Clock gene expression in rheumatoid arthritis and osteoarthritis

Circadian rhythms are controlled and generated by the biological clock located in the hypothalamic suprachiasmatic nucleus (SCN) (1,2). This “master clock” is synchronized to 24h by various environmental factors, primarily the dark-light-cycle but also by regularly occurring social processes, motor activity and food intake (3,4). Patients with RA show modulated circadian rhythms of inflammatory cytokines and cortisol, which may be associated with a modified expression of clock genes (5). The objective of this project is to study the expression and synchronization of clock genes in synovial specimens and fibroblasts from patients with RA or OA. Furthermore, the effect of TNF-α on clock gene expression is investigated.

The expression of 5 different clock genes and Dbp in synovial tissues of RA and OA patients was studied by immunohistochemistry (IHC). All of the clock genes were found to be expressed in the specimens, especially in the intimal layer, but considerable staining was also detected in the subintimal layer (Fig. 1).

Fig.1. Immunohistochemical analysis of clock gene expression in synovial tissues from patients with rheumatoid arthritis (RA) and osteoarthritis (OA).

Double stainings showed that mainly macrophages (CD68 positive) and fibroblasts (CD90 positive) expressed the five clock genes, while T- (CD3 positive) and B- (CD20 positive) lymphocytes showed lesser staining of all of the clock genes (not shown). RA patients showed higher expression of especially Bmal-1, but also Clock and Dbp. Of special interest was the first time detection of the Dbp protein in human samples, which, to our knowledge, has not been performed before.

In accordance with the results from the IHC, quantitative analyses of clock gene expression by real time PCR showed that expression of Bmal-1, Clock and Dbp were higher in RA patients (especially Bmal-1 and Dbp), although the difference did not reach the level of statistical significance, whereas Per1, Per2, and Cry-1 were expressed to a similar degree in OA and RA patients (not shown).

Furthermore, the effect of a 2 h serum shock (a standard method to synchronize clock gene expression) on 24h clock gene expression was studied in fibroblast-like synoviocytes (FLS) derived from RA or OA patients. The transcription factor Bmal-1 showed an expression peak at Zeitgeber Time (ZT) = 8 in both RA and OA SFs (Fig. 2), and tended to higher values in RA FLS as compared to OA FLS. Another interesting observation was that the transcript of Clock was significantly higher expressed in RA FLS than in FLS of OA patients with a peak at ZT = 12 whereas the expression in OA FLS exhibited nearly no rhythmicity. Similar results were obtained when the cells were stimulated with TNFα (not shown).
showed a peak after 8 hours Dbp expression (B) showed similar expressions in RA and OA FLS. and 3 OA patients. Data are mean +/- SEM of three independent experiments. Bmal 1 expression (A) nearly the same characteristics in RA and OA FLS.

Fig 2. mRNA expression profiles over 24h of clock genes induced by serum shock in FLS from 3 RA and 3 OA patients. Data are mean +/- SEM of three independent experiments. Bmal 1 expression (A) showed a peak after 8 hours Dbp expression (B) showed similar expressions in RA and OA FLS. Clock expression (C) showed a very low oscillation in OA FLS in contrast to the expression in RA FLS with a 2.5 fold higher expression at ZT=12. Cry 1 (D), Per1 (E) and Per2 (F) expression showed nearly the same characteristics in RA and OA FLS.

In conclusion, we showed that the most important core clock genes are expressed in synovial tissue of RA and OA patients with the most abundant expression in macrophages and SFs. The expressions were inducible in SFs not only under standard conditions but additionally also with TNF-α. The strong influence of this important cytokine on the expression profiles of the clock genes implies a role of these genes in the progression of RA. Summarizing all these observations, as well as reports from the literature, it seems that inflammation has an measurable effect on the core components of the circadian clock.

References