Hormone concentrations in synovial fluid of patients with rheumatoid arthritis

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Abstract

Objective

Alterations in local concentrations of hormones, affecting directly synovial cells, could be involved in the modulation of the rheumatic inflammatory processes. The aim of present study was to investigate the levels of selected hormones (steroids, peptide and thyroid hormones) in synovial fluid of knee joint of patients with rheumatoid arthritis (RA) and control individuals with non-rheumatic exudate (with osteoarthrosis, OA).

Methods

Thirty-eight patients, 22 female and 16 males, with rheumatoid arthritis (RA) and 12 subjects with osteoarthrosis (OA, control group, 6 females and 6 males) participated in the study. Concentrations of cortisol (CS), 17-β–estradiol (ES), dehydroepiandrosterone (DHEA), progesterone (PRG), aldosterone ALD), prolactin (PRL), insulin (INS), and C-peptide were determined by radioimmunoassay in synovial fluid. Insulin binding to isolated cell membrane of cells from synovial sediment was estimated by using radioiodine labeled insulin. In a group of patients (10 with RA and 4 with OS), the levels of free threeiodothyronine (FT3), TSH and growth hormone (GH) were also determined in synovial fluid.

Results

Increased levels of ES in synovial fluid of RA patients were observed, and higher differences were noted in men. TE concentrations were moderately elevated in synovial fluid of RA patients, however the ratio of ES/TE was significantly higher in male RA compared to OA patients. Higher levels of PRG, ALD and growth hormone were noted in synovial fluid of RA patients. Besides the steroid hormones the presence of insulin and C-peptide was noted in synovial fluid and the correlation between the levels of these two peptides was highly significant.
The concentrations of INS and C-peptide in synovial fluid of patients from RA and OA group were not significantly different, however, highly significant increase of insulin binding to isolated membrane of synovial cells was found. Concentrations of cortisol, dehydroepiandosterone, prolactin, TSH and FT3 in synovial fluid were not significantly different in RA and OA groups.

Conclusions

Besides the steroids also insulin, c-peptide, GH and FT3 were found in synovial fluid. The elevated ALD and GH levels in synovial fluid of RA patients and the presence of INS in synovial fluid with increase of INS binding to plasma membranes of cells from synovial fluid of RA patients suggest that besides the gonadal steroids also these hormones may affect the local inflammatory processes.

Key words

Rheumatoid arthritis, arthrosis, synovial fluid, hormone content.

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Introduction

Rheumatoid arthritis (RA) represents a multifactorial autoimmune rheumatic disease that may originate from the patient's excessive immune and inflammatory response possibly to pathogenic antigen, which is handled without complication in normal individuals (1). Dysfunction of endocrine system is very likely one of the important risk factors involved in the pathogenesis of RA. It was repeatedly demonstrated that steroids (glucocorticoids, estrogens and androgens) and neurohormones are able to modulate tissue specific immune responses (2-4).

Apart from the central neuroendocrine regulatory mechanisms of immune responses, alterations in local concentrations of hormones affecting directly synovial cells, mononuclear cells and lymphocytes could be involved in the modulation of the immune response of tissues and rheumatic inflammatory process (3,5, 6). Besides the gonadal steroids, affecting local RA symptoms by changes in cytokine production, also other hormones (e.g. prolactin, growth hormone, aldosterone, insulin) may play a role in the pathogenic mechanisms underlying RA (7-9). Therefore the studies of the concentrations of hormones in synovial fluid with effects on immune and connective tissues are of key significance with respect to clarification of RA pathogenesis (10). The aim of present study was to investigate the levels of selected hormones (steroids, peptide and thyroid hormones) in synovial fluid of knee joint of patients with RA and control individuals with non-rheumatic exudate (with osteoarthrosis, OA).

Patients and methods

Thirty eight inpatients with rheumatoid arthritis (RA group, 22 females – mean age 47.8 ± 4.6 and 16 males – mean age 52.4 ± 4.9, duration of RA 4.5 ± 1.0 years) admitted to the Research Institute for Rheumatic Diseases (Piestany, Slovakia) and 12 patients with osteoarthrosis (OA group, 6 females – age 61.8 ± 3.6 and 6 males – age 58.4 ± 4.7 duration of disease 2.2 ± 0.5 years) as a control group, participated in the study. All subjects gave written informed consent. The Institute Ethical Committee approved the study.

All patients underwent routine laboratory testing including red blood cell sedimentation rates, acute inflammatory phase reactant (C-reactive protein), rheumatoid factor (as determined by the latex fixation and hemaglutination tests). Moreover, clinical assessment was performed (duration and clinical activity of disease, X-ray stage, previous therapy). The patients were on therapy with non-steroid antirheumatics at the time of investigation, which were withdrawn 3 days before investigations. The part of patients was treated with steroids (18 persons) and the last application of intraarticular glucocorticoids was 7 to 15 months before investigation. Patients on hormonal therapy (estrogens or androgens) and with diabetes mellitus were excluded from the study.

Synovial fluids from RA and OA patients were taken by arthrocentesis of knee joints. The exudates were centrifuged, and differential cell counts were determined in the sediment. Supernatants were kept frozen until hormone analysis. The inflammatory and control exudates were classified according to Rovensky and Lukac (11).

Concentrations of the following hormones were determined in clear supernatant of synovial fluids: cortisol, (CS), prolactin (PRL), aldosterone (ALD), testosterone (TE), 17-β-estradiol (ES), dehydroepiandrosterone (DHEA), progesterone (PRG), insulin (INS), and C-peptide. In addition to these hormones the levels of free threeiodothyronine (FT3), thyrotropin (TSH) and growth hormone (GH) were also determined in samples synovial fluids of 10 RAand 4 OApatients. The concentrations of hormones were measured using radioimmunoassay kits (Immunotech, Marseille, France). The binding of insulin to plasma membrane of cells from synovial fluid was determined according to Macho *et al*. (12).

The results are reported as means ± SEM. Results for each variable were tested for normality distribution using Kolmogorov-Smirnov method. Results not normally distributed were logarithmically transformed for statistical an-

alysis. Wilcoxon rank sum test was used to determine whether two experimental values (in RA and OA groups) were significantly different. Simple linear regression analysis was performed to correlate hormone levels with age or with cell number in synovial fluid. General linear model (univariate) with age adjustment was used for comparison of values in RA and OA groups using SPSS 11.0 software.

Fig. 1. Levels of 17-β-estradiol in synovial fluid of patients with rheumatoid arthritis (RA) and osteoarthrosis (OA).
F: females; M: males; means ± SE. *RA:OA $p < 0.05$; **RA: OA$p < 0.01$.

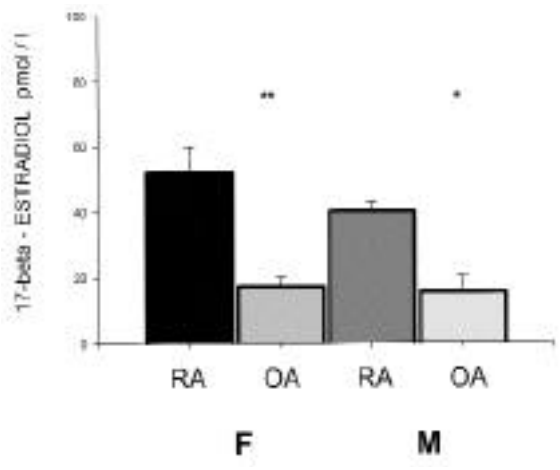
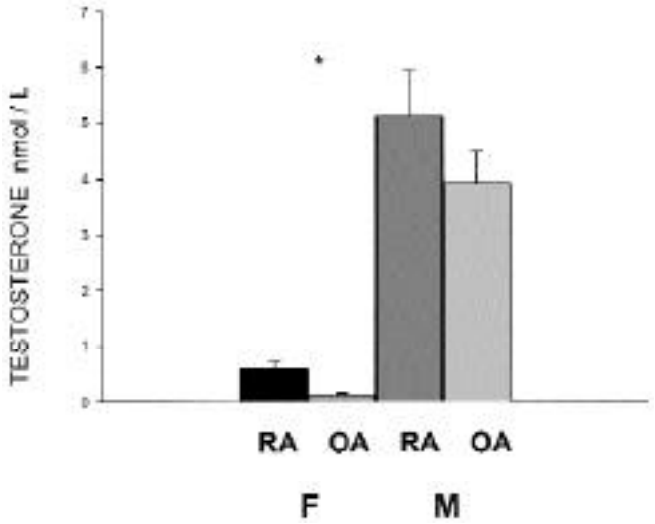


Fig. 2. Testosterone concentrations in synovial fluid of patients with rheumatoid arthritis (RA) and osteoarthrosis (OA).
F: females, M: males, means ± SE, * RA:OA $p < 0.05$.



Results

The comparison of the levels of 17-β-estradiol in synovial fluids of patients with RA to OA showed that the levels of estradiol were increased in RA patients (Fig. 1). The analysis of the correlation of synovial estradiol levels to cell number in synovial fluid (which indicates a degree of inflammatory processes) demonstrated a highly positive correlation ($p < 0.021$). Testosterone concentrations were moderately higher in inflammatory synovial fluids from RA patients as compared to values in OAcontrols (Fig. 2). The ratio of 17-β-estradiol/testosterone concentrations in synovial fluid from knee joints of male RA patients was significantly higher (0.077 ± 0.020) compared to males in OAgroup (0.017 ± 0.08, $p < 0.05$), however; significant differences were not observed in females. Higher concentrations of progesterone were found in synovial fluid of RA patients (Table I) and no differences were noted in estrogen to progesterone ratio in RAand OA patients. The aldosterone concentrations showed higher values in synovial fluids from RA patients (Table I) and positive correlation of aldosterone content and cell number in synovial fluid was noted in RApatients, ($p < 0.05$). A significant increase of growth hormone concentrations was found in synovial fluids from RApatients (Table II). Levels of cortisol, dehydroepiandrosterone, prolactin, TSH and FT3 in synovial fluids were not significantly different in RA and OA groups. However, the estrogen to cortisol ratio was elevated in RApatients compare to OA group (RA 0.425 ± 0.103, OA 0.177 ± 0.080 in men).

The insulin levels in synovial fluid were not significantly different in RA patients as compare to OA, however, insulin binding to isolated membrane of cells from sediment of knee exudates was significantly higher in RA patients when compared to control group (Fig. 3). The concentrations of C-peptide detected in synovial fluids were comparable in RAand OAgroups (Table I). Highly positive correlation ($p < 0.001$) between levels of insulin and C-peptide in synovial fluid was noted.

Discussion

Investigation of local concentration of hormones in inflammation affected joints of RA patients is of key importance for the clarification of RA pathogenesis as well as for the understanding of the mechanisms involved in the development of resistance to antirheumatic therapy (5,6). Besides the actual levels of pro- or anti-inflammatory acting hormones the ratio of the concentrations of estrogen to androgen is, however; important for their modulatory action on the activity of immune system (3). In agreement with previous findings (2, 3, 6) we have observed elevated levels of 17-β estradiol in synovial fluid of knee joint of patients with RA as compared the exudate from patients with osteoarthrosis. In spite of fact that we did not find a decrease of testosterone levels estrogen/androgen ratio was elevated. Also the estrogen/cortisol ratio was increased in RA patients. The estradiol levels correlated with cell number in synovial fluid. These results suggest the prevalence of pro inflammatory steroid hormones in exudates at RA.

The elevation of progesterone levels in synovial fluids of RA patients, observed in our study, did not corresponded with lower plasma levels of progesterone observed in patients with rheu-

Table I. Levels of hormones in synovial fluids of knee joints in patients with rheumatoid arthritis (RA) and in a control group with osteoarthrosis (OA).

	Number	RAsynovial fluid	Number	OAsynovial fluid	
C-peptide	38	1074 ± 292	12	1024 ± 290	
DHEA	38	5.10 ± 1.20	12	3.62 ± 1.28	
PRG	38	2.27 ± 0.73	12	0.85 ± 0.39	§
CORT	38	154 ± 13	12	125 ± 12	
ALD	38	214 ± 26	12	133 ± 18	§
PRL	38	209 ± 22	12	224 ± 36	

C-peptide: in pmol/l; DHEA: dehydroepiandrosterone in nmol/l; PRG: progesterone in nmol/l; CORT: cortisol in nmol/l; ALD: aldosterone in pmol/l; PRL: prolactine in mlU/l. Means ± SE, §RA: OAp < 0.05.

Table II. Hormone levels in synovial fluid from patients with rheumatoid arthritis (RA) and in a control group with osteoarthrosis (OA). GH: growth hormone in milliunits/l; FT3: free threeiodothyronine in pmol/l; TSH: thyreotropin in milliunits/l.

	Number	RAsynovial fluid	Number	OAsynovial fluid	
GH	10	0.21 ± 0.04	4	0.12 ± 0.01	§
FT3	9	3.43 ± 0.15	4	3.71 ± 0.32	
TSH	9	0.39 ± 0.06	4	0.35 ± 0.01	

Means ± SE, §RA:OA p < 0.05.

matoid arthritis (13) and also with anti-inflammatory effect of intraarticulary applied progesterone (14). No correlation of synovial progesterone levels with cell number in synovial fluid was noted and the role of local progesterone in inflammatory process needs further investigation.

The present study demonstrated a significant elevation of aldosterone levels in synovial fluids of patients with RA, and a positive correlation of aldosterone concentrations and cell numbers in knee exudate of RApatients was noted. There are only few observations on the effects of aldosterone on the processes of inflammation. It was demonstrated that aldosterone stimulates inflammatory cell infiltration in kidney (15) and in perivascular spaces of coronary arteries, and spironolactone (an aldosterone receptor blocker) attenuates this pro inflammatory action of aldosterone (16). Spironolactone, at attainable *in vivo* doses, suppressed production of several pro inflammatory cytokines in human leucocytes and showed positive therapeutic effects in patients with chronic arthritis (17). An increase of plasma aldosterone levels was observed in patients with RA(18), and reduction of this elevation of plasma aldosterone in patients with RA was noted after the treatment with nonsteroidal anti-inflammatory drugs (19). The demonstration of increased levels of aldosterone in synovial fluids of RApatients is in agreement with the pro inflammatory action of aldosterone and it suggests a possible role of local aldosterone in inflammation of synovial tissue.

The determination of growth hormone in our study showed higher levels in synovial fluids of RApatients when compared to OA. Elevated growth hormone levels in synovial fluids were found in patients with other forms of inflammatory arthritis suggesting that this hormone may play a role in the pathophysiology of arthritic disorders by stimulatory effect of growth hormone on cytokine release (20, 21).

The role of insulin in inflammatory process is still not clarified. An enhanced susceptibility to infection is well known to occur in poorly controlled diabetes (22). It was demonstrated that metabolism of glucose and glutamine, which are essential for lymphocyte function, was decreased in alloxan-induced diabetes of rats (23). The presence of immunoreactive insulin in synovial fluid was described for the first time in our study. The concentrations of insulin in exudates were even higher as those usually observed in plasma (e.g. OAplasma 0.070 ± 0.010 and synovial fluid 0.202 ± 0.066 nmol/l) and were correlated to c-peptide levels. No significant differences of insulin levels in synovial fluids were observed in RA and OA patients, however, the great differences were noted in insulin bind-

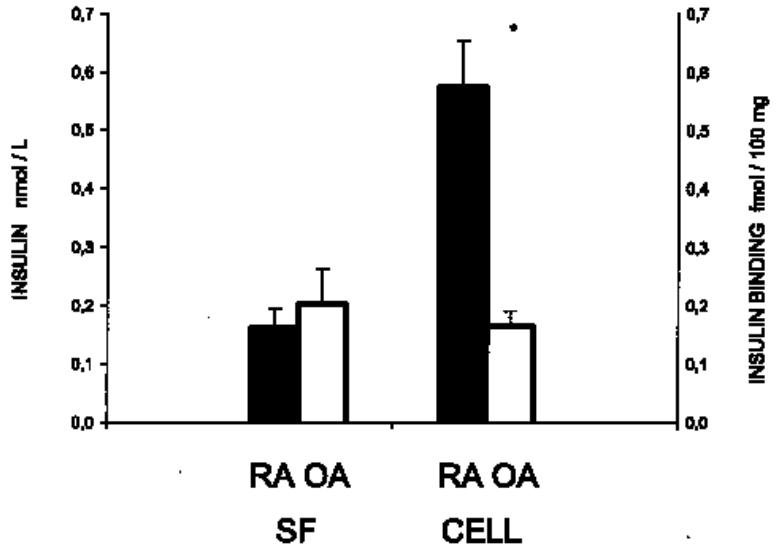


Fig. 3. Insulin levels in synovial fluid (SF) and insulin binding to membrane receptors of cells (CELL) from synovial fluid of patients with rheumatoid arthritis (RA) and osteoarthrosis (OA). Means ± SE, *RA: OAp < 0.05.

ing to membrane receptors of cell from knee exudate of RA and OA patients. Monocytes and macrophages cells, involved in inflammatory reactions, bind and internalize insulin (24). The administration of insulin increased number of GLUT1 and GLUT3 transporters in human circulating mononuclear cells (25), suggesting an influence of insulin on metabolism in white blood cells. It was observed that insulin did not stimulated 2-deoxyglucose uptakes in rheumatoid synovial cells, but the non-rheumatoid synovial cells were highly sensitive to insulin. The lower response of rheumatoid synovial cells to insulin (26) was not probably due to decreased insulin binding, because elevated values of insulin binding to plasma membranes of cells in exudates from RApatients were observed in our study. It was suggested that insulin resistance during inflammatory processes involves a post receptor step at the cellular levels (26). Therefore further studies, including post receptor processes, are necessary to explain the changes in insulin action in rheumatoid synovial cells.

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