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Chronobiology of and Chronotherapy for Rheumatoid Arthritis

Hideto To
University of Toyama
Japan

1. Introduction

Most humans have a relatively regular activity pattern (sleep, labor, and meal etc.). This activity can be roughly classified into the rest phase and active phase, and body temperature, heart rate, blood pressure, and the dominance of the sympathetic and parasympathetic nerves differ in each phase. These variations display daily rhythms, which are known as circadian rhythms.

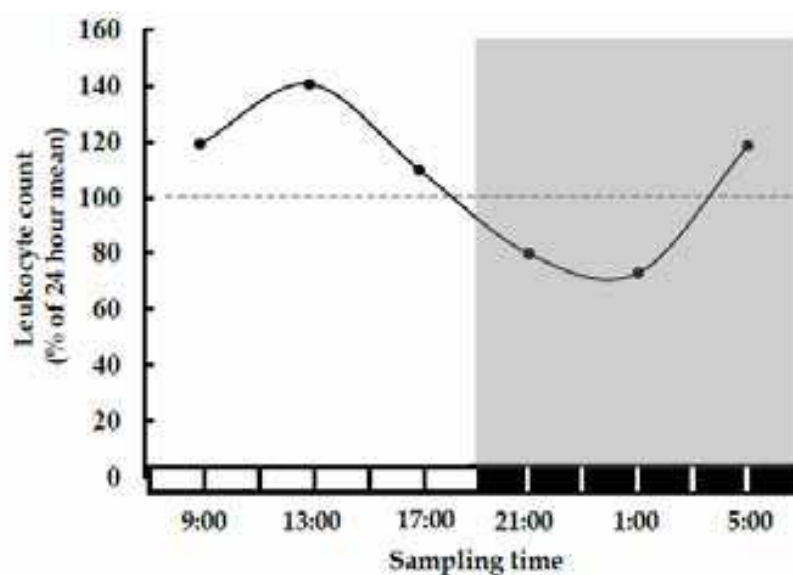


Fig. 1. Circadian rhythm of mouse leukocyte count

For instance, ICR mice, which were housed under standardized light-dark cycle conditions (lights-on and lights-off at 7:00 and 19:00, respectively) at a room temperature of $24 \pm 1^\circ\text{C}$ (range) and a relative humidity of $60 \pm 10\%$ (range) and were allowed free access to food and water displayed a circadian rhythm in their leukocyte counts with a peak at noon and a trough at midnight (Fig. 1). Many organisms display rhythms in various factors (Table 1). In humans, heart rate and blood pressure decline at night. We spend much of our time in a standing position during the day and sleep in a recumbent position at night. It is thought that the wake-sleep transition and endogenous circadian rhythms are responsible for the circadian rhythms in heart rate and blood pressure. Circadian rhythms also exist for the

levels of hormones such as cortisol and melatonin (MLT). The plasma level of cortisol peaks in the morning and that of MLT peaks at midnight. Bone marrow cells, intestinal mucosal cells, and hair matrix cells show relatively active cell division. In the day, there is also a period of time when the number of cells is actively increased by segmentation (DNA synthesis) as well as a period of dormancy. The circadian rhythm of bone marrow cell division involves a peak in the evening and a trough in the midnight, and that of rectal mucosal cell division is higher at 7:00 and lower at 19:00. Thus, various factors in the living body have their own circadian rhythms, and the phases of these cycles vary for each factor.

No.	Factors	Peak period	Trough period	Ref
1	Body temperature	18:00-20:00	3:00-6:00	Lack et al., 2008
2	Heart rate	8:00-18:00	4:00-6:00	Clarke et al., 1976
3	Blood pressure	8:00-20:00	0:00-6:00	Degaute et al., 1991
4	Hepatic blood flow	8:00	14:00	Lemmer et al., 1991
5	Glomerular filtration	16:00	4:00	Koopman et al., 1989
6	Lymphocytes	12:00-17:00	7:00	Miyawaki et al., 1984
7	Cortisol	8:00-13:00	0:00-4:00	Gavrila et al., 2003
	Adiponectin	8:00-13:00	2:00-4:00	
	Leptin-binding protein	8:00-14:00	1:00-5:00	
8	Melatonin	2:00-4:00	9:00-21:00	Kennaway et al., 1998
9	DNA synthesis in the bone marrow	16:00	0:00	Smaaland et al., 1991
10	DNA synthesis in the rectal mucosa	7:00	19:00	Buchi et al., 1991

Table 1. Circadian rhythms of various factors in humans

No.	Factors	Peak period	Trough period	Ref
1	Spontaneous acute dissection and rupture of the thoracic aorta	7:00-12:00	22:00-6:00	Gallerani et al., 1997
2	Myocardial ischemia	8:00	0:00-4:00	Egstrup et al., 1991
3	Ischemic stroke	8:00	18:00-6:00	Argentino et al., 1990
4	Subarachnoid hemorrhage	6:00-20:00	0:00-4:00	Vermeer et al., 1997
5	Asthma	2:00-5:00	7:00-19:00	Dethlefsen et al., 1985
6	Temporal lobe epilepsy	15:00-21:00	0:00-5:00	Quigg et al., 1998
7	Pain sensitivity in teeth	4:00-7:00	11:00-17:00	Pöllmann et al., 1978
8	Migraine	4:00-10:00	21:00-1:00	Fox et al., 1998
9	Stiffness, pain, and functional disability in rheumatoid arthritis	3:00-7:00	13:00-21:00	Straub et al., 2007

Table 2. Circadian rhythms in the risk or frequency of disease occurrence

These circadian rhythms are also associated with the risk or frequency of disease occurrence (Table 2). For example, asthma attacks get worse between midnight and early morning and

are seldom observed in the daytime (Fig.2; Dethlefsen et al., 1985). In addition, the risks of spontaneous acute dissection and rupture of the thoracic aorta, myocardial ischemia, ischemic stroke, and subarachnoid hemorrhage are higher during the active phase than during the rest phase. The variations in heart rate, blood pressure, and blood flow, etc., induced by the wake-sleep transition are considered to affect the risk of such problems occurring. Interestingly, pain such as toothache, migraine, and rheumatoid arthritis pain is more acute in the early morning. Collectively, circadian rhythms are recognized in many diseases, and certain time periods are associated with a high risk or frequency of disease occurrence.

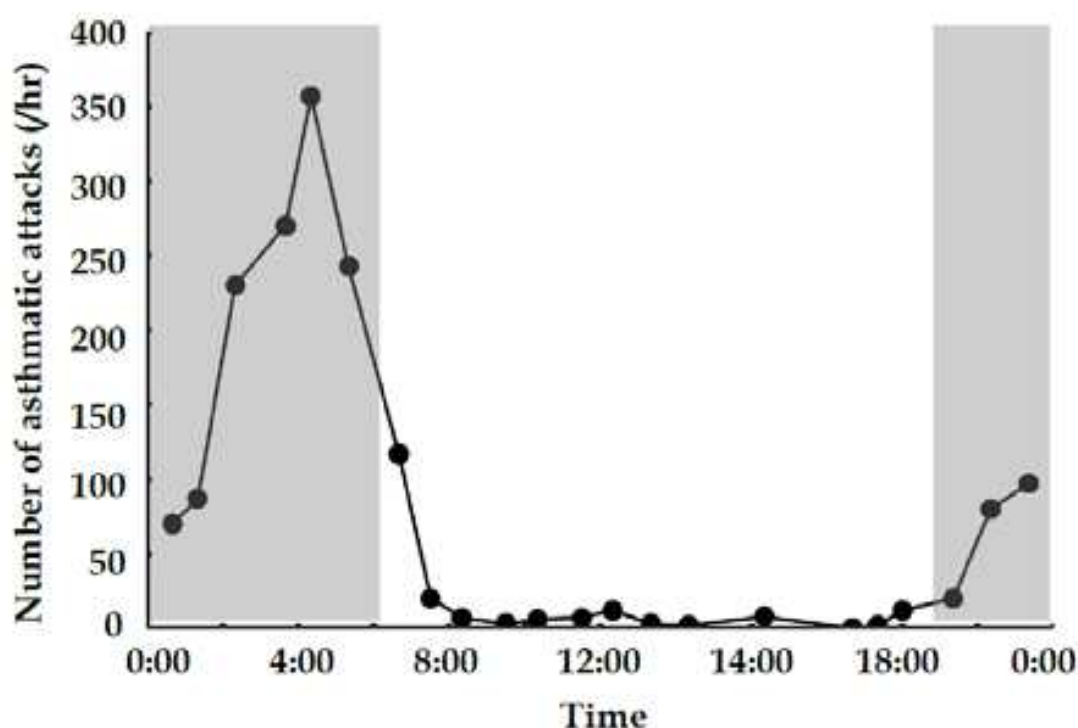


Fig. 2. Circadian rhythm of asthmatic attacks in asthma patients (redrawn from the data of Dethlefsen et al., 1985)

Chronotherapy is defined as the administration of medications in accordance with biological rhythms in order to optimize therapeutic outcomes and/or control adverse effects, and it has been reported that many drugs such as antitumor drugs, antidepressants, and analgesic drugs show rhythm-dependent differences in their effects and pharmacokinetics (Tabuchi et al., 2005; Ushijima et al., 2005; Tampellini et al., 1998). These effects arise from the circadian rhythms found in elements of cellular physiology such as the cell cycle and the expression of receptors, hormones, and enzymes (Iurisci et al., 2006; Matsunaga et al., 2004; Koyanagi et al., 2006). Fig. 3 displays data for the dosing time-dependency of vomiting episodes in urogenital cancer patients treated with cisplatin (Kobayashi et al., 2001). Cisplatin (70 mg/m²) was given to the patients at 5:00 or 17:00. After the cisplatin administration, all episodes of vomiting during a 6-hour period were recorded. Vomiting was markedly decreased in the patients treated at 17:00 compared with that at 5:00 ($P = 0.061$).

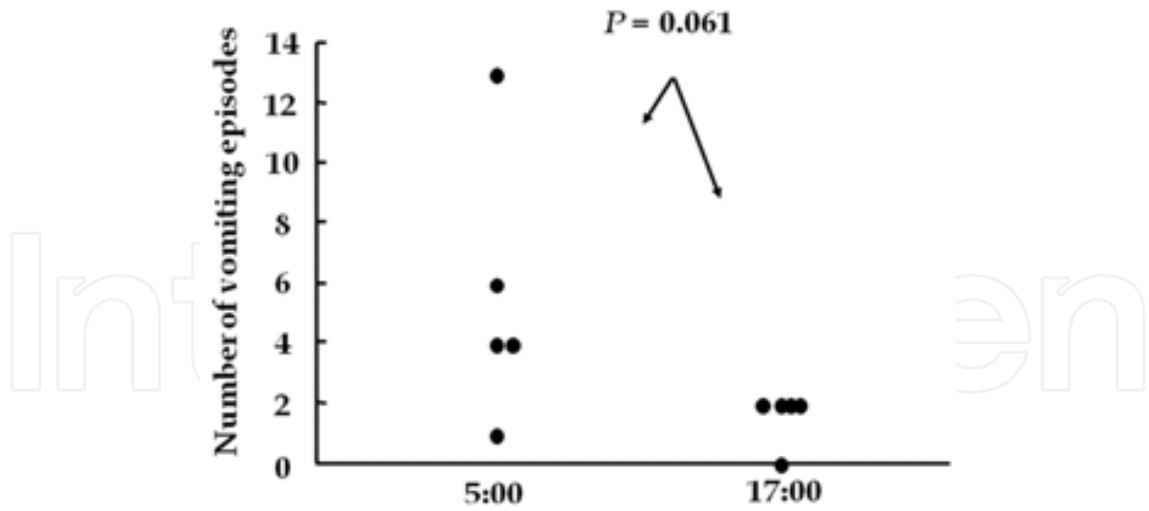


Fig. 3. Number of vomiting episodes according to the time (5:00 vs 17:00) of cisplatin administration in patients with urogenital cancer (redrawn from the data of Kobayashi et al., 2001)

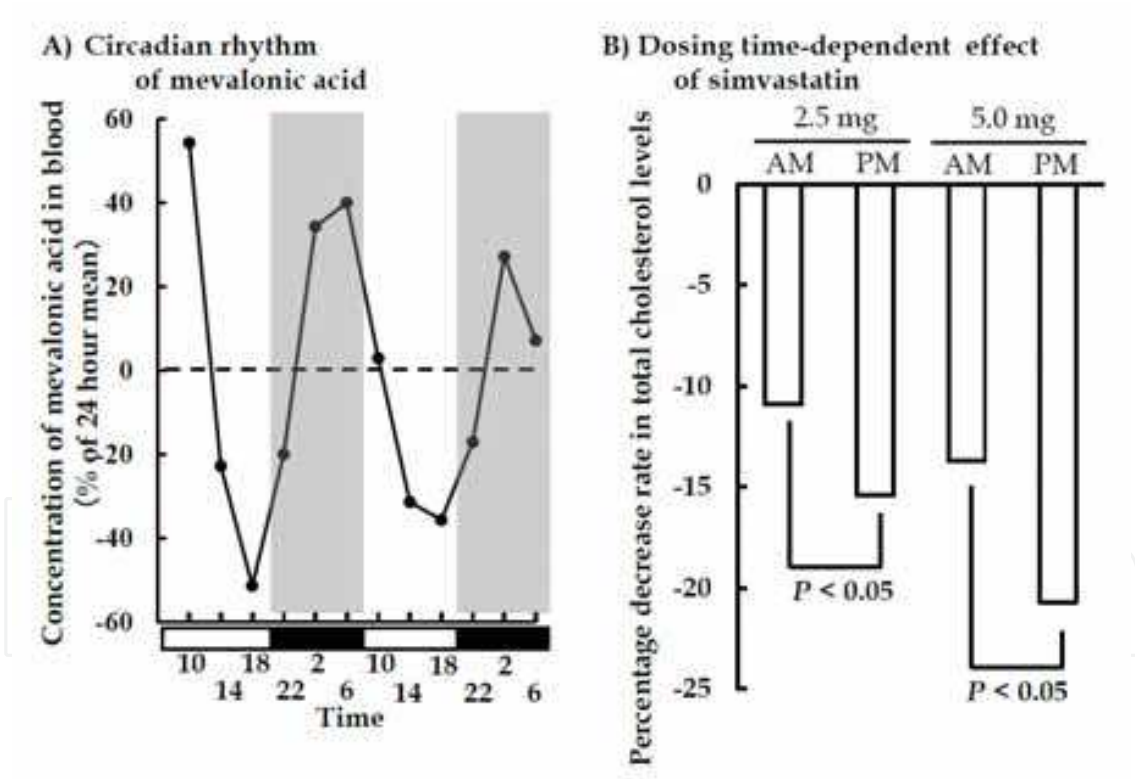


Fig. 4. Circadian rhythm of plasma mevalonic acid levels and the influence of simvastatin dosing time on total cholesterol levels (redrawn from the data of Jones et al., 1992, and Saito et al., 1991)

Moreover, it has been reported that circadian rhythms exist for asthma attacks and cholesterol synthesis, and medicinal treatment based on chronotherapy has been actively applied to the treatment of asthma, hypertension, and hyperlipidemia (D'Alonzo et al., 1995;

Hermida et al., 2010; Haus, 2007; Saito et al., 1991; Smolensky et al., 2007). Hydroxymethyl glutaryl coenzyme A (HMGCoA) reductase participates in the biosynthesis of mevalonic acid and is the rate-limiting enzyme of cholesterol biosynthesis. The plasma mevalonic acid levels in healthy volunteers showed a clear circadian rhythm, with higher levels seen at night and lower levels observed at daytime (Fig. 4A). Simvastatin is an HMGCoA reductase inhibitor. When simvastatin was administered to hyperlipidemic patients once a day in the morning or evening, the evening group displayed a significantly decreased total cholesterol level compared with the morning group (Fig. 4B). Thus, cholesterol biosynthesis may be effectively inhibited when simvastatin is administered in the evening, when the activity of HMGCoA reductase begins to increase.

Taken together, many organisms display circadian rhythms in various factors, and chronotherapy that takes into account circadian rhythms is thought to be a useful therapeutic method.

2. Chronobiology of rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disorder of unknown etiology and a chronic progressive disease that reduces the quality of life of individuals that suffer from the condition (Harris ED Jr., 1990; Gabriel SE., 2001). Although many requirements must be met to establish a diagnosis of RA, morning stiffness is a characteristic feature of RA (Arnett et al., 1988). Tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6), which are inflammatory cytokines, show high concentrations in human blood and synovial fluid, and excess production of these cytokines plays a central role in the pathogenesis of RA (Feldmann et al., 1996; McInnes et al., 2005). Morning stiffness shows a circadian rhythm with a peak in the early morning in RA patients. The IL-6 concentration in blood also shows a circadian rhythm peaking from midnight to early morning, which mirrors the timing of morning stiffness (Crofford et al., 1997).

In this chapter, I would like to introduce the circadian rhythm of rheumatoid arthritis and its mechanism.

2.1 Circadian rhythm of rheumatoid arthritis

RA is an autoimmune disorder of unknown etiology, and morning stiffness is a characteristic of the condition (Arnett et al., 1988). Pain, functional disability, and stiffness show circadian rhythms with a peak in the early morning in many RA patients (Fig. 5) (Bellamy et al., 1991; Kowanko et al., 1982), and the circadian rhythms of pain and stiffness may play a role in local and systemic inflammatory responses. Herold and Günther (Herold et al., 1987) reported that plasma C-reactive protein (CRP) levels, an indicator of inflammatory responses, showed a circadian rhythm with a peak in the early morning and a trough in the evening in RA patients, which matches the rhythms of pain and stiffness. Proinflammatory cytokines, such as TNF- α and IL-6, are secreted from activated monocytes, and macrophages increase CRP levels in hepatocytes. There are clear circadian rhythms in the blood concentrations of these cytokines, with higher levels seen in the early morning in RA patients (Crofford et al., 1997; Perry et al., 2009). Since the circadian rhythms of CRP and cytokines are similar, it is considered that cytokine rhythms contribute to the rhythm of CRP levels.

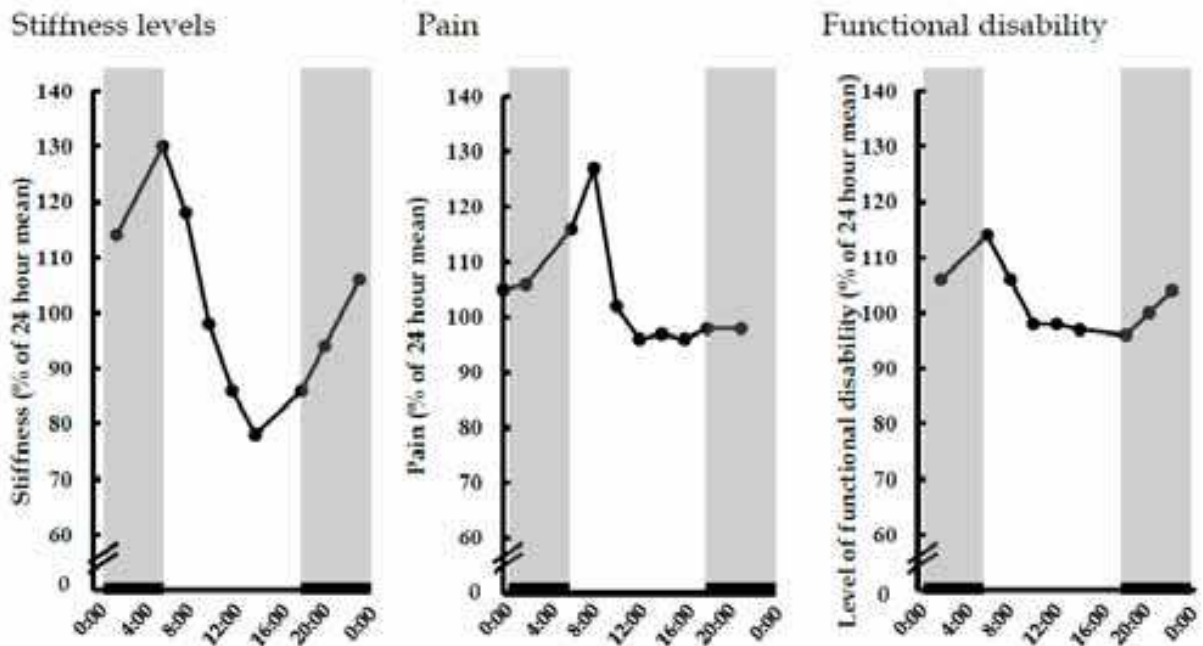


Fig. 5. Circadian rhythms of stiffness, pain, and functional disability in RA patients (redrawn from the data of Strub et al., 2007)

To clarify the relationship between the inflammatory response and cytokines in RA, we studied these circadian rhythms in RA model animals. MRL/lpr mice are an RA model that develop autoimmune disorders that share similarities with human RA and systemic lupus erythematosus (Abe et al., 1980; Koopman et al., 1988). It is difficult to monitor stiffness in RA model animals; however, the circadian rhythm of CRP was found to correspond to that of morning stiffness in RA patients (Herold et al., 1987). Using MRL/lpr mice, we estimated the plasma serum amyloid A (SAA) concentration, which is an acute-phase protein and a sensitive marker of acute inflammatory states, because the CRP level cannot be detected in mice. SAA is also synthesized in the liver upon stimulation by cytokines such as TNF- α and IL-6 (Baumann et al., 1994; Gabay et al., 1999). Before RA onset, there was no significant circadian rhythm in plasma SAA levels (To et al., 2011). However, an obvious circadian rhythm in the plasma SAA concentration involving higher levels in the morning was observed in the MRL/lpr mice that developed RA. There was no significant circadian rhythm in plasma TNF- α levels before RA onset (To et al., 2011). After RA onset, plasma TNF- α levels showed an obvious daily variation, with higher levels in the morning and lower levels at midnight. Collagen-induced arthritis (CIA) represents a true autoimmune reaction against major joint components that is associated with class II major histocompatibility complex genes and pannus formation. CIA model animals display similarities with RA in terms of pathology, immunology, and genetics (Wooley et al., 1984; Holmdahl et al., 1989). It was previously reported that CIA mice showed increased cytokines levels, similar to RA patients (Marinova-Mutafchieva et al., 1997; Mussener et al., 1997). In CIA mice, the SAA level was increased compared with that in the control group and showed a significant circadian rhythm involving higher levels in the morning after RA onset (Fig. 6). The plasma TNF- α concentration also showed a circadian rhythm with a peak in the morning (Fig. 6) (To et al., 2009).

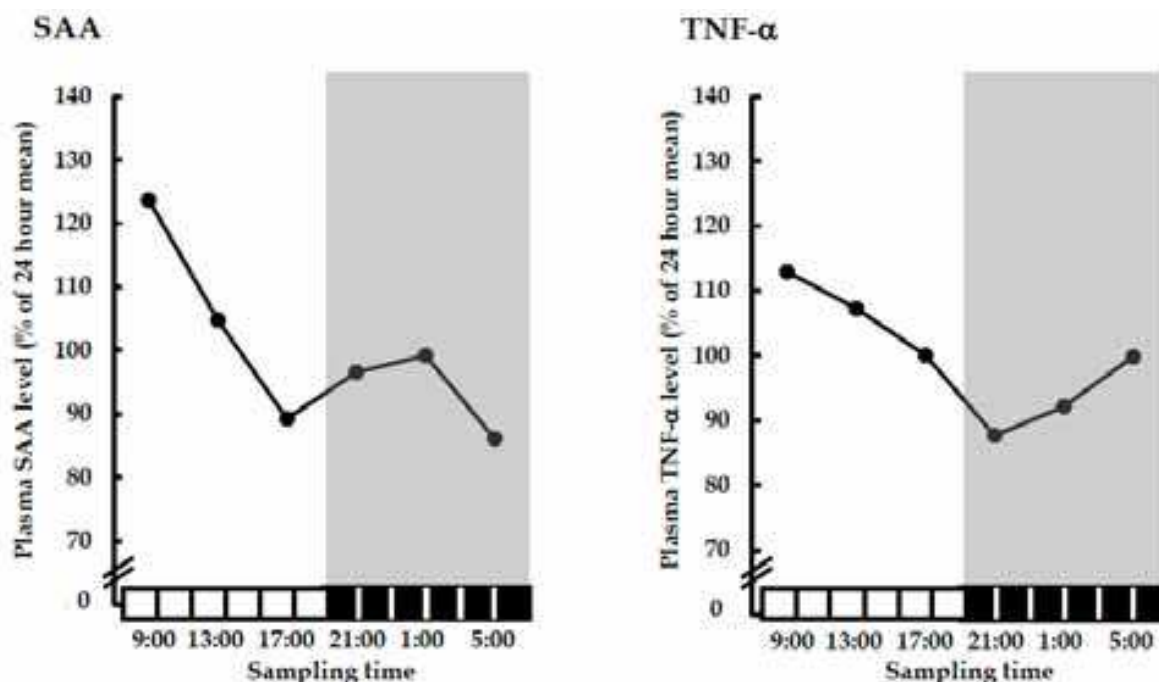


Fig. 6. Circadian rhythm of plasma SAA and TNF- α levels in CIA mice (redrawn from the data of To et al., 2009)

In healthy humans, there is no significant circadian rhythm in plasma IL-6 levels. Moreover, no circadian rhythm is found in patients with various other inflammatory connective tissue diseases. Only RA patients show a clear circadian rhythm in plasma IL-6 concentrations, with higher levels seen from night to early morning and lower levels observed during the daytime (Crofford et al., 1997; Arvidson et al., 1994). Therefore, it is considered that the circadian rhythm in plasma IL-6 levels, which is not present before RA, is a phenomenon peculiar to RA because identical results were obtained in RA patients and RA model animals.

Collectively, the synchronization of the circadian rhythms of the inflammatory response and cytokine levels was also observed in RA patients and RA model animals. It is thought that the inflammatory response contributes to morning stiffness in RA because pain and stiffness develop in the early morning, when the CRP level is higher. Therefore, it is thought that the circadian rhythms of inflammatory cytokines play important roles in the expression of RA symptoms and that these rhythms are important for diagnosing RA.

2.2 Factors affecting the circadian rhythm of rheumatoid arthritis

Although the causes of the cytokine circadian rhythms generated by RA onset have not been completely clarified, it is thought that the MLT and cortisol balance and clock genes are involved in the mechanism.

The circadian rhythms of mammals are mediated by the transcriptional/ posttranscriptional machinery regulating the *clock* genes including *Clock*, *Bmal 1*, *period*, and *Cryptochrome (Cry)* (Sato et al., 2006). Hashiramoto et al. reported that *Cry*, which is one of the *clock* genes, contributes to the induction of arthritis and increases the serum TNF- α level (Hashiramoto et al., 2010). In this study, the number of activated CD3(+) CD69(+) T cells was significantly increased among lymphocytes from *Cry1^{-/-}Cry2^{-/-}* mice. *Cry1^{-/-}Cry2^{-/-}* mice display aggravated

arthritis and increased serum TNF- α concentrations compared with wild type mice. Thus, *Cry* may play an important role in the inducement of RA.

Cutolo et al. proposed that the balance between MLT and cortisol is related to the formation of cytokine circadian rhythms (Straub et al., 2007; Cutolo et al., 2003). MLT has an immunoenhancing effect and induces cytotoxic properties in monocytes etc. (Morrey et al., 1994). Additionally, MLT enhances the expression of inflammatory cytokines (Garcia-Mauriño et al., 1997). In contrast, glucocorticoid suppresses the expression of cytokines such as IL-1, IL-6, and TNF- α . In humans, the MLT level in plasma shows a circadian rhythm with a peak at 3:00 (Kennaway et al., 1998), and the plasma cortisol level shows a circadian rhythm with higher levels seen at 8:00 (Miyatake et al., 1980). The secretion of MLT and cortisol in RA patients differs from that in healthy subjects; i.e., the serum MLT levels of RA patients peak about 2 hours earlier than those in healthy controls (Sulli et al., 2002). Furthermore, the peak cortisol level was found to be lower in RA patients than in the controls (Neeck et al., 1990). These changes in the hormone balance may contribute to the increased cytokine expression seen in RA. It was reported that the serum cytokine and MLT concentrations also showed circadian rhythms with peaks from midnight to early morning, which mirror those of morning stiffness (Arvidson et al., 1997; Sulli et al., 2002). Thus, MLT may be involved in the circadian rhythm of RA symptoms.

We studied the corticosterone concentrations in MRL/lpr mice before/after RA onset. The corticosterone concentration before RA onset showed a significant circadian rhythm with higher levels during the early dark phase and lower levels during the period from the late dark phase to the early light phase (Fig. 7). After RA onset, there was a clear circadian rhythm in the corticosterone level. As rodents are nocturnal animals, the phases of their circadian rhythm for glucocorticoid secretion are shifted by about 12 hours compared with those for humans (Koyanagi et al., 2006). At 21:00, when the corticosterone level peaked, the corticosterone level after RA onset had decreased by 40.9% compared with that before RA onset (Fig. 7). The change in corticosterone secretion in the MRL/lpr mice was similar to that seen in RA patients. On the other hand, the MLT levels in rodents displayed a circadian rhythm with a peak at midnight, as is seen in humans (Conti et al., 1998).

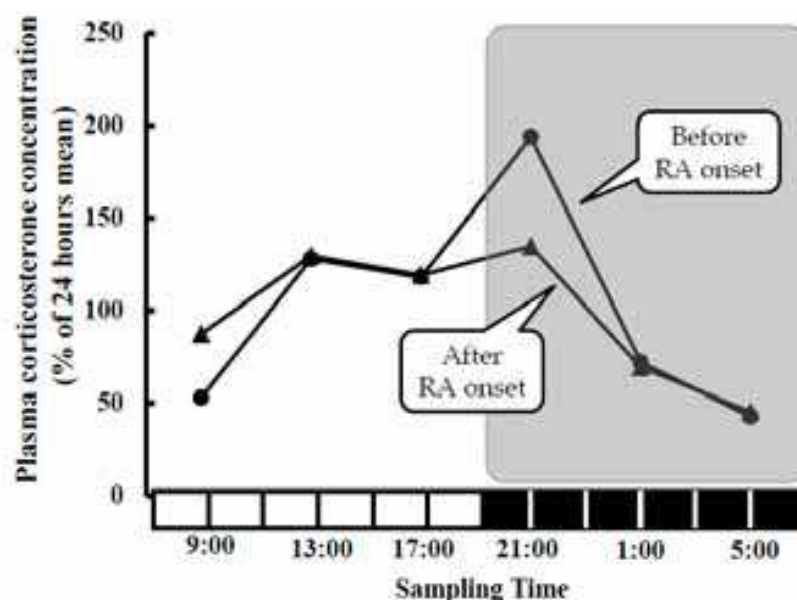


Fig. 7. Circadian rhythms of the corticosterone levels of MRL/lpr mice

Fig.8 shows a hypothesis for the interaction of cytokines and the secretion of MLT and glucocorticoid. Cytokine overexpression is controlled by the secretion balance between the circadian rhythms of MLT and corticosterone. However, the secretion of glucocorticoid declines in RA, and the induction of cytokine expression is increased by MLT. Consequently, the cytokine circadian rhythm follows that of MLT. The phases of the circadian rhythm for glucocorticoid and leukocyte counts, etc., differ by about 12 hours between humans and rodents. However, the phases of the circadian rhythms for the inflammatory response and cytokines are similar between humans and rodents. This may be caused by the similar circadian rhythms for MLT levels between human and rodents. Therefore, it is thought that the circadian rhythm and balance of MLT, which induces the expression of cytokines, and glucocorticoid, which suppresses their expression, participate in the circadian rhythm of RA symptoms.

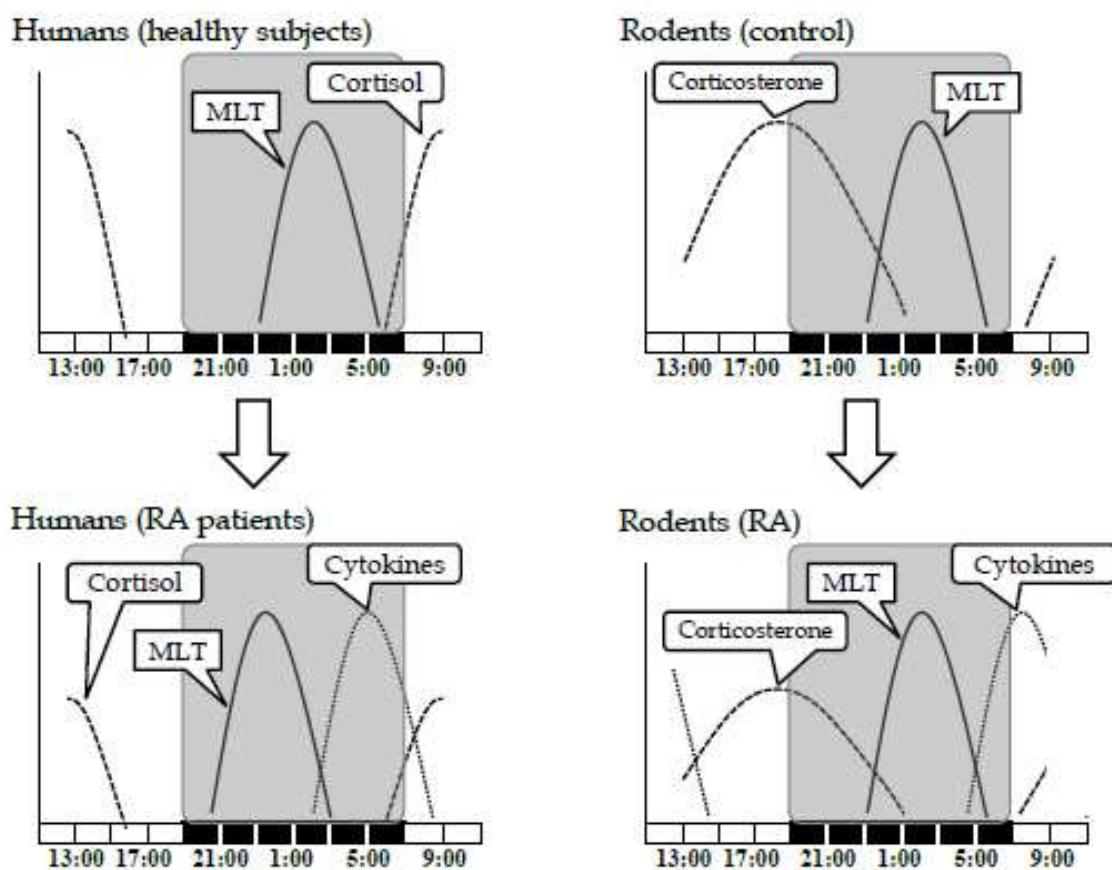


Fig. 8. Interaction of cytokines and the secretion of MLT and glucocorticoid

3. Chronotherapy for rheumatoid arthritis

In the treatment of RA, non-steroidal anti-inflammatory drugs (NSAID) are used to decrease pain; steroids are used to reduce pain and inflammation; and disease modifying antirheumatic drugs (DMARD) are used prior to the development of destructive changes in bones, joints, and organ tissues. In addition, biological DMARD can be used to target specific cytokines.

In this Chapter, I summarize the chronopharmacology and chronotherapies of these drugs.

3.1 NSAID and glucocorticoids

Arthritis develops in many RA patients. Thus, NSAID such as indomethacin are often used as analgesics. Levi et al. reported on the use of chronotherapy involving indomethacin for osteoarthritis (Levi et al., 1985). The patients with osteoarthritis took an indomethacin sustained-release (ISR) oral preparation once a day at 8:00, noon, or 20:00. The evening dosing protocol showed higher safety than the morning dosing protocol.

Glucocorticoids have been used in RA therapy to treat symptoms such as joint stiffness and joint pain. Generally, glucocorticoids are administered in the morning according to the circadian rhythm of endogenous glucocorticoids. De Silva et al. performed a double-blind cross-over study to determine the effect of the timing of prednisolone administration on morning stiffness (De Silva et al., 1984). The duration of morning stiffness was markedly shorter in the night (22:00- 23:00) dosing group than in the morning (6:00- 7:00) dosing group. Moreover, Arvidson et al. also reported that the 2:00-treated group displayed a markedly decreased duration of morning stiffness and joint pain and reduced serum IL-6 levels compared with the 7:30-treated group (Arvidson et al., 1997). From these results, it is thought that administering glucocorticoids at night is useful for the treatment of RA.

Interesting evidence has been reported for glucocorticoid chronotherapy in recent years. Buttgerit et al. developed a new modified-release formulation of prednisone that releases prednisone about 4 hours after ingestion (Buttgerit et al., 2008). When RA patients were randomly given a modified-release tablet at bedtime or an immediate-release prednisone tablet in the morning, the relative change in the duration of joint morning stiffness was significantly higher with the modified-release tablet than with the immediate-release tablet. Furthermore, administering the modified-release prednisone at night did not change the patients' adrenocortical function over 12 months (Alten et al., 2010). These results show that chronotherapy with a modified-release prednisone tablet is safe and effective as a treatment for RA therapy, even though it was thought that administering glucocorticoids at night would have negative effects upon the circadian rhythm of endogenous cortisol and reduce hypothalamic-pituitary-adrenal axis function. Therefore, chronotherapy using the modified-release prednisone tablet is considered to be a useful RA therapy.

3.2 Methotrexate

Methotrexate (MTX) is one of the most commonly used DMARD. It inhibits cytokine production by suppressing lymphocyte proliferation (Williams et al., 2001) and TNF- α transcriptional activity (Becker et al., 1998). MTX induces a high American College of Rheumatology improvement response rate (Choi et al., 2002), inhibits joint inflammation (Kremer et al., 1992; Weinblatt et al., 1992), and conveys a marked survival benefit (Choi et al., 2002) in RA patients although the exact mechanisms underlying its antirheumatic effects are not fully understood (Dolhain et al., 1998; Gerards et al., 2003). Currently, MTX is used as an anchor drug in RA therapy. However, MTX also causes adverse effects, such as myelosuppression and interstitial pneumonitis because it is an anticancer agent. Therefore, it is necessary to design a safe and effective dosing protocol for MTX treatment.

3.2.1 Basic study

In recent years, it has been stated that cytokines are an important factor in the pathogenesis of RA (Chu et al., 1991) and that the levels of proinflammatory cytokines are increased in RA patients. Blood cytokine levels show circadian rhythms in RA patients (Arvidson et al., 1997;

Sulli et al., A, 2002), and these rhythms correspond to those of morning stiffness. We consider that RA therapy associated with cytokine circadian rhythms might be more effective than the RA therapy used commonly in clinical practice.

In previous studies, we revealed that the circadian rhythms of SAA and TNF- α levels, which peak in the light phase, were maintained after RA onset in CIA and MRL/lpr mice (Fig. 6 and 9; To et al., 2009 and 2011). Based on the circadian rhythms of plasma SAA and TNF- α levels in MRL/lpr mice (Fig. 9), MTX was administered three times a week for 2 weeks at 1:00 or 13:00, when the TNF- α level begins to decrease and increase, respectively. The TNF- α levels did not differ between the control and 13:00-treated groups, although they were significantly lower in the 1:00-treated group than in the control group ($P < 0.05$). The SAA concentration in the 1:00-treated group was significantly lower than those in the control and 13:00-treated groups (vs. control: $P < 0.01$, vs. 13:00: $P < 0.05$; Fig. 9) (To et al., 2011).

In the CIA model, clear circadian rhythms in SAA and TNF- α levels were observed, with higher levels seen in the morning and lower levels observed at night (Fig. 6; To et al., 2009), and MTX was intraperitoneally injected at 5:00 or 17:00 every 7 days for 3 weeks. The 5:00-treated group displayed significantly reduced arthritis scores compared with the control and 17:00-treated groups ($P < 0.01$, respectively; Fig. 9). On the other hand, the arthritis scores of the 17:00-treated group did not differ from those of the control group for the entire study period in mice.

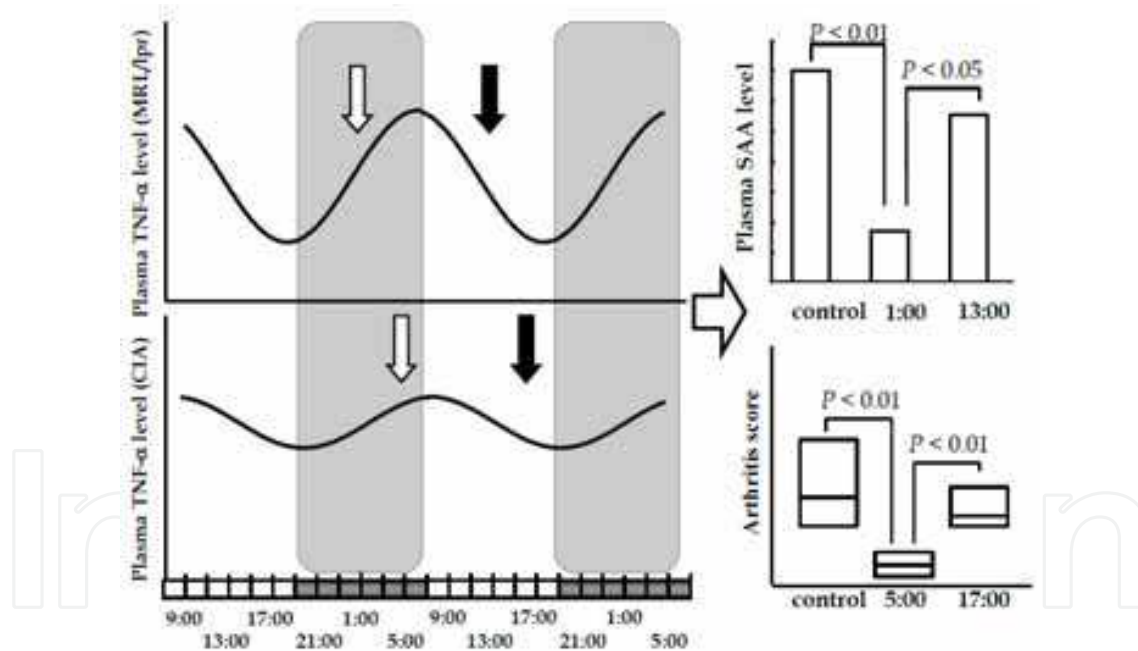


Fig. 9. Influence of MTX dosing time on antirheumatic effects

In these studies, the dosing time-dependent changes in the SAA level corresponded to identical changes in the TNF- α level. It is likely that the SAA level is reduced due to a decrease in the TNF- α concentration after MTX administration from midnight to early morning. In addition, arthritis and inflammation were reduced in the dark phase, when the plasma TNF- α concentration began to increase, in both the CIA model and MRL/lpr mice. These findings reveal that the therapeutic effects of MTX treatment can be improved by administering MTX when the serum TNF- α level begins to increase.

MTX causes myelosuppression and is used as an antitumor drug. It is reported that the toxicity caused by MTX varies significantly in mice depending on the dosing time (Ohdo et al., 1997). In our studies using CIA mice, the MTX dosing groups showed no significant decreases in their leukocyte counts compared with the control group. In RA therapy, MTX is used at very low doses compared with those used in cancer chemotherapy. Its chronotoxicity was studied using 400 mg/kg of MTX to estimate the toxicity of MTX as an antitumor drug (Ohdo et al., 1997). However, no adverse effects were observed in our studies because only 60 mg/kg of MTX was administered to mice. Thus, MTX may reduce plasma TNF- α levels by suppressing transcriptional activity rather than suppressing lymphocyte proliferation.

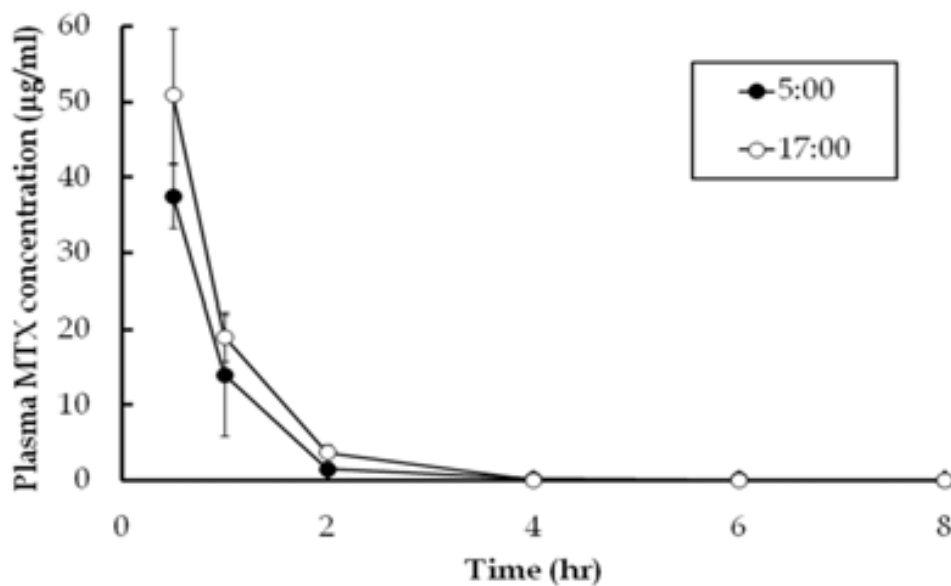


Fig. 10. Plasma concentration of MTX after drug administration at 5:00 or 17:00 (redrawn from the data of To et al., 2009)

To clarify the mechanism underlying dosing time dependency, we investigated the influence of dosing time on the pharmacokinetics of MTX. MTX is largely excreted in urine. Both renal blood flow and the glomerular filtration rate have been found to follow circadian rhythms, with a peak during the active period in animals. The MTX concentration was expected to be higher in the 17:00-treated group than in the 5:00-treated group since MTX was administered during the inactive period. In our study, the plasma MTX concentrations at 0.5 and 2 hour after MTX injection in the 17:00 group were significantly higher than those in the 5:00 group (0.5 hour: $P < 0.05$, 2 hour: $P < 0.01$; Fig. 10). On the other hand, the 5:00 group showed significantly higher plasma MTX levels than the 17:00 group at 4, 6, and 8 hour (4 hour: $P < 0.01$, 6 hour: $P < 0.01$, 8 hour: $P < 0.05$; Fig. 10). However, the area under the plasma-time concentration curve (AUC) was 23,622 $\mu\text{g}/\text{ml}/\text{hr}$ at 5:00 and 32,305 $\mu\text{g}/\text{ml}/\text{hr}$ at 17:00. The AUC in the 17:00-treated group, which showed no decrease in the arthritis score, was 1.28-fold higher than that in the 5:00-treated group, which had a significantly arthritis reduced score. Thus, no relationship was detected between the concentration of MTX and its efficacy. It was reported in previous studies that there were no dosing time-dependent changes in MTX pharmacokinetics in patients with cancer (Balis et al., 1989; Robinson et al., 1989). Moreover, no difference was noted in MTX

pharmacokinetics according to the injection time when MTX was administered to RA patients intramuscularly at 10:00 or 18:00 (Carpentier et al., 1998).

From the results of our studies and the circadian cycles of cytokines in RA patients, it is thought that MTX has a significant dosing-time-dependent anti-inflammatory action and that this effect might be due to the circadian rhythms of cytokine levels rather than the pharmacokinetics of MTX.

It was shown that there were daily variations in the plasma SAA and TNF- α concentrations in CIA and MRL/lpr mice after the onset of RA and that their arthritis score and SAA and TNF- α levels were relieved after the administration of MTX at specific times in synchronization with the circadian rhythm of TNF- α . Therefore, we consider that choosing an optimal dosing time associated with the circadian rhythm of RA symptoms could lead to effective MTX treatment for RA.

3.2.2 Clinical study

From our studies in RA model animals, we anticipated that higher therapeutic effects could be achieved with chronotherapy compared with the current treatment schedule, in which MTX is administered during the night. In RA patients, pain and stiffness are accentuated in the early morning, and proinflammatory cytokines show circadian rhythms that peak from midnight to early morning (Crofford et al., 1997; Perry et al., 2009). Based on our findings in animal studies and the circadian TNF- α rhythms of RA patients, we changed the dosing schedules of RA patients from the standard MTX schedule, in which MTX is administered three times a week (day 1: after breakfast and supper, day 2: after breakfast only), to a chronotherapy schedule, in which the dose and number of doses per week were not changed, but MTX was administered once a day after supper, to examine whether a dosing-time dependency of the therapeutic effects of MTX treatment could be detected in Japanese RA patients. The disease activity score (DAS) 28, modified health assessment questionnaire (MHAQ) score, and adverse effects were assessed. DAS28 is a composite score based on tender and swollen joint counts (28 joints), the patient's global assessment of their disease activity (100 mm Visual Analog Scale (VAS): 0 = no activity, 100 = extreme activity), and the CRP level. DAS28 values were calculated as follows:

$$\text{DAS28 (CRP)} = 0.56 \times \sqrt{(\text{TJC28})} + 0.28 \times \sqrt{(\text{SJC28})} + 0.014 \times \text{GH} + 0.36 \times \ln(\text{CRP}+1) + 0.96,$$

where TJC = tender joint count, SJC = swollen joint count, GH = general health, and CRP = CRP level in mg/l. A score of ≥ 4.1 indicates high disease activity, a score > 2.7 and ≤ 4.1 indicates moderate disease activity, a score < 2.7 indicates low disease activity, and a score < 2.3 indicates disease remission (van Gestel et al., 1996). The European league against rheumatism (EULAR) response states were classified as follows: good responders were patients displaying an improvement of > 1.2 and a present score of ≤ 2.7 ; moderate responders were patients displaying an improvement of > 0.6 and ≤ 1.2 and a present score of ≤ 4.1 , or an improvement of > 1.2 and a present score of > 4.1 ; and non-responders were any patients displaying an improvement of ≤ 0.6 or patients displaying an improvement of > 0.6 and ≤ 1.2 and a present score of > 4.1 (Fransen et al., 2005).

The chronotherapy administered MTX after supper proved highly effective despite the dose and the number of doses remaining the same, although many patients did not derive a therapeutic benefit from the therapy. As the half-life of MTX is about 2 hours, the MTX had disappeared from the plasma by 10 hours after its administration. Therefore, it was thought

that the plasma concentration produced by administering MTX after supper was insufficient. We therefore administered MTX before bedtime once a day to a patient in whom MTX was ineffective in the previous study. Consequently, the DAS28 of the patient decreased from 5.45 (4 months) to 3.25 (7 months) over the study (moderate response) (Fig.11). From this result, it was considered that a higher therapeutic effect can be achieved by administering MTX before bedtime compared with after supper.

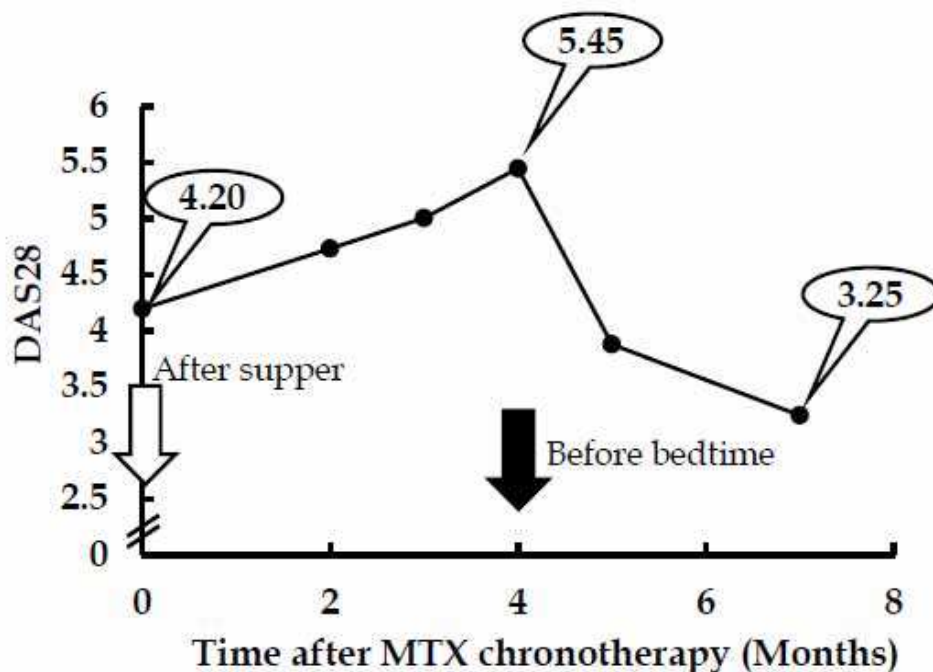


Fig. 11. Influence of MTX chronotherapy on DAS28 score (case data)

We studied the effects of MTX chronotherapy before bedtime, and the dose and the number of doses per week were not changed. Twenty-two rheumatoid arthritis patients between 41 and 78 years of age were enrolled, and 77% received MTX chronotherapy for the entire 3 months of the study. Fig. 12 shows the change in DAS28 in a female RA patient to whom MTX was administered at bedtime. After the start of the chronotherapy, her DAS28 value was markedly decreased by 1.5 at only one month, and the effect was maintained throughout the 3 months of the study. In particular, all of her joint swelling disappeared, and the tenderness in many of her joints was relieved after the chronotherapy.

The chronotherapy improved the DAS28 score in 14 of 17 patients (82.4%), and the mean DAS28 value was significantly decreased by 0.460 at one month, 0.506 at two months, and 0.521 at three months after the start of the chronotherapy ($P < 0.05$ and $P < 0.01$, respectively) (Fig. 13; To et al., 2011). In particular, despite the dose and the number of doses remaining the same, 23.5% of patients attained clinical remission. DAS28 is calculated from the following 4 parameters: the tender and swollen joint counts, the patient's global assessment of disease activity, and the CRP concentration. The median tender joint count changed little throughout the study. On the other hand, the swollen joint count was

markedly decreased in all patients after 3 months chronotherapy. The CRP level continued to improve throughout the 3 month study period and had improved by 64.2% after the chronotherapy compared with the baseline. The patient's global assessment of disease activity is susceptible to patient bias because each patient evaluates their own degree of illness. In this study, the patients understood that the method of MTX administration had changed. The patient's global assessment of disease activity did not show definite changes, even though a placebo effect of the chronotherapy was anticipated. Therefore, it was considered that the placebo effect did not contribute to the observed significant decrease in the DAS28 score.

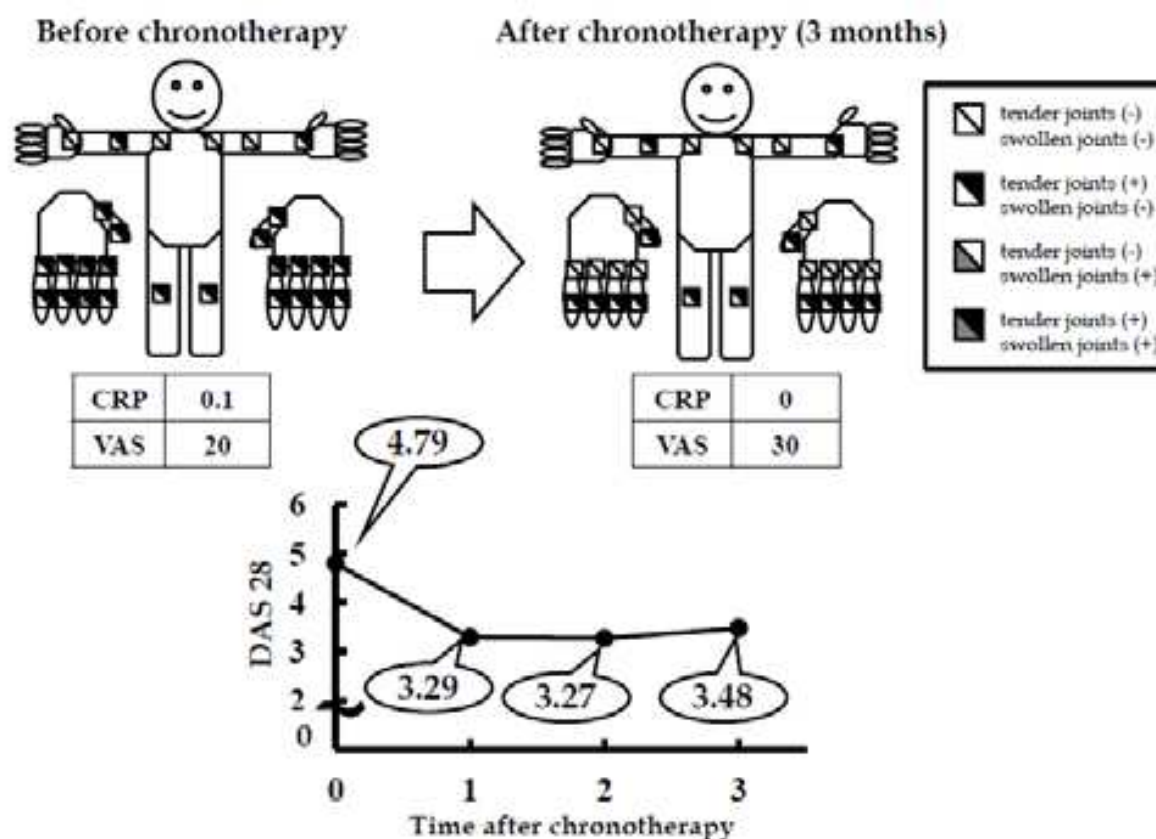


Fig. 12. Influence of MTX chronotherapy (before bedtime) on DAS28 score (case data)

The MHAQ is used to estimate the functional capacity of RA patients. It assesses the ability of patients to perform daily activities using eight questions. The final MHAQ score is the mean score of the eight questions and ranges from 0 to 3, with higher levels reflecting greater disability (Pincus et al., 1983). It was revealed that chronotherapy improved the functional capacity of the RA patients although we could not clarify the factors responsible for the improvement in the MHAQ score from the data obtained in this study. Almost all patients had mild leukopenia although the incidence of leukopenia higher than Grade 1 increased from 11.8% to 23.5% throughout the study. Moreover, there were no severe adverse effects in 17 patients.

From these results, it was demonstrated that MTX chronotherapy is safe and markedly improves the disease activity and functional capacity of RA patients.

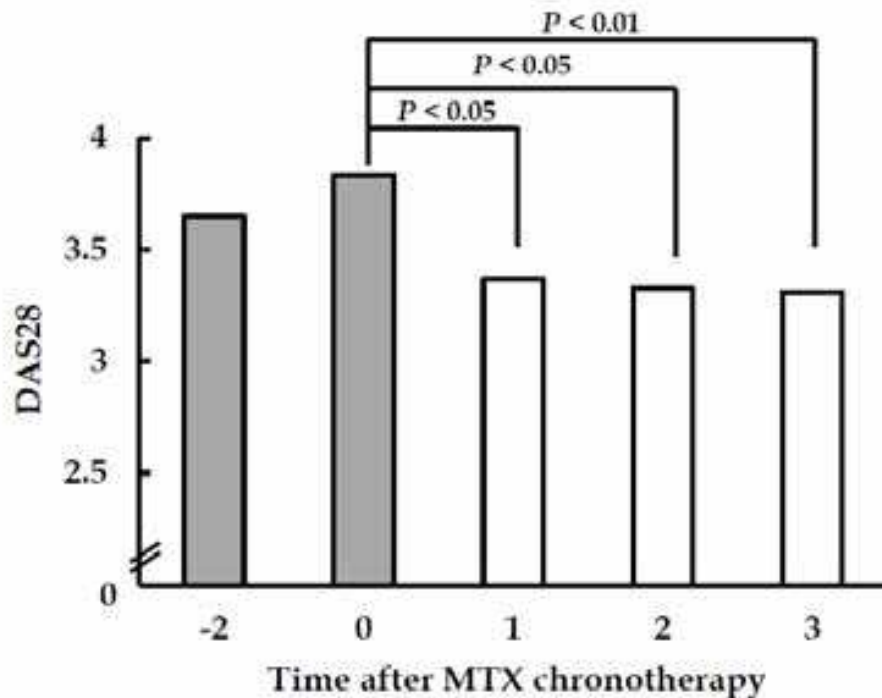


Fig. 13. Efficacy of 3 months MTX treatment in Japanese RA patients (redrawn from the data of To et al., 2011)

4. Conclusion

RA is an autoimmune disorder of unknown etiology, and morning stiffness is a well-known characteristic of the condition. Inflammatory responses show circadian rhythms in RA patients, and these rhythms correspond to that of morning stiffness. Moreover, it is considered that cytokine rhythms contribute to the rhythm of inflammatory responses since the circadian rhythms of inflammatory responses and cytokines are similar. The symptoms of RA such as disease activity and the patients' functional capacity, arthritis, pain, etc. were relieved after the administration of anti-antirheumatic drugs such as MTX, steroids, NSAID, etc. at specific times in synchronization with the circadian rhythm of cytokines and the inflammatory response. Choosing an optimal dosing time that is associated with the circadian rhythms of RA symptoms is, therefore, expected to lead to more effective medical therapy for RA (Fig. 14).

Presently, we are performing basic and clinical studies to prove the utility of chronotherapy involving various anti-antirheumatic drugs. From these studies, we hope to be able to propose safe and effective RA therapies. Furthermore, the mechanism regulating the generation of circadian rhythms in RA is being studied. It is expected that new drugs targeting RA will be discovered and that new RA therapies that regulate the abovementioned circadian rhythms will be developed once the mechanism responsible for their generation has been clarified.

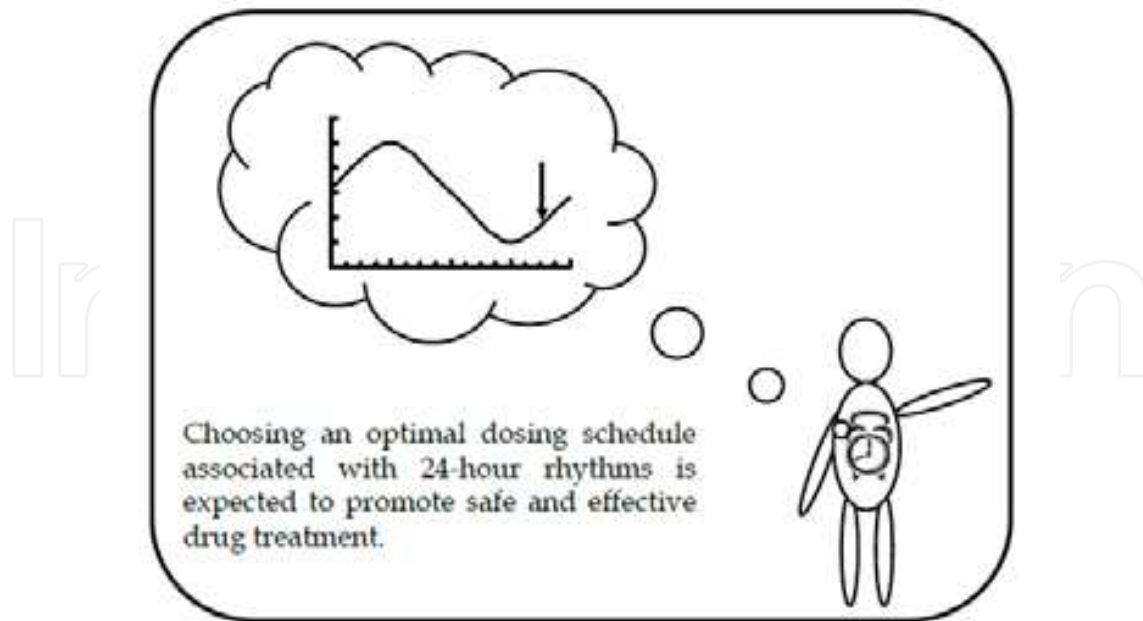


Fig. 14. Concept of chronotherapy

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Autoimmune disorders are caused due to break down of the immune system, which consequently fails in its ability to differentiate "self" from "non-self" in the context of immunology. The diseases are intriguing, both clinically and immunologically, for their diversified clinical phenotypes and complex underlying immunological mechanisms. This book offers cutting-edge information on some of the specific autoimmune disease phenotypes, respective diagnostic and prognostic measures, classical and new therapeutic options currently available, pathogenesis and underlying mechanisms potentially involved, and beyond. In the form of Open Access, such information is made freely available to clinicians, basic scientists and many others who will be interested regarding current advances in the areas. Its potential readers will find many of the chapters containing in-depth analysis, interesting discussions and various thought-provoking novel ideas.

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Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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