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ORIGINAL ARTICLE

Circadian pattern and the effect of standardized physical exercise on procollagen IIA N-peptide (PIIANP) in rheumatoid arthritis at different stages and in healthy individuals

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Abstract

Background: Variant collagen IIA is re-expressed in diseased cartilage. Low procollagen IIA N-peptide (PIIANP) levels in serum have recently been reported in rheumatoid arthritis (RA). We investigated circadian rhythmicity and effect of physical activity on PIIANP in early and longstanding RA and in healthy subjects.
Methods: Patients with early and longstanding RA and controls were included. Fasting and serial blood samples were collected during 24 h. PIIANP response to physical activity was studied before and serially after standardized exercise.
Results and conclusion: In RA at different stages and healthy individuals, PIIANP exhibited no circadian rhythmicity, and PIIANP in serum was not influenced by physical activity.

Keywords: *Rheumatoid arthritis; collagen; PIIANP; diurnal variation; standardized physical activity*

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory joint disease with autoimmune traits, which causes cartilage depletion and bony erosions if left untreated. To study the effect of targeted therapies, it is essential to apply markers that reflect specific aspects of joint pathology, e.g. cartilage, bone and soft tissue metabolism (Garnero et al. 2000).

Collagen II is the major structural protein in cartilage. There is evidence, that soluble fragments of collagen II released by enzymic cleavage during fibrillogenesis or degradation of mature collagen II fibres can be used to assess the rate of collagen II synthesis and breakdown in RA and osteoarthritis (OA) (Christgau et al. 2001). As a result of alternative splicing there are two forms of procollagen II (Ryan & Sandell 1990). Type IIA procollagen is synthesized primarily by immature cartilage (Lui et al. 1995, Oganesian et al. 1997, Sandell et al. 1991, Zhu et al. 2001) and as cartilage matures, procollagen IIA expression decreases in favour of procollagen IIB (Lui et al. 1995, Zhu et al. 1999). However, type IIA procollagen is re-expressed in damaged cartilage, e.g. in OA (Aigner et al. 1999). As type IIA procollagen is expressed in diseased cartilage in particular, the procollagen IIA N-peptide (PIIANP) is suggested to reflect the anabolic capacity by chondrocytes (Aigner et al. 1999). We and others have previously reported that PIIANP in serum is decreased in RA compared with healthy subjects (Christensen et al. 2009, Rousseau et al. 2004). However, when the results of molecular marker measurements are interpreted, some issues in addition to anthropometric factors and technical assay performance should be addressed, including possible circadian

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changes and the effect of physical activity. Recently, Quintana et al. reported that PIIANP increases in the morning when OA patients arise from bed (Quintana et al. 2008). Similar studies have not been carried out in other chronic joint diseases including RA. This study was undertaken in order to investigate whether PIIANP in the circulation exhibits within- and between-day changes at an early or late stage of RA compared with healthy subjects and whether PIIANP is influenced by a standardized exercise programme in healthy and RA sufferers.

Methods and materials

Patients and controls

Circadian variation

The study comprised 33 subjects including nine randomly selected, newly diagnosed and untreated RA patients (disease duration <6 months) (ERA), nine patients with longstanding disease (5–15 years) and erosions by X-ray in at least one joint/joint area (LRA) and 15 healthy control subjects (Table 1). RA was classified according to the American College of Rheumatology criteria (Arnett et al. 1988). Exclusion criteria were glucocorticoid therapy within 4 weeks before inclusion, past or present malignancy, liver and/or kidney disease, severe heart (NYHA class >II) or lung disease, hypertension (>140/90 despite antihypertensive treatment), advanced atherosclerotic disease, pregnancy and lactation, anticoagulant therapy, concomitant inflammatory rheumatic diseases, infection with parvovirus B19 or hepatitis B or C, non-compliance, arthroplasty or other major surgery within 8 weeks before inclusion. ERA patients were disease-modifying antirheumatic drugs (DMARD) and steroid naïve and only non-steroidal anti-inflammatory drugs (NSAIDs) or mild analgesics were allowed. LRA patients were on methotrexate (MTX) monotherapy (six patients), MTX in combination therapy with another DMARD (one) and sulfasalazine monotherapy (two). Glucocorticoids were not allowed in any group. Patients with LRA were also excluded if their DMARD therapy had changed within 3 months before inclusion. Fifteen healthy control subjects were recruited among hospital staff, friends and family members. No current medication beside over-the-counter drugs was allowed.

Baseline blood samples were collected at 10.00 h on day one after 1–2 h of normal activities including dressing and breakfast. Subsequently, samples were obtained every 3 h until 22.00 h. Fasting samples were drawn the following day at 07.00 h before rising from bed and subsequently at 10.00 h. Thus, seven samples were obtained per individual. During the daytime the participants were permitted to do normal activities but they did not participate in heavy physical activities such as exercises.

Physical exercise

A total of 34 individuals were included: ten DMARD- and steroid-naïve patients with disease duration <6 months (ERA), and ten patients with longstanding RA with disease duration 5–15 years and erosive disease by X-ray in at least one joint/joint area (LRA). RA was classified according to the 1987 ACR criteria (Arnett et al. 1988). Patients with LRA received MTX monotherapy (five patients), MTX combination therapy (three), ciclosporin (one) and sulfasalazine (one). NSAIDs and over-the-counter analgesics were allowed in both groups. Exclusion criteria were as described above. The healthy control group consisted of 14 separately recruited healthy subjects as detailed above.

The exercise programme was carried out between 06.30 h and 09.45 h. Using a test-bike (Monarch Viktergometer model 90814 E), the participants underwent a 5-min warm-up session, aiming to reach a submaximal level at 70–80% of their maximal pulse capacity (maximum pulse: 220 minus age). Subsequently, the submaximal level was maintained for 20 min with 4-min cycles with increasing loads of 0.5, 0.7, 0.9 and 1.1 kg, respectively.

Baseline blood samples were collected immediately before starting the warm-up session and physical activity programme, upon termination and subsequently after 1 and 3 additional hours.

Joint counts (0–40) (van Riel & Scott 2004), the Health Assessment Questionnaire (HAQ score, 0–3) (Thorsen et al. 2001) and the visual analogue scale (1–10) (VAS pain, global and doctor) were recorded in the RA subsets. Radiographs of hands, wrists and forefeet were assessed by one experienced radiologist and scored according to the Larsen method (Larsen et al. 1977).

Laboratory analyses

Blood samples were centrifuged at 2500***g*** for 10 min and stored at -80°C. C-reactive protein (CRP) (<10 mg l^{-1}) and erythrocyte sedimentation rate (ESR) were measured by standard methods. IgM-rheumatoid factor (RF) (cut-off level: 17 IU ml^{-1}) was measured by an enzyme-linked immunosorbent assay (ELISA) as reported in (Høier-Madsen et al. 1986)

PIIANP was measured by a competitive ELISA (Millipore/LINCO Research, USA) as described previously (Sharif et al. 2007). Interassay coefficients of variation were 16.3% and 6.4% for low (59–122 ng ml^{-1}) and high (424–880 ng ml^{-1}) concentration controls, respectively. Intra-assay coefficients of variation were below 5%. All analyses were done in duplicate using kits with

the same lot number. Serial samples from the same patient were analysed simultaneously.

Ethics

The trial was approved by the local ethics committee (J. no. M-2359-02) and fulfilled the Declaration of Helsinki and the International Conference on Harmonisation 1996 revised guidelines for Good Clinical Practice.

Statistics

Comparison of unpaired data was done using Fisher's exact test, the Mann–Whitney *U*-test or the Kruskal–Wallis test. To approximate normal distribution, PIIANP was logarithmically transformed before inclusion in the subsequent analyses. Linear regression was applied to analyse PIIANP during 24 h using baseline levels at 10.00 h as a reference. In addition, a time-adjusted mixed model was used to calculate within- and between-subject variations. Linear regression analyses with robust standard errors were used to analyse the course of PIIANP during physical activity. In both the diurnal variation and physical activity study, the slope of PIIANP was computed for each control and RA subject using linear regression analysis. Subsequently, the unpaired *t*-test was used to compare the mean slopes between groups. Results are presented as median (interquartile range) except for PIIANP (median (95% confidence interval (CI)); *p*-values <0.05 were considered statistically significant. All analyses were performed using STATA version 9.2 (www.stata.com).

Results

Diurnal variation

PIIANP measurements from three LRA patients and three controls at 10.00 h on day two were not available. Patients with ERA had higher swollen joint counts and VAS doctor score than LRA sufferers. Control subjects were younger than patients with LRA. In the LRA cohort, RF-positive patients had significantly lower PIIANP compared with RF-negative patients (763 ng ml^{-1}, 95% CI 342–946 vs 1192 ng ml^{-1}, 95% CI 1083–1301, p=0.04). There was no correlation between PIIANP and disease activity measures including VAS scores in either RA group.

PIIANP exhibited no diurnal variation in any of the three cohorts (p >0.05 at all time points compared with baseline). Within-subject variations were 2.43% (95% CI 2.03–3.03), 2.62% (95% CI 2.13–3.22) and 2.96% (95% CI 2.48–3.39) for ERA patients, LRA patients and controls, respectively. Between-subject variations were 3.71% (95% CI 2.20–6.23), 7.84% (95% CI 4.76–12.9) and 7.97% (95% CI 5.46–11.6) for the ERA cohort, LRA group and controls, respectively. When comparing the PIIANP slopes in each cohort, there was no difference between ERA and LRA patients (p=0.49), between controls and LRA (p=0.28), or between controls and ERA (p=0.58).

Using linear regression, no variation in PIIANP from 10.00 h day 1 to 10.00 h day 2 was found in ERA (beta coefficient -0.02 (-0.18–0.13), p=0.77), LRA (-0.11 (-0.29–0.07, p=0.22) and controls (0.09 (-0.06–0.24), p=0.25).

Table 1. Diurnal variation. Demographics, clinical and laboratory characteristics of patients with rheumatoid arthritis (RA) and controls at inclusion.

Variable	Early RA (n = 9)	Longstanding RA(n = 9)	p-Value	Controls (n = 15)
Sex M/F (% female)	2/7 (78%)	3/6 (67%)	1.00	6/10 (60%)[a]
Disease duration (months)	3 (2.5–3.5)	105 (82–118)	<0.001	0
Age (years)	63 (44–72)	62 (54–65)	0.93	48 (40.5–56)*[b]
IgM-RF positive (%)	6/9 (67%)	7/9 (78%)	1.00	–
Erosive disease (%)	1/8 (13%)	9/9 (100%)	<0.001	–
Swollen joint count (0–40)	11 (9–12)	4 (2–8)	0.01	–
Tender joint count (0–40)	14 (10–15)	10 (2–11)	0.17	–
ESR (mm h^{-1})	15 (10–30)	20 (9–25)	0.96	–
CRP (mg l^{-1})	12 (9–16)	15 (3–17)	1.00	–
VAS doctor (0–100 mm)	41 (37–56)	29 (6–33)	0.04	–
VAS global (0–100 mm)	37 (9–50)	24 (19–34)	0.89	–
VAS pain (0–100 mm)	48 (9–62)	34 (28–35)	0.63	–
HAQ score (0–3)	0.50 (0.25–1.0)	0.63 (0.38–1.13)	0.63	–
PIIANP (ng ml^{-1})	923 [701–1465]	840 [532–1076]	0.35	736 [518–1182][a]

p-Values calculated using Mann–Whitney *U*-test and Fischer's exact test. [a]Not significant compared with either RA group. *p <0.01 compared with longstanding RA patients; [b]not significant compared with patients with early RA. Data are median (interquartile range) except for PIIANP (median [95% confidence interval]).

RF, rheumatoid factor; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; VAS, visual analogue scale; HAQ, Health Assessment Questionnaire; PIIANP, N-terminal propeptide of collagen IIA.

Physical activity

One ERA patient and one LRA patient were considered outliers (PIIANP <100 ng ml^{-1} and >4000 ng ml^{-1}, respectively) and were therefore excluded. There was no difference between these patients and the rest of the cohort with respect to disease activity measures (data not shown).

Patients with ERA had higher swollen joint counts and higher VAS doctor scores compared with patients with LRA. Neither ERA nor LRA patients differed from controls with respect to age and gender. There was no difference in age and gender between RA groups.

PIIANP tended to be lower in RF-positive than in RF-negative LRA patients (776 ng ml^{-1} (494–1336) vs 1202 ng ml^{-1} (1177–1575), p=0.07). PIIANP was not associated with disease activity measures (data not shown).

Using linear regression analysis, PIIANP did not differ from pre-exercise level during or after exercise (data not shown) and when comparing the slopes of PIIANP between groups no difference was observed (between RA groups (p=0.99), between LRA patients and controls

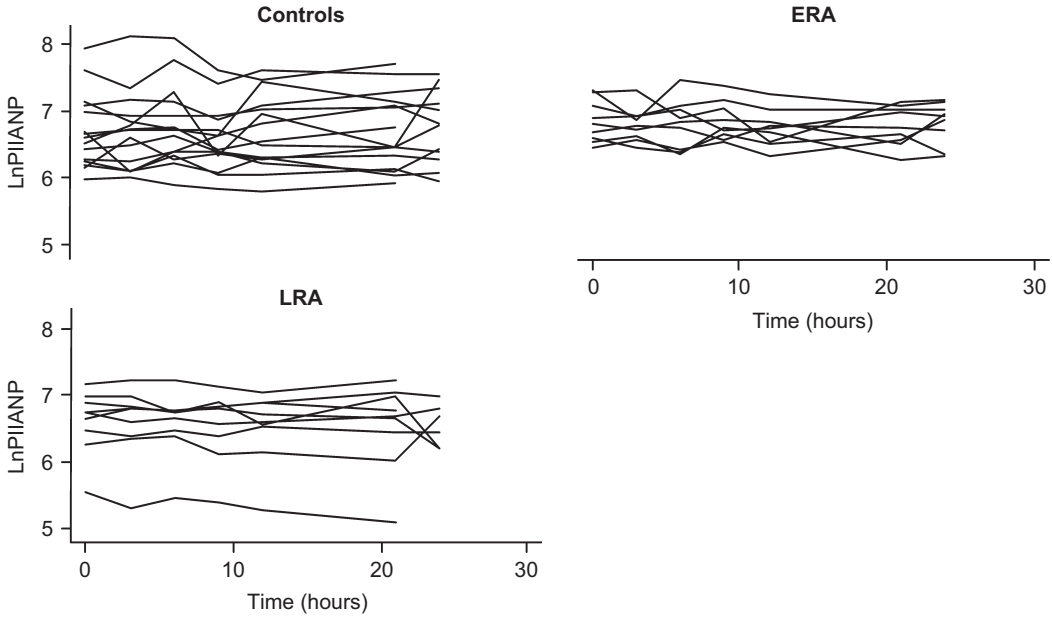

Figure 1. Procollagen IIA N-peptide (PIIANP) during 24 h observation (baseline, 10.00 h) in healthy individuals with early (ERA) and longstanding (LRA) rheumatoid arthritis. The y-axis is ln-scale.

Table 2. Physical activity. Demographics, clinical and laboratory characteristics of patients with rheumatoid arthritis (RA) and controls at inclusion.

Baselinevariable	Early RA (n = 9)	Longstanding RA(n = 9)	p-Value	Controls(n = 14)
Sex M/F (% female)	1/8 (89%)	3/6 (67%)	0.058	4/10 (40%)[a]
Disease duration (months)	3 (2.5–3.3)	35 (30.3–52)	<0.001	0
Age (years)	44 (38–50)	55 (50–62)	0.07	50 (38–55)[a]
IgM-RF positive (%)	6/9 (67%)	6/9 (67%)	1.00	–
Erosive disease (%)	2/9 (22%)	10/10 (100%)	0.002	–
Swollen joint count (0–40)	9 (8–11)	5 (2–7)	0.02	–
Tender joint count (0–40)	10 (5–14)	8 (5–11)	0.33	–
ESR (mm h^{-1})	14 (10–15)	18 (14–30)	0.13	–
CRP (mg l^{-1})	5 (2–12)	15 (5–25)	0.18	–
VAS doctor (0–100 mm)	37 (33–40)	28 (7–31)	0.02	–
VAS global (0–100 mm)	22 (9–35)	26 (14–33)	0.76	–
VAS pain (0–100 mm)	28 (15–49)	18 (15–39)	0.38	–
HAQ score (0–3)	0.25 (0–0.5)	0.5 (0.125–0.625)	0.53	–
PIIANP (ng ml^{-1})	1020 [780–1402]	872 [677–1324]	0.76	629 [425–1069][a]

p-Values calculated using Mann–Whitney U-test and Fischer's exact test

[a]Not significant compared with either RA group. Data are median (interquartile range) except for PIIANP (median [95% confidence interval]).
RF, rheumatoid factor; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; VAS, Visual Analogue Scale; HAQ, Health Assessment Questionnaire; PIIANP, N-terminal propeptide of collagen IIA.

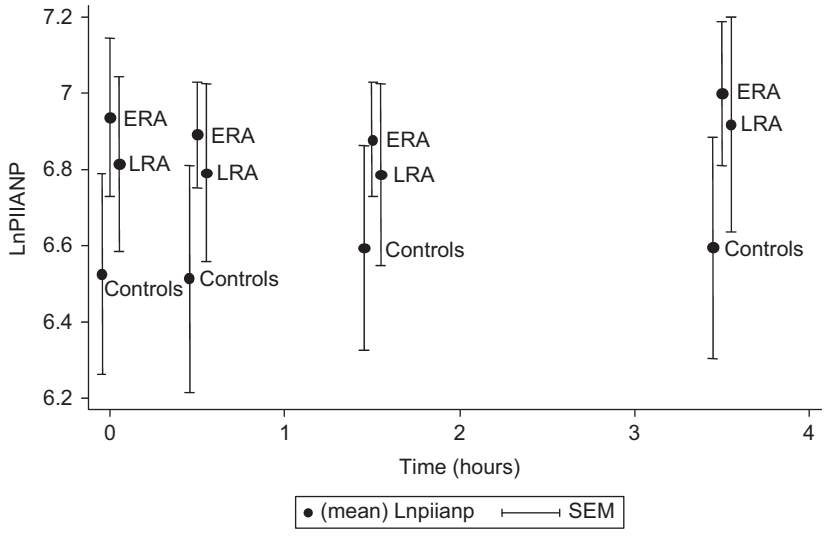

Figure 2. Procollagen IIA N-peptide (PIIANP) before (0), during (0–0.5 h) and after (>0.5 h) of controlled physical activity. ERA, early rheumatoid arthritis; LRA, longstanding rheumatoid arthritis; Lnpiianp, logarithmically transformed PIIANP; SEM, standard error of the mean.

(p=0.79), and between ERA patients and healthy individuals (p=0.87)).

Discussion

RA is a chronic and often progressive disease with distinctive features at early and advanced stages. This is the first study comparing circadian changes and effects of physical activity on PIIANP, an anabolic marker of cartilage collagen metabolism, in RA at an early and advanced stage using the same experimental set-up in both subsets. Of particular note, the study on PIIANP in DMARD- and steroid-naïve patients may imply a closer reflection of the underlying disease mechanisms compared with drug-treated RA patients. As in a previous study, PIIANP was decreased in longstanding seropositive RA (submitted for publication). By contrast, PIIANP remained stable assessed by seven consecutive within-day measurements, and likewise there were no significant changes during or following a standardized physical exercise programme. These findings apply to both RA subsets and healthy control individuals. This observation is at variance with a recent report by Quintana et al. including patients with OA at different stages defined by Kellgren–Lawrence score (Quintana et al. 2008). In that study PIIANP increased by 4.5% within 1 h after arising from bed followed by a decrease to baseline during daytime (Quintana et al. 2008). The authors suggested that PIIANP accumulates in the joint during rest, and that it is mobilized via the lymphatics to the circulation during day-time physical activity by analogy with hyaluronic acid and cartilage oligomeric matrix protein (COMP) (Andersson et al. 2006a, b, Engström-Laurent & Hällgren 1987, Kong et al. 2006, Quintana et al. 2008). Besides the possibility that cartilage collagen metabolism differs between OA and RA, the differences between the present result and those by Quintana et al. may relate to different experimental circumstances. In the present study, we chose 3-h intervals between blood drawings during the first 12 h followed by fasting blood collection early next morning before mobilization and then a final blood sample after exactly 24 h. This procedure has the advantage that it is feasible for patients and volunteers. By this approach, however, short-lived changes reflecting normal or diseased pathways may be missed. This is underscored by studies on hyaluronic acid, which increases shortly after arising from bed (Engström-Laurent & Hällgren 1987, Manicourt et al. 1999).

The study has some limitations. First, each subset comprised a relatively small number of subjects. However, the reliability of our observations is underscored by the low within- and between-subject variations and no day-to-day variation in either the RA cohorts or the control group. Furthermore, the upper limit of the CIs of the within-subject variations in the three cohorts was below 3.39%, which further strengthens our results because within-subject variations below 5% can be considered insignificant. Second, unlike the study by Quintana et al. we did not account for potential confounding by dietary collagen intake. However, based on their results, this source of variation seems to be negligible (Quintana et al. 2008). Third, RA is a heterogeneous disease including, for example seropositive and seronegative disease and patients with or without extra-articular manifestations. In this study, we confirmed our previous observation that patients with autoantibodies

had decreased PIIANP compared with seronegative patients (submitted for publication). The present sample sizes do not allow a comparison between seropositive and seronegative patients with respect to diurnal variation and physical activity.

In conclusion, this study demonstrates that PIIANP does not exhibit sustained responses to within-day rhythmic signals or submaximal physical activity. There were no qualitative differences between RA patients with early and advanced disease or healthy subjects.

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