous administration of cortisol in 12 patients with RA and nine healthy controls. Data are expressed as mean (SD)

Plasma and salivary cortisol were analysed by radioimmunoassay. Briefly, freshly thawed samples of saliva were centrifuged (2000 *g*, 15 minutes) and cortisol concentrations were measured in 200 μ l of supernatant or in 50 μ l of plasma. [3 H]Cortisol was used as the radioligand, and antiserum prepared to cortisol-21-hemisuccinate-bovine serum albumin was kindly provided by C Oliver, Marseille, France. Dextran coated charcoal was used to separate free and bound fractions.⁸

The statistical analysis was performed using the SIGMASTAT 2.0 program. A comparison of hormone concentrations was made by two way analysis of variance with consecutive post hoc tests used to determine the differences between groups. The plasma cortisol concentrations were entered in the following biexponential equation: $\text{cortisol}_{\text{plasma}} = a \times e^{-bt} + c \times e^{-dt}$ using regression procedure. From the *b* and *d* variables the half time of each portion of the curve ($t_{1/2}1$, $t_{1/2}2$) was calculated as $\ln 2/b$ and $\ln 2/d$. The area under the plasma concentration-time curve (AUC) was calculated by

Table 1 Cortisol levels (at 0830) after overnight dexamethasone suppression, and basic pharmacokinetic parameters expressed as mean (SD). Significance was set at $p < 0.05$ (Mann-Whitney rank sum test)

	RA	Controls	p Value
Cortisol (ng/ml)	18 (25.22)	21.13 (3.6)	0.69
$t_{1/2}1$ (h)	0.27 (0.06)	0.21 (0.06)	0.48
$t_{1/2}2$ (h)	5.17 (1.95)	4.46 (1.33)	0.84
AUC (ng×h)/ml	1489 (229)	1153 (138)	0.32
Total body clearance (l/h)	14.44 (1.15)	14.38 (1.66)	0.35

the SIGMAPLOT program (SPSS Inc, USA). The total body clearance was calculated as the dose (20 mg) divided by the AUC. All data are expressed as means (SD). Significance was set at $p < 0.05$.

RESULTS

Dexamethasone administration suppressed plasma cortisol concentrations to an almost undetectable level in all subjects, except one patient with RA. In this patient, a raised concentration of plasma cortisol was verified by repeated analysis despite the fact that the cortisol concentration in saliva sample measured simultaneously was not raised.

Figure 1 shows that after intravenous administration of 20 mg of hydrocortisone the disappearance curve conformed to the biexponential plasma concentration profile. The first slope of the curve represents the distribution phase with mean half time $t_{1/2}1$. The second portion of the curve represents the elimination of cortisol from plasma with half time $t_{1/2}2$ (table 1). No significant difference in the disappearance curve of cortisol in plasma was found in patients with RA in comparison with the control group. The concentration of salivary cortisol was unexpectedly increased in two patients in 360 minutes. Neither the mean concentration of salivary cortisol nor the salivary to plasma cortisol ratio in 360 minutes reached statistical significance. Table 1 shows the basic pharmacokinetic parameters, which were similar in patients and controls.

DISCUSSION

Changes in cortisol concentrations in plasma observed under pathological conditions are usually attributed to alterations in cortisol release. The well known fact that hormone levels in plasma are determined also by the rate of its elimination is often neglected. An appropriate approach to study the rate of cortisol distribution and elimination is repeatedly to measure its level in plasma after administration of cortisol in subjects pretreated with dexamethasone to block endogenous cortisol secretion.⁹ Pharmacokinetic parameters, such as the half times of both the distribution and elimination part of the cortisol disappearance curve, observed in this study are similar to those reported previously.¹⁰ However, no differences were noticed in the profile of plasma cortisol concentrations in premenopausal patients with RA. Thus, our hypothesis that RA could be accompanied by increased cortisol clearance failed to be verified.

The biologically active fraction of cortisol in plasma is the free hormone, which is not bound to plasma proteins. The main binding proteins are cortisol binding globulin (CBG) and albumin. Interestingly, IL6 was shown to inhibit the synthesis of CBG in cultured hepatoblastoma derived cells.¹¹ If IL6 acted in the same way in vivo, those patients with RA, in whom IL6 concentrations are raised,^{3 5 12} would exhibit an increased free fraction of cortisol. This possibility was evaluated in our study by measurement of salivary cortisol. It has been shown that during the elimination phase, salivary cortisol represents well

the concentration of the free fraction of the hormone in plasma.⁹ The results show that salivary cortisol disappearance in patients with RA was proportional to that in healthy controls. Although the ratio of salivary and plasma cortisol tended to be higher in patients with RA, this difference did not reached statistical significance. Thus, the shift between the free and bound fractions of plasma cortisol does not seem to be present in patients with RA.

The suggestion has been made that glucocorticoid hypocompetence may occur in a subset of women with premenopausal onset of RA and thus might contribute to the development of RA.¹³ Some authors reported lower cortisol levels and reduced cortisol responses to surgical stress in patients with RA than in control patients with osteomyelitis and osteoarthritis,¹² or subtle changes in cortisol response during an insulin tolerance test.² On the other hand, basal levels of cortisol and adrenocorticotropic hormone as well as subsequent response to corticotrophin releasing hormone stimulation in patients with newly diagnosed RA did not differ significantly from those in healthy controls.¹⁴ Raised cortisol levels were even reported in premenopausal female patients with RA previously not treated with glucocorticoids.¹⁵ However, based on the cortisol levels it is difficult to interpret the relative adrenal hypocompetence because of the negative feedback control of the HPA axis. Nevertheless, cortisol concentrations in patients with RA are thought to be relatively low during ongoing inflammation.^{5–16} As shown in the present study, changes in cortisol elimination from plasma do not seem to be a contributing factor to cortisol availability, at least not in female patients with premenopausal onset of RA. Further studies are needed to verify whether these findings can be generalised also to other patients with RA.

In conclusion, present investigations showed that the profile of disappearance of total cortisol from plasma, and salivary cortisol levels during the elimination phase after its intravenous administration, are unchanged in premenopausal women with RA. Alterations in cortisol clearance are not likely to have a role in cortisol availability in patients with RA.

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