PEPTIDE HORMONES AND HISTAMINE IN PLASMA AND SYNOVIAL FLUID OF PATIENTS WITH RHEUMATOID ARTHRITIS AND OSTEOARTHROSIS

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Objectives. Hormones other than adrenal and gonadal steroids may play also a significant role in the pathogenesis of rheumatoid arthritis. The aim of this study was to investigate the levels of selected peptide hormones and histamine in synovial fluid of knee joints and in plasma of patients with rheumatoid arthritis and with osteoarthrosis.

Methods. The concentrations of insulin, C-peptide, prolactin, growth hormone, free triiodothyronine (FT3), thyrotropin (TSH), and histamine were determined in synovial fluid and plasma of 27 patients with rheumatoid arthritis (RA) and in 12 patients with osteoarthrosis (OA).

Results. The presence of peptide hormones in synovial fluid was demonstrated. The levels of TSH and growth hormone were lower in synovial fluid than in plasma in both groups, while those of prolactin were comparable in synovial fluid and in plasma. The levels of C-peptide ($p<0.05$), insulin and FT3 were higher in synovial fluid than in plasma of OA patients, but lower in synovial fluid of RA patients as compared to their levels in plasma. Significant positive correlations between the levels in plasma and synovial fluid were observed in prolactin ($p<0.001$, $r=0.741$) and TSH ($p<0.05$, $r=0.88$) only. After age adjustment, no significant differences in synovial fluid and in plasma levels of all hormones were found between OA and RA patients. The levels of histamine in plasma were similar in RA and OA patients, in synovial fluid of both groups histamine was found in almost undetectable amounts.

Conclusions. The selected peptide hormones, e.g. insulin, C-peptide, prolactin, growth hormone, FT3 and TSH, are present in synovial fluid of RA and OA patients, some of them in the concentrations comparable to these in plasma. The role of the locally present hormones in pathogenesis of RA has to be investigated in further studies and analyses.

Key words: Peptide hormones – Histamine – Synovial fluid – Rheumatoid arthritis – Osteoarthrosis

The role of endocrine system in the onset and development of multifactorial autoimmune diseases, such as rheumatoid arthritis (RA), has been documented by clinical experience in patients with changes in plasma hormone levels (i.e. higher prevalence of RA in women /3:1/, increased incidence of RA in males after 45 years of age, improvement of disease during pregnancy, onset or worsening after the puerperium, influence on inflammatory activity in RA by glucocorticoids). However. hormones other than adrenal and gonadal steroids may play a significant role in the pathogenesis of RA (TORPY and CHROUSOS 1996; CUTULO and WILDER 2000). Further investigation of the participation of the endocrine system in this process may open up new therapeutic approaches for RA.

Several hormones (gonadal and adrenal steroids, prolactin, catecholamines, corticotrophin releasing hormone) affect the immune system centrally or through an influence on local productions of cytokines, on microcirculation and on the functions of immune cells on site of inflammatory processes (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). The strongest attention in development of RA has been paid to the role hypothalamic-pituitary-adrenocorticotropic axis (HPA). In pharmacological doses, glucocorticoids exhibit their immuno-suppressive effect by inhibiting the production of pro-inflammatory cytokines and by increasing the production of anti-inflammatory cytokines (BARNES 1998). On the other side, prolactin (PRL) and growth hormone (GH) show a stimulatory effect on the immune system by antagonizing the action of glucocorticoids in the body (WALKER and JACOBSON 2000). It has been observed that patients with hyperprolactinemia have reduced response to glucocorticoid therapy (ROVENSKY et al. 2001). The role of PRL in the pathogenesis of RA, however, remains still unclear.

Apart from the central neuroendocrine regulatory mechanisms that influence the immune system function, direct action of hormones on mononuclear cells, lymphocytes, synovial cells, etc. could be involved in the onset and development of rheumatoid arthritis and in the modulation of the immune response of tissues to selected antigen stimuli (CUTULO et al. 1992, 2002). Investigations of local effects of hormones on immune and connective tissues are therefore of main importance for the clarification of RA pathogenesis and of the mechanisms of resistance in RA patients to antirheumatic therapy (CUTULO et al. 2003). The knowledge of the local hormonal effects on immune and connective tissues seems to be of key significance with respect to pathogenesis of local inflammatory processes.

The aim of our study was therefore to compare the levels of selected peptide hormones assessed in synovial fluid to those observed in plasma and to investigate the differences of the hormone levels in inflammatory and non-inflammatory exudate of knee joints.

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 their written informed consent. The Ethical Committee of the Institute approved the study design. Twenty-seven patients with rheumatoid arthritis (RA group, 17 females and 10 males, mean age 52.5 ± 2.5 years, duration of disease 4.5 ± 1.1 years) and 12 patients with osteoarthrosis (OA group, 6 females and 6 males, mean age 60.9 ± 3.0, duration of disease 2.0 ± 1.0 years) participated in the study. All patients underwent routine clinical and laboratory investigations, laboratory data including erythrocyte sedimentation rate, C-reactive protein, 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 therapy were recorded. The patients with RA were on therapy with nonsteroid antirheumatics, which were withdrawn minimum 3 days before blood and synovial fluid collection. Patients on hormonal therapy (glucocorticoids, androgens or estrogens), with diabetes mellitus or endocrine disorders were excluded from the study.

Blood for hormone assays was sampled after an overnight fast. Plasma was separated and frozen until the levels of hormones were determined. Synovial fluids from RA and OA patients were taken during therapeutic arthrocentesis of knee joint. After centrifugation of exudates supernatants were kept frozen until hormone analyses were performed. Concentrations of prolactin (PRL), insulin, and C-peptide were determined in plasma and in clear supernatant of exudates. Besides these hormones also the levels of free triiodothyronine (FT3), thyrotropin (TSH), growth hormone (GH) and histamine were determined. The concentrations of 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 experimental values (in RA and OA groups) were significantly different. Simple linear regression analysis was performed to correlate hormone levels within plasma and synovial fluid. General linear model (univariate) with age adjustment was also used for comparison of values in RA and OA groups using SPSS 11.5 software.

Results

Hormones in synovial fluid. The presence of peptide hormones and histamine was observed in synovial fluids of individuals suffering from RA as well as in OA patients (Table 1). The mean concentrations of TSH and GH in synovial fluids of knee joints of OA and RA patients were lower (32% and 55%) as compared to their content in plasma (Figure 1). The TSH levels in synovial fluid of OA patients were significantly lower ($p<0.05$) than the levels in plasma. The mean levels of

Table 1
Levels of insulin, C-peptide, PRL, GH, FT3, TSH, and histamine in plasma and synovial fluid of knee joints of RA and OA patients. Data are expressed as means ± SE.

	PLASMA		SYNOVIAL FLUID	
	RA	OA	RA	OA
Insulin (pmol/l)	123 ± 20	99 ± 38	154 ± 34	202 ± 66
C-peptide (pmol/l)	1463 ± 272	920 ± 166	737 ± 161*	1229 ± 259
PRL (ng/ml)	6.05 ± 1.17	8.49 ± 2.21	7.06 ± 0.96	7.15 ± 1.11
GH (mU/l)	0.29 ± 0.07	0.33 ± 0.14	0.18 ± 0.03	0.13 ± 0.01
FT3 (pmol/l)	5.37 ± 1.35	2.92 ± 0.13	3.51 ± 0.24	3.72 ± 0.33
TSH (mU/l)	1.12 ± 0.47	1.23 ± 0.04	0.42 ± 0.10	0.30 ± 0.06*
Histamine (nmol/l)	368 ± 83	384 ± 106	2.80 ± 1.52	1.19 ± 0.57

PRL-prolactin, GH-growth hormone, FT3-free triiodothyronine, TSH-thyreotropin.
* p<0.05 - plasma vs. synovial fluid.

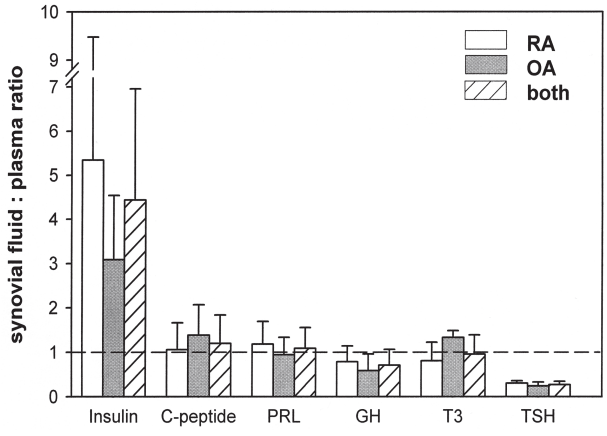

Fig 1 The synovial fluid : plasma ratio of insulin, C-peptide, PRL, GH, FT3, TSH and histamine in RA (white bars) and OA patients (grey bars) and in both patients group together (patterned bars). Data are means ± SE of ratios calculated for each subject.

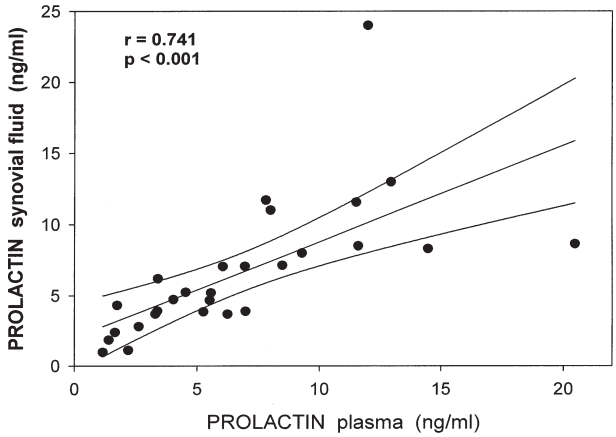

Fig. 2 Correlation of the levels of prolactin in synovial fluid and in plasma of both RA and OA patients. Correlation coefficient (r = 0.741, p<0.001).

free-triiodothyronine and C-peptide were lower in synovial fluid of RA patients (65% and 50%, respectively) as compared to their levels in plasma, but higher in synovial fluid of OA patients (127% and 134%, respectively) as compared to their levels in plasma (Table 1). The levels of PRL in synovial fluid were not significantly different from those in plasma. The mean levels of insulin were slightly higher in synovial fluid as compared to those in plasma. Significant and positive correlation between the concentrations of hormones in plasma and synovial fluids of OA and RA patients was observed in the hormones PRL (p<0.001, r=0.741, Figure 2) and TSH (p<0.05, r=0.88); however, no significant relationship was observed for insulin, C-peptide, GH, triiodothyronine and histamine. The lack of correlation could be probably related to wide variation in the values of ratios of concentrations of these hormones in plasma (PL) and synovial fluid (SF) of individual patients. The calculations of SF:PL ratio of hormone levels in each patient showed higher mean of ratio for insulin levels in SF with great variation (Figure 1). Similarly the great variations of individual values of SF:PL ratio were noted for C-peptide, free triiodothyronine and growth hormone.

Plasma and synovial fluid hormone levels in RA and OA. Slightly elevated plasma FT3 levels were noted in RA patients as compared to controls. However, the concentrations of FT3 in synovial fluids were similar in RA and OA patients (Table 1). Synovial fluid and plasma levels of GH were not significantly different in RA and OA groups. No significant differences between levels of PRL, insulin, TSH, C-peptide in

synovial fluid and in plasma were found when OA and RA patients were compared. The concentrations of C-peptide were significantly higher in plasma than in synovial fluid in RA patients (Table 1, $p<0.025$), but lower in OA patients, however this difference did not reach statistical significance.

Besides the peptide hormones also the content of histamine in plasma and synovial fluid was determined. The levels of histamine in plasma were similar in RA and OA patients. The levels of histamine in synovial fluids were very low and the assessment reached the limits of detection by radioimmunoassay method (Table 1).

Discussion

Besides the plasma, various contents of hormones were demonstrated in several body fluids like saliva, tears, and synovial fluid (Haeckel and Hanecke 1993; Lawrence 2002). These hormones are required for the proper function of the epithelial layer of organs from which they are secreted (e.g. oral mucosa, cornea, synovial cells). It was suggested that the determination of hormones, antibodies, certain drugs, which could reflect blood concentrations, could be explored in the early diagnosis of diseases, and as a means of monitoring general health (Lawrence 2002).

Local manifestations of RA are influenced by action of hormones with immunomodulatory effects. Therefore, investigations of concentrations of hormones in synovial fluid of affected joints in RA patients are of key importance for the clarification of RA pathogenesis. The hormonal levels in synovial fluid could be a way for the understanding of mechanisms involved in the development of resistance to antirheumatic therapy (Cutolo et al. 1992, 2002; Chrousos 2001).

Prolactin plays an important local immunoregulatory role in both human and animal lymphocytes. PRL modulates the expression of genes and the production of growth factors and cytokine receptors (Gutierez et al. 1994). It was suggested that PRL plays a role in the pathogenesis of RA, since plasma levels of PRL are elevated in RA patients, and T- and B-lymphocytes have high-affinity PRL receptors. We have found similar levels of PRL in plasma and synovial fluid, and no significant differences between RA and OA patients were noted. No significant difference between serum PRL values in patients with RA and normal blood donors was presented by Berczi et al. (1987), but they observed that the bioactivity of PRL was significantly decreased in RA patients, when compared to values obtained in age and sex matched controls. However, the determination of PRL levels and PRL bioactivity was not performed in synovial fluid in this study.

The role of insulin in the inflammatory process is still not clear. An enhanced susceptibility to infection is known to occur in poorly controlled diabetes (Otton et al. 2002). It was demonstrated that metabolism of glucose and glutamine, which are essential for lymphocyte function, was decreased in alloxan-induced diabetes of rats. Insulin administration in vivo or hormone added to the culture medium of freshly obtained lymphocytes from diabetic rats reversed the changes of metabolism of glucose, pyruvate, and glutamine (Otton et al. 2002), suggesting important effects of insulin on lymphocyte metabolism and functions. 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 than those observed in plasma but interestingly no correlation with C-peptide concentrations were found. Both insulin and C-peptide are secreted from cells producing insulin, and their presence in synovial fluid may suggest a possible local production. Extra pancreatic insulin-producing cells were described in several organs (liver, spleen, thymus, adipose tissue, and bone marrow) of diabetic mice (Kojima et al. 2004). The expression of the insulin-producing gene has to be examined also in synovial tissues. No significant differences were observed in insulin content in synovial fluid and in plasma between RA and OA patients. However, the increase of insulin binding to membrane receptors of cell isolated from knee exudate of RA patients was noted (Macho et al. 1999). Monocytes and macrophage cells, involved in local inflammatory reactions, bind and internalize insulin. It was observed that basal glucose uptake is increased in synovial cell culture from patients with RA in comparison to those with osteoarthritis (Estrada et al. 1994, Hernvann et al. 1991). Insulin did not stimulate uptake of 2-deoxyglucose in rheumatoid synovial cells, however, the non-rheumatoid synovial cells showed high response to insulin. The lower response of rheumatoid synovial cells to insulin (Estrada et al. 1994) was not due to decrease of insulin binding, because elevated values of insulin binding to plasma membranes of cells in exudates from RA patients were observed in our previous study (Macho et al. 1999). It was suggested that insulin resistance during inflammatory processes involves post-receptor steps at the subcellular levels (Aussel et al. 1987). Therefore, further investigations including determination of post-receptor processes are necessary to explain the changes in insulin action in rheumatoid synovial cells.

Elevation of FT3 plasma levels in RA patients, observed in our investigations, is in agreement with results of ZENOVKO et al. (1998), which describe the tendency of plasma FT3 to increase in RA patients without glucocorticoid therapy. Further, our results demonstrated that in synovial fluid there are no differences in FT3 levels in synovial fluids of RA and OA patients. Only a one third of TSH levels in plasma is transferred to synovial fluid and no differences in TSH levels in plasma or in synovial fluid were noted between RA and OA patients.

We found similar baseline levels of GH in plasma of RA and OA patients. This is in agreement with previous observation of SVENSON et al. (1987), TSATSOULIS et al. (1999), ROVENSKY et al. (2001). However, DENKO and MALEMUD (2004) found elevated GH plasma levels in patients with RA as compared to age-matched normal subject. Also DENKO et al. (1996) did not find differences in GH content in synovial fluid and in plasma of male patients with RA. Slightly lower GH levels in synovial fluid than in plasma were noted in OA patients in our observation. The comparison of GH levels in synovial fluid of RA and OA patient showed slightly higher levels of GH in RA patients as compared to OA patients, suggesting that local GH level could play a role in pathophysiology of arthritic disorders (DENKO et al. 1996). It was demonstrated in experimental animals that increase of GH could be an adaptive mechanism involved in the regulation of local inflammatory processes (BLUET-PAJOT et al. 1996).

In conclusion, our study showed that besides glucocorticoids, estrogens and androgens, also insulin, C-peptide, PRL, GH, FT3 and TSH are present in synovial fluid of RA and OA patients, some of them in to those in plasma comparable concentrations. However there were no marked differences in peptide hormone concentrations in synovial fluid between RA and OA patients, as it was observed for gonadal steroids (ROVENSKY et al. 2004). It seems possible that locally present peptide hormones play a minor role in pathogenesis of local inflammatory processes at RA in comparison to steroids. However, the differences in insulin binding on membrane of synovial cells in RA and OA patients (ROVENSKY et al. 2005) suggest, that in spite of similar levels in synovial fluid it could be change in the local effects of hormones in RA and OA patients. To confirm this speculation, further studies on direct effects of peptide hormones on synovial tissue are necessary.

Acknowledgment

This study was supported by grants VTP 21-06-01/98and by APVT-21-008602. We wish to express sincere gratitude to Dr. S. Wimmerova for help with statistical analyses and to Mrs. E. Visztova for her precise laboratory work.

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