GONADAL AND ADRENAL STEROID HORMONES IN PLASMA AND SYNOVIAL FLUID OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Objectives. Gonadal and adrenal steroids were shown to affect multiple immune processes including inflammatory response. These effects were documented, specifically, through an influence on local productions of cytokines and the functions of synovial cells at the site of inflammatory processes. The aim of this study was to investigate the levels of selected hormones in synovial fluid of knee joints of patients with rheumatoid arthritis (RA) and with osteoarthrosis (OS, control group).

Methods. The concentrations of cortisol (CORT), 17-ß-estradiol (ES), dehydroepiandrosterone (DHEA), testosterone (TE), progesterone (PRG), and aldosterone (ALD) were determined in plasma and synovial fluid.

Results. Significant positive correlations between the levels in plasma and synovial fluids were observed in hormones ES, PRG, TE, DHEA and ALD. In most hormones, the levels in synovial fluids were similar as in plasma; however, the content of ALD was higher in synovial fluid as compared to plasma. Higher levels of ES (in females), DHEA (in males), and ALD were observed in plasma and synovial fluids of RA patients as compared to OS patients. After adjustment to age, no significant RA vs. OS difference was noted in ES, TE, DHEA, PRG, and CORT in plasma and synovial fluid. Age-adjusted ALD concentration tended to be higher in synovial fluid of RA patients as compared to OS patients. The ratio of ES/TE concentrations in synovial fluid was significantly higher in male RA patients compared to OS group. Also the ES/CS and ES/DHEA ratios in synovial fluid were elevated in RA patients in comparison to controls.

Conclusions. These results demonstrated the prevalence of pro-inflammatory hormones in synovial fluid of RA patients, suggesting the possible role of these steroid hormones in inflammatory processes.

Key words: Steroid hormones - Synovial fluid - Plasma - Human subjects - Rheumatoid arthritis - Osteoarthrosis

Rheumatoid arthritis (RA) is the most common inflammatory joint disease characterized by proliferative, invasive synovitis and a chronic T-cell response that has escaped normal control mechanisms. Inflammation of the synovial membrane surrounding a joint leads to swollen, tender, and stiff joints. In contrast to RA, osteoarthritis (OS) is a disease affecting joint structures and develops as a consequence of injurious activities acting on a vulnerable joint with modest secondary inflammatory response.

Gonadal and adrenal steroids have been shown to affect multiple immune processes including inflammatory response (BARNES et al. 1998; CASTAGNETTA et al. 1999; ELENKOV AND CHROUSOS 2002; GUTIEREZ et al. 1994; HAMANO et al. 1998; IMRICH 2002; LI et al. 1993; MORISHITA et al. 1999). It has been proposed that physiologic levels of estrogens stimulate immune responses, whereas androgens suppress inflammatory reactions (CASTAGNETTA et al. 2003). The role of steroid hormones in the development, onset, and progression of autoim-

mune diseases such as RA has been also indicated by abundant clinical data (e.g. higher prevalence in females, increased incidence in post-partum and post-menopause, anti-inflammatory effects of glucocorticoids).

On the systemic level, studies in RA patients have suggested that the activity of the hypothalamic-pituitary-adrenal (HPA) axis is inappropriately normal with respect to the ongoing inflammation (Masi et al. 1999; Straub et al. 2002) and is not capable of exerting control over inflammatory responses. Inappropriately normal cortisol (CORT) and low levels of adrenal androgen dehydroepiandrosterone sulphate (DHEAS) observed in this group of RA patients were regarded as a signs of adrenal hypocompetence (Masi et al. 1999). Several works showed a correlation between the RA activity and testosterone (TE), dehydroepiandrosterone (DHEA) and DHEAS plasma levels (Masi 1995, Khalkhali-Ellis et al. 1998).

Apart from the central neuroendocrine regulatory mechanisms, local effects of steroids on immune and connective tissue during inflammation is of key importance for the clarification of RA pathogenesis (Cutolo et al. 1992; Cutolo et al. 2002; Cutolo et al. 2003). Adrenal and gonadal steroids transported from peripheral blood into the joint structures undergo series of poorly elucidated modification, which may further affect their immunomodulating properties in target tissue. Local conversion of DHEA in cells of synovial tissue may lead to an increase of estrogens important for local immunomodulation in RA synovitis (Cutolo et al. 2004). Therefore, the steroid content in the synovial fluid may differ from that in plasma and conversion of steroids in the synovial tissue may depend on presence or absence of inflammatory process.

The aim of our study was to analyze plasma and synovial fluid concentrations of selected steroids in patients with inflammatory (RA) and non-inflammatory (OS) joint diseases.

Subjects and Methods

The patients admitted to the National Institute for Rheumatic Diseases (Piestany, Slovakia) were informed about the purpose of the study and gave written informed consent. The Ethical Committee of the Institute approved the study. Basic characteristics of the RA and OS patients are shown in Table 1. All RA patients underwent routine clinical and laboratory investigations. Laboratory data including erythrocyte sedimentation rate, C-reactive protein, and rheumatoid factors (latex test and hemaglutination test) in plasma were obtained before examination. In clinical assessment, the duration and clinical activity of disease, X-ray stage, and antirheumatic therapy were recorded. The patients with RA were on therapy with nonsteroidal antiinflammatory drugs, which were withdrawn at least 3 days before blood and synovial fluid collection. Patients on hormonal therapy (androgen or estrogen), with diabetes mellitus, or endocrine disorders were excluded from the study.

Table 1
Basic characteristics of the RA and OS patients and synovial fluids (means±SE)

	RA	OS
Number and gender (M/F)	13/18	6/6
Age (years)	47±5	62±5
Disease duration (years)	4.5±1.1	2.0±1.0
ESR mm/h	41±4	10±5 §
CRP mg/ml	22.6±4.45	4.5±1.7 §
SF nuclear cells	18974±3909	381±72 §
SF LD activity (μkat/l)	6.76±0.86	2.68±0.30 §
SF proteins (g/l)	41.5±1.62	34.8±192 §

§ = RA vs. OS $p < 0.05$,
ESR – erytrocyte sedimentation rate, CRP – C-reactive protein, SF – synovial fluid, LD lactate dehydrogenase.

Blood samples for hormone assays were taken after an overnight fast. Plasma was separated and kept frozen until the levels of hormones were determined. Synovial fluids from RA and OS patients were collected during therapeutic arthrocentesis of a knee joint. After centrifugation of exudates, supernatants were kept frozen until hormone analysis was performed. Concentrations of the following steroid hormones were determined in plasma and in exudates supernatant: cortisol (CORT), aldosterone (ALD), testosterone (TE), 17-β estradiol (ES), dehydroepiandrosterone (DHEA), progesterone (PRG). The concentrations of the hormones were measured using commercially produced radioimmunoassay kits (Immunotech, Marseille, France).

Results for each variable were tested for normality distribution using Kolmogorov-Smirnov method. Results not normally distributed were logarithmically transformed for statistical analysis. Wilcoxon rank sum test or t-test was used to determine whether two exper-

Table 2
Levels of 17-beta estradiol (ES, pg/ ml), testosterone (TE, ng/ml), dehydroepiandrosterone (DHEA, ng/ml), and progesterone (PRG, nmol/l) in plasma and synovial fluid of RA and OS patients (mean ± SE)

	Plasma				Synovial fluid			
	RA		OS		RA		OS	
	F	M	F	M	F	M	F	M
ES	52±9	54±5	30±5 §	33±14	46±9	42±4	17±2§	15±4§
TE	0.11±0.04	2.26±0.31	0.08±0.01	1.61±0.41	0.15±0.03	1.62±0.22	0.03±0.01	1.15±0.15
DHEA	1.15±0.30	1.25±0.36	0.68±0.19	0.53±0.06§	0.83±0.18	1.95±0.60	0.45±0.06	0.44±0.15§
PRG	0.22±0.07	0.32±0.06	0.25±0.10	0.30±0.14	0.85±0.34	0.51±0.19	0.34±0.24	0.20±0.07

§=RA vs. OS $p<0.05$.

imental values (in RA and OS groups) were significantly different. Simple linear regression analysis was performed to correlate hormone levels within plasma and synovial fluid or pleural exudates. General linear model (univariate) with age adjustment was also used for comparison of values in RA and OS using SPSS 11.0 software (SPSS Inc. USA).

Results

As expected, marked RA vs. OS differences were found in sex steroids. Plasma and synovial fluid estradiol levels were higher ($p<0.05$) in RA females as compared to OS females. Plasma estradiol tended to be higher and synovial fluid estradiol was significantly higher ($p<0.05$) in RA males as compared to OS males. Dehydroepiandrosterone levels in plasma as well as in synovial fluid were higher ($p<0.05$) in RA males compared to OS males (Table 2). No significant differences in progesterone plasma levels were noted. Comparison of progesterone levels in synovial fluid of RA and OS patients showed a moderate increase of progesterone levels in RA patients, when total numbers of both gender were compared (RA 0.75±0.23, OS 0.27±0.11 ng/ml, $p<0.05$). However, at the distribution of progesterone levels according to gender (Table 2) the elevation was not significant due to relatively high individual variations of progesterone values in synovial fluid. Concentration of cortisol and aldosterone tended to be higher in plasma and were significantly ($p<0.05$) higher in synovial fluid in RA patients compared to OS group (Table 3). The levels of gonadal steroids in synovial fluids showed a negative correlation with age, therefore the levels in RA and OS patients were adjusted to age. After adjustment for age, no significant RA vs. OS difference was found in ES, TE, DHEA, PRG, CORT or ALD in plasma. There was no significant difference in age-adjusted concentrations of ES, TE, DHEA, PRG and CORT in synovial fluid. Age-adjusted concentration of aldosterone tended to be higher ($p=0.058$) in synovial fluid of RA patients as compared to that of OS patients.

Table 3
Levels of cortisol (CORT) and aldosterone (ALD) in plasma and in synovial fluid of knee joints of RA and OS patients (means ± SE)

	Plasma		Synovial fluid	
	RA	OS	RA	OS
CORT (nmol/l)	163±27	195±53	157±15	113±13 §
ALD (pmol/l)	204±49	124±17	214±26	133±18 §#

§ = RA vs. OS $p<0,05$; # age-adjusted RA vs. OS difference $p=0.058$.

The synovial fluid: plasma ratios of all steroids measured in our study were comparable in RA and in OS patients. Significant positive correlations between the concentrations of estradiol, testosterone, dehydroepiandrosterone, progesterone and aldosterone in plasma and in synovial fluid were found in both groups of patients (Fig 1 and Fig 2).

The ES/TE ratio in synovial fluid was significantly higher (0.077±0.020) in RA males compared to OS males (0.017±0.008, $p<0.05$), but not in females. The ES/CORT ratio in synovial fluid was also elevated in male RA patients as compared to OS males (0.425±0.103 in RA males and 0.177±0.031 in OS males). ES/DHEA ratios were higher in total number of RA patients as compared to total patients in OS group (0.250±0.120 in RA and 0.044±0.010 in OS).

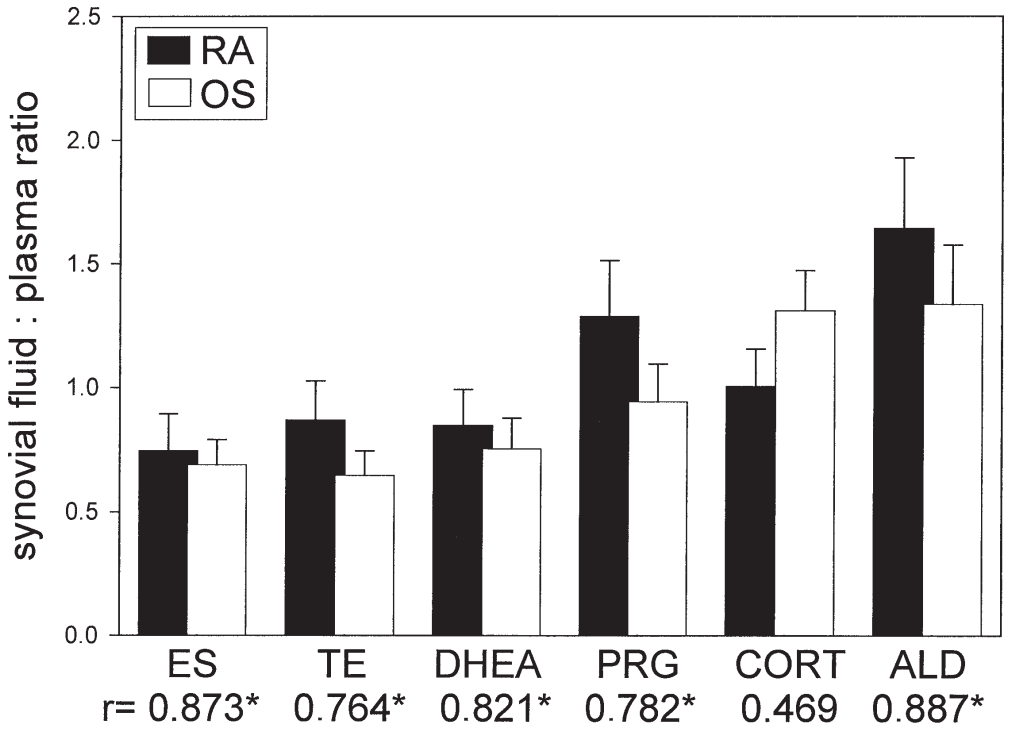

Fig.1. The synovial fluid : plasma ratio of 17-beta-estradiol (ES), testosterone (TE), dehydroepiandrosterone (DHEA), progesterone (PRG), cortisol (CORT) and aldosterone (ALD) in RA (black bars) and OS patients (white bars). Data are means ± SE, r = correlation coefficients of hormones in plasma vs. synovial fluid in all subjects. * significant ($p < 0.05$) correlation.

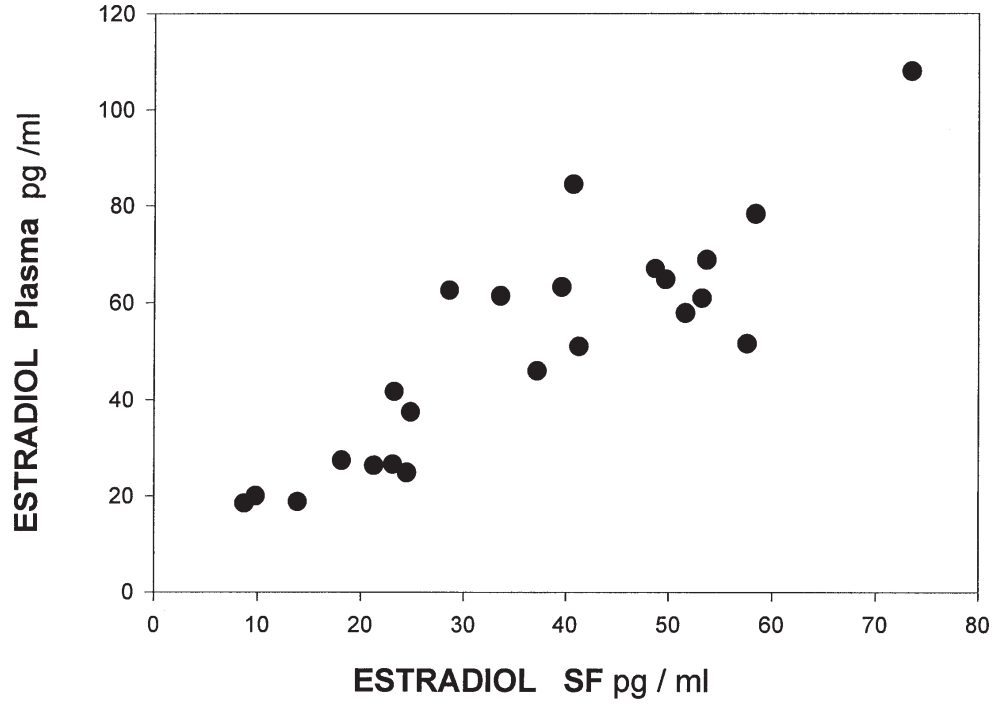

Fig.2. Correlation of the levels of 17-beta-estradiol in plasma and in synovial fluid. Correlation coefficient r = 0.873, < 0.05

Discussion

An analysis of steroid hormones in synovial fluid of affected joints is of key importance towards a clarification of RA pathogenesis and for understanding of antirheumatic therapy resistance development (Cutolo et al. 1996; Chrousos 2001; Cutolo et al. 2002). In the present study, we assessed concentrations of steroids in synovial fluid of joints in relation to their plasma levels in patients with RA and OS. We found highly significant positive correlations between steroid levels in plasma and in synovial fluid, except of cortisol, in both groups of patients. Our results also indicate that a steroid content in synovial fluid is proportional to that in plasma, regardless of ongoing inflammatory disease i.e. RA, or low inflammatory i.e. OS. This may suggest that the inflammatory process in RA patients does not considerably affect steroid levels in synovial fluid.

Besides the actual levels of pro- or anti-inflammatory acting hormones, the estrogens-to-androgens ratio is weighty for their immunomodulatory actions (Cutolo et al. 2004). In the present study we found elevated ES/TE and ES/CORT ratios in patients with RA as compared to OS patients. These results suggest the relative prevalence of pro inflammatory steroid hormones in exudates of RA patients.

On the other hand, marked differences in several steroids were found in RA compared to the OS group. Since levels of androgens and estrogens show important decline with age, differences in age-stratification in RA and OS groups must be taken into account. Lack of significant differences between RA and OS groups after adjustment to age suggests the observed changes in plasma and synovial fluid are very likely due to different ages of patients in the RA group as compared to the OS group.

In our study, higher estradiol concentrations were found in the plasma and synovial fluid of RA patients compared to OS patients, and these results are in agreement with previous findings (Cutolo and Wilder 2000). Elevated estradiol levels and lower testosterone and dehydroepiandrosterone concentrations in plasma and synovial fluid were reported in RA patients (Cutolo et al. 1992; Masi 1995). On the other hand, no significant difference in testosterone and higher plasma dehydroepiandrosterone were noted in our male RA patients when compared to the OS group. Low serum dehydroepiandrosterone sulphate were frequently reported in premenopausal females with RA by several research groups (Khalkhali-Ellis et al. 1998; Dessein et al. 2001; Cutolo et al. 1999; Masi et al. 1995) as well as by our group (Imrich et al. in press). In contrast, no changes of plasma dehydroepiandrosterone levels were observed in patients with juvenile rheumatoid arthritis (Khalkhali-Ellis et al. 1998), and Straub et al. (2002) even found a relative increase of serum dehydroepiandrosterone levels in patients with RA as compared to patients with reactive arthritis or to healthy subjects.

Lower levels of adrenal and gonadal androgens were described in plasma and synovial fluids when RA patients were compared to healthy subjects (Cutolo et al. 1992; Cutolo et al. 2004). Our results of higher dehydroepiandrosterone in synovial fluid of males are not in line with previous observations (Cutolo et al. 2003). In our study, however, the non-inflammatory exudates from patients with OS were used for comparison to the RA group.

The presence of hypothalamic-pituitary-adrenal axis dysfunction has been suggested in the subset of patients during the preclinical phase (Masi 2000). No changes in cortisol plasma levels with elevated ACTH concentrations indicating defective adrenal gland function in patients with RA were suggested (Gudbjorson et al. 1996). The presence of relative adrenal insufficiency in relation to ongoing inflammation in RA was mainly related to altered adrenal responsiveness to ACTH stimulation (Cutolo et al. 1999; Gudbjorson et al. 1996) or to adrenal androgen deficiency (Masi et al. 1999). In our observation, no significant changes in plasma cortisol were found. Tendency towards higher cortisol levels was noted in the synovial fluid of patients with RA, but the ratio of estrogen to cortisol concentrations showed the prevalence of proinflammatory estrogens in RA patients.

For the first time, we demonstrated a significant elevation of aldosterone levels in the synovial fluids of patients with RA compared to OS patients. There are only few observations of the effects of aldosterone on the processes of inflammation. It was demonstrated that aldosterone stimulates inflammatory cell infiltration in the kidneys and in perivascular spaces of coronary arteries. Spironolactone (aldosterone receptor blocker) attenuates this pro-inflammatory action of aldosterone (Sun et al. 2002; Gerling et al. 2003). Spironoloactone at in vivo attainable doses suppressed production of several pro inflammatory cytokines in human leucocytes (Bendtzen et al. 2003) and showed positive therapeutic effects in patients with chronic

arthritis. An increase of plasma aldosterone levels was observed in patients with RA (Ornat 1991), and reduction of this elevation of plasma aldosterone in patients with RA was noted after the treatment with nonsteroidal anti-inflammatory drugs (Altman et al. 1992). The demonstration of increased levels of aldosterone in the plasma and synovial fluid of RA patients in our study is in agreement with the pro-inflammatory action of aldosterone, and it suggests a possible role of local aldosterone in the inflammation of synovial tissue deserving further investigation.

In conclusion, we found that levels of steroids in synovial fluid are proportional to their plasma levels without respect to inflammatory process in the joint. On the other hand the ES/TE and ES/CORT ratio was higher in RA compared to OS patients, suggesting relative excess of proinflammatory estrogens in synovial fluid. For the first time we report here higher aldosterone levels in RA patients as compared to patients with OS.

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