

# Circadian rhythms of nocturnal hormones in rheumatoid arthritis: translation from bench to bedside

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Translation from basic research to clinical medicine is complex and needs to be carefully performed. In addition, translational research is needed using human subjects who meet current diagnostic criteria.

Recently, circadian rhythm science has pointed the way to rational intervention on nocturnal hormone production to modulate the immune/inflammatory reactions in patients suffering from rheumatoid arthritis (RA).<sup>1,2</sup> These approaches have been introduced into practice with increasing degrees of interest.<sup>3</sup>

We will discuss two recent examples of therapeutically modulating melatonin (MLT) and cortisol as nocturnal hormones implicated in RA clinical circadian rhythms.

## CIRCADIAN RHYTHMS AND MORNING STIFFNESS IN RHEUMATOID ARTHRITIS

It has been known for many decades that disease symptoms in RA follow obvious circadian rhythms, with an increase of activity in the early morning hours, abatement during the day, and a smaller new increase in the early evening (fig 1A).<sup>3,4</sup>

A number of papers have reported a temporal relationship between elevated levels of pro-inflammatory cytokines and symptoms of RA, such as morning stiffness.<sup>4-9</sup> Several of these cytokines are highly elevated in patients with active RA in the early hours of the day but after noon they are almost non-existent. Their release pattern and serum concentrations, respectively—possibly triggered by MLT and other hormones or a subordinate neuroendocrine centre in the hypothalamus<sup>4</sup>—follow a strict 24-h daily cycle (fig 1B).

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The closest similarity with the daily pattern of RA symptoms, such as morning stiffness, joint pain and functional disability, seems to exist for interleukin (IL)-6.

Pro-inflammatory hormones start to rise before the onset of RA symptoms and before endogenous cortisol in these patients is activated to counteract the inflammatory cascade of disease symptoms. It should be also noted that rhythmic fluctuations of the nocturnal secretion and the peripheral metabolism of endogenous cortisol, as well as changes in the activation of biologically inactive to active cortisone in the synovial cells, may play a role in the pathophysiology of RA.<sup>9,10</sup>

In patients with RA, inflammation induced changes in synovial fluid composition, oedema of the synovium and periarticular structures, as well as redistribution of interstitial fluid while sleeping, contribute to clinical stiffness of the joints that is most pronounced in the morning.<sup>11,12</sup> Morning stiffness is also considered to be causally related to the circadian rhythms of pro-inflammatory factors we have discussed above (fig 1).

These processes are closely linked to regulatory interactions between the endocrine, nervous and immune systems, with distinct 24-h daily rhythms.<sup>10</sup>

The role of IL6 in the regulation of inflammatory and immune responses, particularly in RA, is well established but an increased production of other pro-inflammatory cytokines such as tumour necrosis factor (TNF), IL1, IL8, IL12 and IL17 by primary and secondary immune cells has also been reported.<sup>13</sup>

It has been also discussed that RA is characterised by an inadequate anti-inflammatory response that may contribute to morning stiffness. This lack of anti-inflammatory response is not yet totally understood.

## MELATONIN IN INFLAMMATION AND RHEUMATOID ARTHRITIS

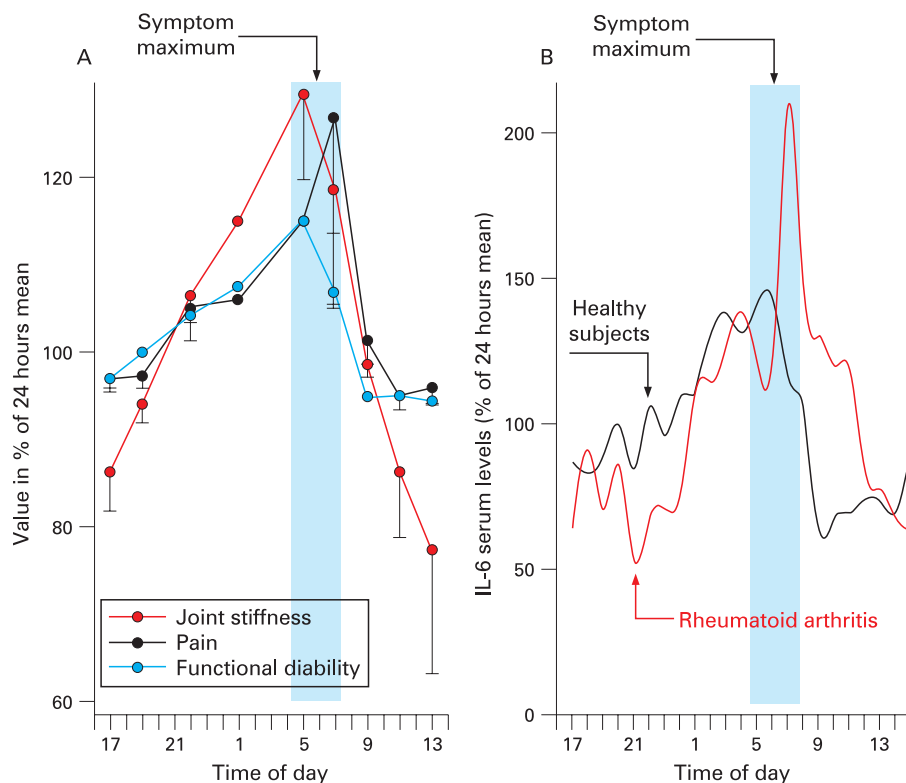
A positive genetic relationship between MLT and RA has been recently suggested.<sup>14</sup>

In 2002, our first study evaluated MLT levels in patients with RA with a focus upon analyses of circadian variations.<sup>15</sup> MLT serum levels at 8 pm and 8 am were found to be significantly higher in patients with RA than in healthy controls ( $p < 0.05$ ). The differences were more evident in patients who were older than 60 years. In patients with RA and in healthy subjects, MLT levels increased progressively from 8 pm to the early morning hours; however, they reached peak levels at midnight in patients with RA, which was at least 2 h earlier than in controls. Subsequently, MLT concentrations in RA reached a plateau that lasted for 2–3 h; this was not observed in controls. The study confirmed that the nocturnal rhythm of MLT occurs also in patients with RA, but with an earlier peak and a broader plateau in the early morning.

In human peripheral blood mononuclear cells, at physiological concentrations MLT has been reported to stimulate the production of interferon- $\gamma$  (IFN $\gamma$ ), IL1, IL2, IL6 and IL12, but not IL4.<sup>16,17</sup> In addition, MLT was found to enhance production of inflammatory cytokines from cultured human monocytes/macrophages, including IL12 and turning the MLT/IL2 connection towards the enhancement of T cell immunity.<sup>18</sup> MLT was found to be detectable at high concentration in synovial fluids from patients who had RA, and binding sites for MLT were present in synovial macrophages.<sup>19</sup>

Interestingly, IFN $\gamma$ , IL1, IL6, IL2, IL12 and tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) production, (Th1 cytokines/promoting cytokines) reach their peak during the night and early morning, shortly after MLT serum levels are highest and plasma cortisol is lowest. Therefore, we believe that MLT upregulates cytokine production and immune function.<sup>20</sup> Accordingly, among the signs of joint inflammation in patients with RA, the intensity of pain varies as a function of the hours of the day; pain is greater after waking in the morning than in the afternoon or evening (fig 1A).<sup>4</sup>

Circadian changes are also observed in joint swelling and finger size in the early morning in patients affected by RA. Studies on experimental granulomatous lesions confirmed that the rhythm of the inflammatory reaction is due to the rhythmic MLT release by the pineal gland.<sup>21</sup> Therefore, MLT may activate the inflammatory response during the night, at least in RA, which is mainly considered to be a Th1 cytokine driven immune disease.



**Figure 1** Circadian rhythm of symptoms and interleukin (IL-6). A. The graph depicts the circadian rhythm of joint stiffness, pain and functional disability. B. The circadian rhythm of IL6 secretion in healthy subjects (black line) and patients with rheumatoid arthritis (red line). The data are given as percentages of the 24-h mean value of the respective variable.

However, MLT is also one of the most efficacious antioxidant compounds in mammals being at least as active as vitamin E in its ability to scavenge hydrogen peroxide and the highly destructive hydroxyl and peroxy nitrite anions.<sup>22</sup>

**MELATONIN FROM BENCH TO BEDSIDE IN RA**

An earlier study in patients with RA suggested that blood MLT levels were found decreased consistent with the possibility that the loss of its antioxidant activity could contribute to the disease.<sup>23</sup> As until recently there was still a need to obtain clinically-based evidence about the possible role of MLT as disease-promoting or -protecting hormone in RA, a double-blind placebo controlled study investigating the effects of MLT administration in patients with RA was initiated.<sup>24</sup>

The results obtained were somewhat disappointing and surprising, as the authors stated in the discussion.<sup>25</sup> MLT decreased the levels of lipid peroxidation, but increased erythrocyte sedimentation rate and neopterin levels compared with patients treated with placebo, consistent with an antioxidant effect but also suggesting some pro-inflammatory activity.

In addition, no improvement of RA disease was observed, and in some cases the disease appeared to be worse.

Recently, serum TNF $\alpha$  was found to be higher in Northern European patients with RA than in their controls and was found to be significantly correlated with the increased serum MLT concentrations, at least during the winter.<sup>26</sup> Therefore, increased serum concentrations and circadian rhythm of MLT and a “relative adrenal insufficiency” in chronic RA (low cortisol), allows Th1 type cytokines to be produced in higher amounts during the late night under the enhancing effect of pineal hormone.<sup>3</sup>

In conclusion, the translation from basic research to clinical medicine clearly showed that MLT treatment does not improve RA.<sup>27</sup>

**IMPROVING MORNING STIFFNESS: SUCCESSFUL TARGETING OF PATHOPHYSIOLOGICAL RHYTHMS**

Cortisol secretion and glucocorticoid receptor density has been reported to be altered in patients with RA.<sup>28</sup> Furthermore, circadian changes of peripheral metabolism of endogenous glucocorticoids may also contribute to the early

morning manifestation of the disease symptoms in RA.<sup>4</sup> Based on these considerations, it has been suggested that the usual administration of glucocorticoids between 6 and 8 am is not optimal.<sup>4</sup> This could simply be too late since the night-time pathophysiological processes have already lead to inflammation, pain and subjective symptoms.<sup>3-5 7 29-31</sup> Consequently, it has been hypothesised that it could be easier to prevent the circadian increase of pro-inflammatory cytokine levels and, therefore, the consequently observed clinical signs and symptoms of the disease than to treat these signs and symptoms once they are established in the morning.

In an early study, patients with active RA were woken at 2 am in order to take their usual glucocorticoid drug. It turned out that this procedure indeed had better effects on severe morning RA symptoms than the same dose of drug did if administered at 7.30 am.<sup>7</sup> However, regular waking of the patients is impractical for the therapeutic regimen and will on its own influence the hypothalamic-pituitary-adrenal (HPA) axis.<sup>32</sup>

These observations led to the development of a new modified-release prednisone tablet formulation.<sup>4</sup> This oral prednisone tablet is taken at bedtime and releases the active drug at about 2 am. This results—but with a delay of about 4 h—in a pharmacokinetic profile and a total drug exposure almost identical to that of conventional prednisone.

The efficacy and safety of this novel medication has been recently investigated in a study of 288 patients with active RA, half of which were randomised to receive a modified-release prednisone tablet, and the other half a standard prednisone tablet. Morning stiffness of the joints was measured in both groups and the target variable for the statistical analysis was the mean relative change of duration of morning stiffness at 12 weeks. This mean relative change was much higher for the modified tablet group (-22.7%) vs the standard tablet group (-0.4%) (p = 0.045).

In terms of the actual time the duration of the morning stiffness was reduced for both treatments vs baseline, but the reduction was also much higher in the modified group (44.0 min) compared to baseline. The absolute difference in minutes between the treatments at 12 weeks was 29.2 min (p = 0.072) in favour of the modified tablet. The safety profile was the same for both treatments.<sup>4</sup>

It was concluded that the new modified-release formulation is clinically

and statistically better than the conventional immediate-release preparation with regard to morning stiffness of the joints. Furthermore, the effects of the new tablet taken at night were achieved in addition to the established clinical control of the disease resulting from treatment with conventional immediate-release prednisone.

This result is considered to be relevant for daily clinical practice.<sup>32</sup> The reason is that severe morning stiffness limits function, especially grip strength. Morning stiffness is part of the American College of Rheumatology (ACR) classification and remission criteria and also part of the early referral recommendation for newly diagnosed rheumatoid arthritis.<sup>33–35</sup>

However, it is currently—in contrast to eg, pain, functional limitations, quality of life and ability to work—relatively neglected in clinical studies.

From another recently published study it has been concluded that, in patients with early RA, the degree of morning stiffness appears to reflect functional disability and pain better than traditional markers of inflammation such as joint counts and erythrocyte sedimentation rate (ESR).<sup>36</sup> It has also been shown that the variables swollen joint count and morning stiffness are more predictive for a change in treatment than, for example, C-reactive protein or tender joint count.<sup>37</sup> We conclude from these data that morning stiffness has a considerable impact on the patient's quality of life and that it is worth developing treatment strategies that more intensively aim at reducing its duration. Nevertheless, further studies are warranted to investigate the long-term effects of new treatment regimens such as those relating to timing of basic inflammatory processes and the entire range of RA symptoms, including pain.<sup>38–39</sup>

## CONCLUSIONS

Successful or unsuccessful application of pathophysiological concepts in RA resulted in the abandoning of therapeutic strategies or successful translation from bench to bedside. By considering a supposed beneficial role of MLT in RA therapy, the translation from basic research to clinical medicine clearly showed that MLT treatment worsens the disease.

By contrast, the night-time administration of prednisone based on circadian rhythms established a clinical improvement of the disease superior to morning administration. Further steps along this route will include studies to answer the question whether the new time-related

approach demonstrates that a given glucocorticoid dose is more effective and/or less deleterious. In addition, it should also be shown whether this strategy has benefits in other rheumatic and non-rheumatic diseases that are characterised by severe inflammatory early morning symptoms.<sup>4–32</sup>

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