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Circadian rhythm of serum cytidine deaminase in patients with rheumatoid arthritis during rest and exercise

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SUMMARY Circadian rhythm of serum cytidine deaminase and C reactive protein was assessed in 11 inpatients with rheumatoid arthritis who were crossed between 24 hours of bed rest and 24 hours of normal ward activity. Blood was taken at six hourly intervals and the results analysed by fitting sine waves with an assumed period of 24 hours to the measured concentrations. Cytidine deaminase after activity, but not at rest, showed circadian variation, with a 24 hour mean level of 17·4 units (normal 3–13 units) and an amplitude of 1·1 units. The circadian variation, defined as the curve's peak to trough difference as a percentage of the 24 hour mean, was 12·3% and occurred at 1208 hours. C reactive protein showed no significant circadian rhythm, in keeping with published findings. The timing of the peak in serum cytidine deaminase concentrations after a period of morning physiotherapy, but not during the bedrest morning, suggests that exercise accounts for the circadian rhythm, probably by increasing the lymphatic clearance from inflamed joints.

Key words: chronobiology, diurnal variation, nucleoside aminohydralase, joint inflammation.

It is not known why clinical measures of disease activity in rheumatoid arthritis (RA), such as grip strength, pain, stiffness, and articular index, show greatest activity in the early hours of the morning.[1 2] Similar changes in blood total protein count, α_1-antitrypsin, orosomucoid, caeruloplasmin, and transferrin[2 3] suggest that there is diurnal variation of inflammatory markers, possibly related to circulating cortisol concentrations, though similar changes in C reactive protein (CRP), plasma viscosity, serum sulphydryl, and serum histidine have not been demonstrated.[2–4] Other factors may be important—for example, diurnal variation in the erythrocyte sedimentation rate can be explained by food ingestion.[5]

Cytidine deaminase is a cytoplasmic enzyme found in high concentrations in polymorphs. It is released from dead and damaged polymorphs in inflamed rheumatoid joints and drains down its concentration gradient from synovial fluid to the blood.[6] The serum concentration of cytidine deaminase reflects input from all inflamed joints. It is an integrated measure of joint inflammation that has been shown to correlate with existing measures of joint inflammation[7] and to reflect longitudinal changes in a patient's condition.[8]

This paper reports a study that was designed to assess circadian variation in serum cytidine deaminase in a group of patients with RA studied at rest and during activity. CRP was also measured as a control marker of inflammation known not to show circadian rhythm.[2]

Patients and methods

With ethical committee approval and written informed consent, eight female and three male inpatients of mean age 62 years (range 40–71) with

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definite or classical RA[9] volunteered for the study. Patients with infections or recent changes in treatment were excluded. Patients were randomly allocated to 24 hours' bedrest or normal ward activities on the first day, crossing to the other regimen for the second day. Blood was taken at 0600, 1200, 1800, and 2400 on each day. During the rest day the patients stayed in bed but were allowed up to the toilet. During the activity day the patients were encouraged to get up after the 0600 venepuncture and attended physiotherapy during the morning.

Clotted blood samples were centrifuged at 1000 *g* for 10 minutes. One aliquot of serum was stored at −20°C for cytidine deaminase estimation (carried out within one month of collection) and one aliquot at 4°C for CRP estimation. Cytidine deaminase was assayed by the method of Jones *et al*[10] and CRP by nephelometry.[11]

Data or data transformations (as logs and percentages of the 24 hour mean) were assessed by analysis of variance[12] and by fitting sine curves with an assumed period of 24 hours by least squares regression techniques.[13] The amplitude of the sine waves so derived was tested against zero—that is, with the null hypothesis that the data did not vary significantly with time of day. The interday differences were compared by Student's *t* test.

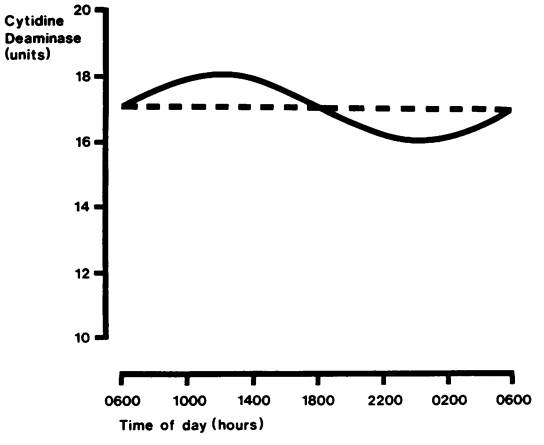

Fig. 1 *Circadian rhythmicity of cytidine deaminase in 11 diurnally active patients with rheumatoid arthritis.*

Results

There was no significant circadian variation of CRP concentrations. Cytidine deaminase values after activity, but not at rest, showed circadian rhythm ($p=0{\cdot}048$) (Fig. 1) with a mesor (24 hour mean level) of 17·4 units and an amplitude of 1·1 units. The circadian variation, defined as the fitted curve's acrophase (peak) to bathyphase (trough) difference as a percentage of the 24 hour mean, was 12·3%. The acrophase occurred at 1208 and the bathyphase at 0008.

There were no statistically significant interday differences for CRP (Table 1). The serum cytidine deaminase concentration was higher at 1200 on the exercise day than on the rest day ($p=0{\cdot}048$), but there were no other significant differences (Table 1).

Discussion

The results suggest that serum cytidine deaminase concentrations follow a circadian rhythm in ambulatory hospital inpatients with RA but not in patients resting in bed, whereas CRP shows no such variation, in keeping with published findings.[2 3]

A common pattern of change in human biological systems is a cycle repeated every 24 hours. One way of studying such circadian rhythms is by fitting a sine wave. The technique is useful in supplying the time of the maximum and minimum values, allowing comparison between rhythms, but may artificially reduce the amplitude of the variation and exclude other potential patterns. These possible short-

Table 1 *Mean and standard deviation (SD) concentrations of cytidine deaminase and C reactive protein in 11 inpatients with rheumatoid arthritis during 24 hours bed rest or 24 hours of normal ward activity*

Time	*C reactive protein (mg/l)**				*Cytidine deaminase (units)†*			
	Rest		*Exercise*		*Rest*		*Exercise*	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
0600	4·4	3·6	5·0	4·1	15·1	8·3	17·0	13·1
1200	4·4	3·7	5·5	4·9	14·5†	6·9	18·2	10·5
1800	4·5	4·0	5·5	4·7	15·7	6·6	17·1	8·9
2400	4·6	4·0	5·4	4·5	16·8	8·5	16·1	7·4

*C reactive protein—normal range 0·8–8·0 mg/l; cytidine deaminase—normal range 3–13 units.
†$p<0{\cdot}05$ (paired *t* test) compared with the exercise day.

comings should be borne in mind when the interpretation of any chronobiological study is considered.[14]

Most workers attribute circadian rhythms in clinical and serological measures of inflammation to a cyclical variation in circulating cortisol concentrations that peak early in the morning. It has been suggested that the morning exacerbation of symptoms experienced by many patients with RA is related to these changes,[15] though the definitive criteria for proof of a causal relation have never been met.[16] The results presented here, however, suggest that exercise is an important factor in the diurnal variation of serum cytidine deaminase. In patients with RA there is a steep gradient between inflamed synovial fluid cytidine deaminase and serum cytidine deaminase (up to 20:1),[6] suggesting that cytidine deaminase originates from joints and drains into the blood. Small proteins, such as cytidine deaminase, are cleared from joints via the synovial lymphatic system, and the lymphatic flow is powered mainly by joint motion.[17] It follows that patients with RA would be expected to have higher concentrations of serum cytidine deaminase after exercise than while resting. The inpatients studied underwent a period of moderately vigorous physiotherapy between 0830 and 1000 but spent the rest of the day according to the ward routine, which involves little ambulatory activity. The peak in serum cytidine deaminase concentrations around noon, and the lack of circadian variation during either day for CRP, which originates from the liver and is therefore unaffected by joint motion, is in keeping with this hypothesis.

The number of circulating polymorphs shows a circadian rhythm with a peak in the early evening in resting patients with RA[2] as well as in normal controls.[14] The pattern of cytidine deaminase variation shown here does not reflect this cycle, suggesting that there is no exercise related alteration in the numbers of blood polymorphs undergoing lysis.

Although statistically significant, the cyclical differences in serum cytidine deaminase were small (mean difference between acrophase and bathyphase 2·2 units). In RA, in which serum cytidine deaminase concentrations up to 50 units (normal 3–13 units) are possible, these differences are unlikely to be clinically important. Workers studying small difference in serum cytidine deaminase concentrations, such as those produced by the withdrawal of non-steroidal anti-inflammatory drugs,[8] should, however, endeavour to collect samples under controlled conditions.

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