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ORIGINAL ARTICLE

Cyclic adenosine 5′-monophosphate in synovial fluid of rheumatoid arthritis and osteoarthritis patients

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Abstract The relationship between synovial fluid (SF) cAMP level and IL-18 and PGE2 SF levels in rheumatoid arthritis (RA) and osteoarthritis (OA) patients, and between SF cAMP level and disease as well as inflammatory activity in RA were investigated in 17 RA and 19 OA patients. Erythrocyte sedimentation rate (ESR), serum (S) C-reactive protein (CRP) level and SF IL-18 level were higher in RA than in OA patients. SF PGE2 level was similar in both groups. SF cAMP level was higher in OA than in RA patients. In RA patients, SF cAMP level showed negative correlation with Disease Activity Score including a 28-joint count and S CRP, ESR and SF IL-18 level. The results suggest that cAMP promotes anti-inflammatory response in RA and OA patients, which is higher in the latter. Promotion of anti-inflammatory response by cAMP elevating agents might be useful in the treatment of RA.

Keywords Rheumatoid arthritis · Osteoarthritis · Synovial fluid · Cyclic AMP · Prostaglandin E2 · Interleukin-18

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Introduction

A complex cytokine network produced by cells from inflamed synovia is involved in the pathogenesis of rheumatoid arthritis (RA). The network is composed of positive and negative feedback pathways regulating cytokine expression and action [1, 2].

Interleukin (IL)-18 is a proinflammatory cytokine with a prominent role in the pathogenesis of RA synovitis and tissue destruction in RA [3]. A number of proinflammatory cytokines [4, 5] including IL-18 [6, 7] can be detected in higher levels in synovial fluid (SF) of RA patients as compared with osteoarthritis (OA) patients.

Prostaglandin E2 (PGE2) contributes to a number of reactions involved in RA synovitis [8]. However, it has not been extensively studied in SF of RA patients. A higher level of SF PGE2 was found in RA than in OA patients, with no correlation with the levels of various cytokines [9]. The improvement of synovitis in RA patients by various drug treatments was accompanied by a decrease in the SF level of PGE2 [10–12].

Cyclic adenosine 5′-monophosphate (cAMP) mediates a number of various anti-inflammatory pathways resulting in inhibition of tumor necrosis factor alpha (TNFα) and IL-1, and stimulation of IL-10 production [13]. In vitro and in vivo studies demonstrated an inhibitory effect of cAMP on numerous and various reactions involved in immune and inflammatory responses, suggesting a central role of cAMP in anti-inflammatory mechanisms [14].

There are only a few studies exploring cAMP in SF of RA patients. There was no correlation between SF cAMP level and disease activity [15, 16], however, the treatment increasing the SF cAMP level ameliorated the symptoms of joint inflammation [16].

Considering the complex relationship of various participants involved in the pathogenesis of RA synovitis, we studied the relationship of SF cAMP primarily with two proinflammatory mediators, IL-18 and PGE2 in SF, and with the disease and systemic inflammatory activity in RA and OA patients.

Methods

The study included 17 RA and 19 OA consecutive patients with knee joint effusion and indication for arthrocentesis. RA patients (male/female 3/14, median age 53, range 39–66) met the American College of Rheumatology 1987 classification criteria [17]. All patients were treated continuously with some of the disease modifying anti-rheumatic drugs alone or in combination with other anti-rheumatic drugs: nonsteroidal anti-inflammatory drugs (NSAIDs) ($n = 5$), cyclo-oxygenase (COX)-2 inhibitors ($n = 10$) and methylprednisolone ($n = 4$).

In OA patients (male/female 10/9, median age 54, range 21–69), knee OA was diagnosed based on radiography and clinical findings. They were treated at the time of arthrocentesis with NSAIDs ($n = 13$) or COX-2 inhibitors ($n = 4$). The written informed consent was obtained from the patients involved in the study. The study was approved by Ethical Committee of the Dubrava University Hospital, Zagreb.

Disease activity in RA patients was evaluated by Disease Activity Score including a 28-joint count and S CRP level (DAS28-CRP) [18] using DAS28-CRP Calculator [19].

In both RA and OA patients SF was obtained by a therapeutic knee arthrocentesis in volume between 30 and 80 mL, depending on the volume of knee effusion. To reduce the viscosity of SF prior to analysis, SF samples were treated with hyluronidase (20 units/mL, at 37°C for 30 min) using Hylorunidase Sigma, type 1–2, H-3506 [20].

Erythrocyte sedimentation rate (ESR) was measured by a standard technique [21] and serum (S) C-reactive protein (CRP) was measured by an immunoturbidimetric assay [22] using Olympus autoanalyzer. SF IL-18, PGE2 and cAMP levels were measured by enzyme-linked immunosorbent assay (ELISA) using IL-18 kit from R&D (Cat. No. 7620), PGE2 Correlate-EIA (Cat. No. 900–001) and cAMP Correlate-EIA (Cat. No. 900-067) from Assay Designs, respectively. The manufacturer's instructions were strictly followed [23–25] and plates were read on a Dynatech M5000 reader. Statistics was done using NCSS 2000 program attached to the book [26]. As higher values for proinflammatory variables and lower values for anti-inflammatory variables in RA then in OA group could be expected, the differences between mean in RA versus OA group were analyzed by one-tailed t test. The correlations between various parameters within groups were analyzed by Pearson correlation. P values less then 0.05 were considered significant.

Results

ESR, S CRP level and SF IL-18 level were higher in RA than in OA patients. SF PGE2 SF level was similar in both groups. SF cAMP level was higher in OA than in RA patients (Table 1).

In RA patients, SF cAMP level showed negative correlation with DAS28-CRP, ESR and SF IL-18 level (Fig. 1a–c). The correlation coefficients are shown on Table 2. There were no statistically significant correlation coefficients between SF cAMP level and S CRP level in RA patients and between SF cAMP and S CRP, ERS, SF IL-18 and SF PgE2 in OA patients.

Discussion

Differences between the RA and OA groups of patients (higher ESR, S CRP level, SF IL-18 level and lower SF cAMP level in RA than in OA patients) reflected a higher inflammatory activity in RA than in OA. These results are in concordance with the higher SF levels of various proinflammatory cytokines found in RA as compared with OA patients [3–6].

Similar SF PGE2 levels in RA and OA patients could be explained by medication with drugs lowering PGE2 synthesis, administered to patients of both groups, and by the anti-inflammatory effects of PGE2. Generally, PGE2 is considered as a proinflammatory mediator [8] but in fact, it exerts dual pro- and anti-inflammatory effects depending on target cells, receptor subtype and context of activation [27–30]. PGE2 contributes to switching on the resolution of inflam-

Table 1 Difference between rheumatoid arthritis and osteoarthritis patients

Parameter	RA, $n = 17$	OA, $n = 19$	P
S CRP (mg/L)	37.94 (33.85)	10.89 (9.72)	0.001315
ESR (mm/h)	48.1 (14.10)	29.2 (11.57)	0.000048
SF IL-18 (pg/mL)	2624.10 (1248.21)	903.28 (603.08)	0.000003
SF PGE2 (pg/mL)	2001.70 (1304.08)	1619.48 (1214.55)	0.192152
SF cAMP (pmol/mL)	0.53 (0.414)	2.65 (4.53)	0.031484

Values are mean (SD), one-tailed t test. *RA* rheumatoid arthritis, *OA* osteoarthritis, *S CRP* Serum C-reactive protein, *ESR* erythrocyte sedimentation rate, *SF* synovial fluid, *IL-18* Interleukin-18, *PGE2* prostaglandin E2, *cAMP* cyclic adenosine 5′-monophosphate

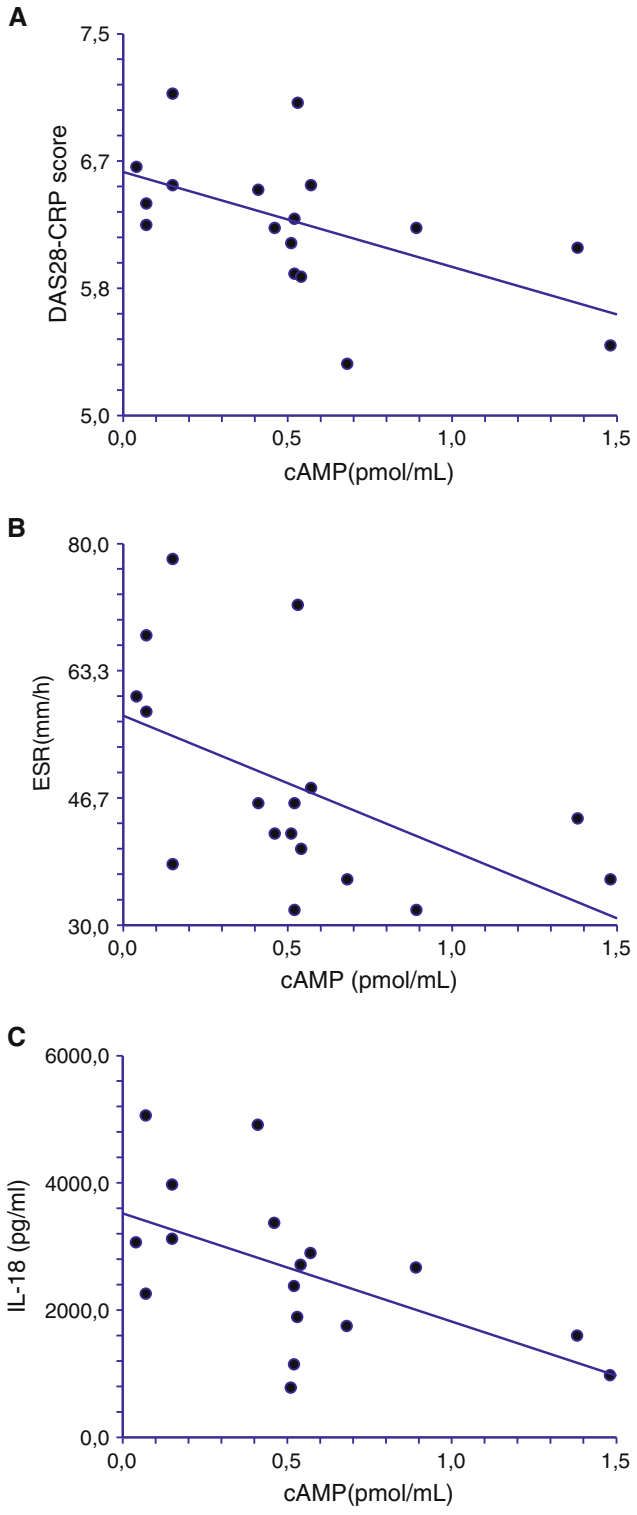

Fig. 1 Relationship between synovial fluid cyclic adenosine 5′-monophosphate (cAMP) level and (**a**) Disease Activity Score including a 28-joint count and serum C-reactive protein level (DAS28-CRP). **b** Erythrocyte sedimentation rate (ESR), and (**c**) synovial fluid interleukin-18 (IL-18) level in rheumatoid arthritis patients ($n = 17$). For correlation coefficients, see Table 2

Table 2 Correlation between SF cAMP level and various variables in rheumatoid arthritis and osteoarthritis patients

Variables	*n*	*r*	*P*
RA patients			
SF cAMP verus DAS28-CRP	17	−0.5566	0.0203
SF cAMP versus CRP	17	−0.2051	0.4210
SF cAMP versus ESR	17	−0.5214	0.0319
SF cAMP versus SF IL-18	17	−0.5657	0.0176
SF cAMP versus SF PGE2	17	0.0997	0.7238
OA patients			
SF cAMP versus CRP	19	0.34489	0.1610
SF cAMP versus ESR	19	0.31810	0.1832
SF cAMP versus SF IL-18	19	−0.2674	0.2684
SF cAMP versus SF PGE2	19	0.3508	0.1409

r Pearson correlation coefficient, *SF* synovial fluid, *cAMP* cyclic adenosine 5′-monophosphate, *RA* rheumatoid arthritis, *OA* osteoarthritis, *DAS28-CRP* Disease Activity Score including a 28-joint count and serum C-reactive protein (CRP) level, *ESR* erythrocyte sedimentation rate, *IL-18* Interleukin-18, *PGE2* prostaglandin E2

mation [31], so PGE2 need not correlate with chronic inflammatory response.

In contrast to previous studies [15, 16], we found negative correlation between SF cAMP level and disease activity as assessed by DAS28-CRP and ESR.

The lack of correlation between SF cAMP and S CRP levels could be explained by dual opposite effects of cAMP in the regulation of IL-6 expression, which is a major stimulator of CRP synthesis [32]. The expression of IL-6 is enhanced by TNFα, IL-1β and PGE2. The latter acts through cAMP signaling [33, 34]. On the other hand, an increase in cAMP suppresses the expression of TNFα [35–37] and could be assumed that the net effect of cAMP on IL-6 synthesis and consequently CRP synthesis depends on balance of these two processes.

Relationship between cAMP and PGE2 and IL-18 SF levels was not investigated. The majority of PGE2 effects are mediated by cAMP signaling [27] and positive correlation between cAMP and PGE2 may be expected. The lack of correlation between cAMP and PGE2 SF levels might be due to the PGE2 lowering medication administered to all patients, variability in PGE2 signaling depending on target cells, receptor subtype and co-stimulation [27], and effects of a number of G-protein coupled receptor ligands other than PGE2 that also act through cAMP signaling and contribute to the RA pathogenesis [38].

IL-18 is a proinflammatory cytokine with a powerful Th1 promoting activity [39]. The negative correlation between cAMP and IL-18 SDF levels might be explained by the immunomodulatory function of cAMP. Suppressing

T-cell activation, cAMP prevents inappropriate immune response and autoimmunity [40]. Elevation of cAMP differently affects cytokine production in effector T cells, suppressing it in Th1 cells and increasing it in Th2 cells [41]. cAMP elevating agents including PGE2 drive Th1/Th2 balance toward Th2 response [38, 42]. PGE2 participates in negative feedback loops regulating cytokine expression. TNFα and IL-1β increase COX-2 expression and PGE2 synthesis [8]. PGE2, in turn, down-regulates TNFα and IL-1β expression [42, 43]. In contrast to IL-1β, IL-18 does not increase COX-2 expression and PGE2 synthesis [44]. Consequently, a negative correlation between cAMP and IL-18 might be expected.

In conclusion, assuming that essential molecular mechanisms regulating immune and inflammatory responses are common to all relevant cell types, the data derived from experimental settings (cell culture, experimental animals) can be translated to clinical setting (our study). Taking together, cAMP appears as a pivot mediator of anti-inflammatory response. Elevation of SF cAMP level accompanied by lower DAS28-CRP score, lower ESR and lower SF IL-18 level in RA, and a higher SF cAMP level in OA than in RA suggest that cAMP promotes anti-inflammatory response in both RA and OA patients, which is higher in the latter. Promotion of anti-inflammatory response by agents elevating cAMP might be useful in the treatment of RA.

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