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CIRCADIAN VARIATIONS IN THE SIGNS AND SYMPTOMS OF RHEUMATOID ARTHRITIS AND IN THE THERAPEUTIC EFFECTIVENESS OF FLURBIPROFEN AT DIFFERENT TIMES OF DAY

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1 Seventeen patients with rheumatoid arthritis were studied in a double-blind crossover trial contrasting three different times of administration of twice-daily flurbiprofen.

2 Twelve of these patients were also studied when taking the same dose of flurbiprofen as a split dose four times a day.

3 Symptoms and signs of the disease were self-assessed throughout the day for several days on each regimen and the information was analysed for rhythmicity.

4 Twice a day flurbiprofen may be more effective than four times daily flurbiprofen, and the regimen without an evening dose was the least effective of three twice-daily treatments tested.

5 Circadian rhythms of grip strength and finger joint size were demonstrated, and were similar on all treatment regimens.

6 These rhythms have a similar pattern to those detected during studies of immune responses, and it is suggested that morning stiffness in rheumatoid arthritis is not only the result of nocturnal inactivity, and may respond to appropriately timed medication given to decrease inflammation or to suppress other aspects of the immune response.

Introduction

There is much evidence that the toxicity, pharmacokinetic properties and therapeutic effectiveness of many drugs vary with the time of day of administration, often due to changes in the body's physiological state at different times of day (Haus, Halberg, Kuhl & Lakatua, 1974; Scheving, Von-Mayersbach & Pauly, 1974; Reinberg, 1976). Dubois & Adler (1963) found that a single morning dose of glucocorticoid was as effective as larger divided doses in controlling rheumatoid symptoms. Inflammatory processes in man and in laboratory animals vary in intensity throughout the day, and reflect circadian variations in cellular components of immunity (Bartter, Delea & Halberg, 1962; Abo, Kawate, Itoh & Kumagai, 1979; Abo & Kumagai, 1978; Szabo, Kovats & Halberg, 1978; Williams, Kraus, Dubey, Yunis & Halberg, 1979). Carrageenin-induced oedema of the rat's paw is a commonly used model of inflammation for evaluation of anti-inflammatory agents, and shows circadian variations in the magnitude of swelling and also in the percent reduction of this swelling by indomethacin (Labrecque, Dore, Laperriere, Perusse & Belanger, 1978; Labrecque, Laperriere, Perusse & Belanger, 1978). Indomethacin (3 mg/kg) reduced the oedema by 39.7% at 08.00 h but was ineffective at 20.00 h. We (Pownall & Knapp, 1980) have also shown that 1 mg/kg flurbiprofen reduced the carrageenin-induced oedema of the rat paw compared to vehicle-treated animals by between 209% and 67% depending on the time of testing. Using methylprednisolone to suppress the cell-mediated immune response to the chemical sensitiser, oxazolone, Kabler, Knapp & Pownall (1978) & Pownall & Knapp (1979) have shown that at 10.00 h the steroid is immunosuppressive whereas at 22.00 h it is not. The immune response in untreated rats varies in magnitude by over 100% depending on the time of challenge (Pownall & Knapp, 1978). In order to evaluate properly the therapeutic effectiveness of a drug, the circadian variations in the pathological processes, and in the efficacy of the drug on these processes should first be investigated.

In man, maximal immune responses are often seen in the night and early morning hours. The delayed hypersensitivity reaction to purified protein derivative of tuberculin is greater when tested at 07.00 h than at 22.00 h (Cove-Smith, Kabler, Pownall & Knapp, 1978), which correlates well with the known rhythmicity of T-cells, macrophages and monocytes on which the response depends. Immediate-type hypersensitivity reactions to house dust and grass pollens are most intense at 23.00 h when the skin is

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maximally sensitive to histamine-mediators (Lee, Smolensky, Leach & McGovern, 1977) and when B-cells are most numerous (Abo & Kumagai, 1978).

Morning stiffness in rheumatoid arthritis is well known and differences in grip strength and joint size measured at different times of day have been reported. Wright (1959) and Wright & Dowson (1969) described changes in grip strength in patients with rheumatoid arthritis and in healthy persons, over 24 h, with minimim grip in the early hours of the morning. Other workers have found that grip strength of patients with rheumatoid arthritis increased during the day but that the differences were not significant (Boardman & Hart, 1967; Lee, Baxter, Dick & Webb, 1974), probably because the mean grip of groups of patients were compared rather than considering each as an individual.

Heyman (1974) found that the finger joint size of normal persons was larger in the morning than the evening. Hand volume apparently varies during the day (Wright & Dowson, 1969), but the authors gave no details.

Circadian variations in the symptoms of arthritis may be partly a result of rhythmic changes in the vigour of immune processes and in synovial inflammation.

Current work with the oxazolone model in rats suggests that the rhythmicity of adrenopituitary production of corticosteroids might be a modulating influence on the intensity of the immune response, and time-dependent variations in their effect on adrenopituitary function are now well known. The effect of the timing of administration of steroidal and non-steroidal anti-inflammatory drugs on therapeutic effectiveness and toxicity is of potential importance and a chronotherapeutic study of flurbiprofen has been carried out in patients with rheumatoid arthritis. Preliminary observations on five patients have been published (Kowanko, Swannell, Mahoney, Pownall & Knapp, 1980).

Methods

Patient selection

Patients with rheumatoid arthritis, who had active synovial swelling of the small joints of the hands, were considered for the study. They were either in the early stages of the disease or were stable on gold or penicillamine treatment. Those taking corticosteroids were excluded. The patients were all taking, or about to start taking, 200 mg flurbiprofen daily for their arthritis. The usual contra-indications for flurbiprofen were observed and patients were of either sex and any age. During the trial the use of other anti-inflammatory drugs such as indomethacin and aspirin was forbidden. Extra analgesia was provided by paracetamol if necessary. The patients also had to be capable of using simple measuring equipment at home.

Study design

Each patient was aware that there was not to be a change in treatment, only a change in timing of treatment. They were each studied on four occasions: during a run-in period and during each of the three twice-daily treatment periods which differed in the time of administration of two 100 mg doses of flurbiprofen. During the run-in period patients were studied while taking their usual non-steroidal anti-inflammatory drugs according to regimens chosen arbitrarily by themselves or their doctors. Twelve of the patients were taking 50 mg flurbiprofen four times a day during the run-in period. For the three twice-daily treatment periods patients were issued with three bottles of tablets, A, B and C, and were instructed to take two tablets from A at 08.00 h, two of B at 13.00 h and two of C at 23.00 h. Sufficient tablets were supplied for 14 days. One of the bottles contained placebo tablets identical in all outward appearances to the active 50 mg flurbiprofen tablets contained in the other two bottles. Each patient was studied on each of the three possible twice-daily treatment periods (Table 1). In this way each patient acted as his own control. The order of these test periods was randomised for each patient, and the trial was conducted double-blind.

Data collection

Patients were given careful instructions in the use of the equipment and practised under supervision. Measurements were made and recorded by the patients at home.

Measurements of finger joint size and grip strength were made at 08.00 h, 11.00 h, 13.00 h, 15.00 h, 20.00 h and 23.00 h for two or three consecutive days, during the run-in period and during the second week of each treatment period.

Interphalangeal joint circumference was measured using a plastic loop connected to a spring-loaded pointer moving on a scale graduated in 1 mm steps.

Table 1 Twice daily treatment regimens

Period	*08.00 h*	*13.00 h*	*23.00 h*
FFP	Flurbiprofen 100 mg	Flurbiprofen 100 mg	Placebo
FPF	Flurbiprofen 100 mg	Placebo	Flurbiprofen 100 mg
PFF	Placebo	Flurbiprofen 100 mg	Flurbiprofen 100 mg

Table 2 Analysis of variance of patients' assessments of grip strengths and finger joint sizes

Source of variation	*DF*	*Left grip strength* *MS*	*VR*	*P*	*DF*	*Right grip strength* *MS*	*VR*	*P*	*DF*	*Joint size* *MS*	*VR*	*P*
Between patients	15	164117	2079.7	<0.005	15	152653	1936.9	<0.005	16	125058	3940.3	<0.005
Within patients, between regimens	30	1018	12.9	<0.005	30	1081	13.7	<0.005	30	773	24.4	<0.005
Within patients, regimens												
between days	2	434	5.7	<0.005	2	116	2.0	N.S.	2	174	5.5	<0.005
between times within times	5	2929	37.1	<0.005	5	2611	33.1	<0.025	5	1282	40.4	<0.005
between days	10	68	0.9	NS	10	88	1.1	NS	10	16	0.5	NS
Residual	714	79			714	79			735	32		

DF = degrees of freedom; MS = mean square; VR = variance ratio; *P* = probability; NS = not significant

The proximal interphalangeal joint sizes of all the fingers and the distal interphalangeal joint size of both thumbs were measured with the digit extended.

Grip strength was measured using an inflatable grip test bag and a pressure gauge scaled in 2 mm Hg increments. The bag was inflated to 20 mm Hg and then, with the arm resting on a table, the bag was squeezed as hard as possible. The highest reading of three attempts was recorded for each hand.

The patients' self-assessments of finger joint size and grip strength were reproducible from day to day at the same time as tested by analysis of variance (Kowanko, Pownall & Knapp, in preparation). (Table 2).

Subjective assessments of overall stiffness and overall pain were made at the same times as the objective measurements by marking numbered scales from 0 (none) to 10 (most severe) according to the degree of stiffness or pain experienced at the time of assessment.

Paracetamol tablets were provided for extra pain relief where required. The date, time and number of paracetamol tablets taken was recorded.

Blood samples were taken at the end of the run-in period and each of the three test regimens for liver function tests, blood cell counts and erythrocyte sedimentation rate estimations.

Data analysis

The data were analysed by standard statistical techniques (Snedecor & Cochran, 1967) and also by the single and group mean cosinor analyses developed by Halberg, Tong & Johnson (1967). These allow variations in the observed data during the course of the day to be related mathematically to clock time. Essentially the technique involves fitting a sine curve, in these studies with a period of 24 h, to the observed data. Three parameters from this form of analysis can then be used to compare drug regimens. They are the mean level of the sine wave, the amplitude (half the peak-trough difference) and the timing of the peak of the sine curve.

Results

Nineteen patients were recruited into the trial. There were three withdrawals due to gastro-intestinal intolerance and one withdrawal due to a flare-up in the arthritis symptoms. Two of these patients withdrew after completing half the trial and their results are included in the analyses, making a total of 17 patients analysed. Twelve of these were taking 50 mg flurbiprofen four times a day and gold during the run-in period, five were taking other non-steroidal anti-inflammatory drugs. One patient's grip was too strong for the grip meters.

Table 3 Matrix of probabilities of significant differences in mean grip strength between different study periods using Wilcoxon's paired test (NS, $P > 0.05$)

Study periods	*Run-in (all patients)*	*Run-in (patients taking flurbiprofen four times a day*	*FPP*	*FPF*	*PFF*
FFP	$P < 0.05$	$P < 0.005$			
FPF	$P < 0.05$	$P < 0.005$	NS		
PFF	$P < 0.01$	$P < 0.001$	NS	NS	

Blood tests

No significant differences between treatment regimens was noted in any of the blood tests performed. Erythrocyte sedimentation rate varied greatly but no trends were apparent.

Grip strength

The mean grip strength for each hand of each patient was calculated for the run-in period and each of the test periods. Wilcoxon's two-tailed test for paired measurements was used to compare regimens. There were no significant differences between the twice daily treatment periods. Highly significant differences were found between the run-in period and each of the twice daily regimens. When the sub-group of 12 patients who took flurbiprofen four times a day during the run-in period was analysed, the differences were even more significant. Table 3 shows the *P*-values obtained from paired tests between mean grip strengths in different study periods.

Circadian variations in grip strength at the measurement times daily were readily apparent. Each two or three day measurement period was therefore considered as a sine wave with a 24 h periodicity.

Significant ($P < 0.05$) circadian rhythms in grip strength were calculated in 54% of the data sets, 68% had sine waves fitted with $P < 0.10$.

Paired non-parametic tests between these sine waves did not reveal any differences in amplitude or time of peak grip strength.

Group mean cosinor techniques (Halberg *et al.*, 1967) were applied to the parameters derived from the single sine waves. All the group cosinors were significant with $P < 0.05$, but no differences in amplitude or time of peak grip strength between regimens were noted. The mean level of the run-in group was lower than the levels of the three twice daily regimens, which is a reflection of the lower mean grip strength in the run-in group.

Table 4 shows the group grip strength data for the left and right hands.

Finger joint sizes

The patients recorded measurements for each of the ten interphalangeal joints. The sum of ten joints was used in analysing the results. The mean sum of joints for each patient during the run-in and each of the test periods was calculated. Wilcoxon's paired tests were used to compare the study periods. Table 5 shows that no significant differences in mean sum of joints were

Table 4 Group mean cosinor results for grip strengths

Study period	*Run-in (all patients)*	*FFP*	*FPF*	*PFF*
Number in group	Left 16	15	16	15
	Right 16	15	16	15
Amplitude (95% confidence limits)	Left 7.7(1.1,15.3)	7.7(3.3,12.5)	7.2(3.3,11.1)	6.3(2.0,10.7)
	Right 5.8(1.4,10.3)	7.7(3.6,12.4)	7.8(3.4,12.0)	5.5(2.4,8.8)
Clock time of peak grip (95% confidence limits)	Left $17^{20}(11^{46},18^{32})$	$18^{05}(15^{31},19^{24})$	$16^{52}(14^{55},18^{08})$	$17^{19}(15^{47},19^{15})$
	Right $16^{37}(13^{01},19^{02})$	$17^{29}(14^{59},18^{40})$	$16^{34}(15^{26},17^{47})$	$16^{35}(13^{59},19^{05})$
Mean level (mm Hg)	Left 117	130	125	131
	Right 118	130	125	130

Table 5 Matrix of probabilities of significant differences between mean sum of ten joints in different study periods (NS, $P > 0.05$)

Study periods	*Run-in (all patients)*	*Run-in (patients taking flurbiprofen four times daily)*	*FFP*	*FPF*	*PFF*
FFP	$P < 0.01$	$P < 0.05$			
FPF	$P < 0.01$	$P < 0.05$	NS		
PFF	$P < 0.01$	$P < 0.01$	NS	NS	

observed between the twice daily regimens, but that the run-in period differed significantly from all the regimens.

Most patients did not have synovial swelling in all ten finger joints. Affected finger joints were determined by clinical examination. It was thought that the sum of affected joints might be a more sensitive index than the sum of ten joints. Mean sums of affected joints were calculated and statistical tests confirmed the results obtained using the sum of ten joints.

Sine waves were fitted to the sum of joints data, and 39% were significant at $P < 0.05$, 49% fitted with a $P < 0.10$. Paired non-parametric tests between the study periods did not reveal any changes in amplitude or time of peak of the rhythms. Group mean cosinor analysis confirmed that no differences existed between the regimens. The results are shown in Table 6.

Forty-seven percent of sine waves fitted to the sum of affected joints data were significant at $P < 0.05$, 53% at $P < 0.10$, comparatively more than for the sum of ten joints. However, no significant differences in amplitude or time of peak were shown using paired tests between individuals on different treatment regimens. Group mean cosinor results using the sum of affected joints data confirmed that there were no differences in joint sizes between the regimens.

Extra analgesia

The number of paracetamol tablets taken by patients for pain relief was recorded. Four patients did not take any. There was no significant difference between the number of tablets taken during the three regimens.

Subjective pain and stiffness

Patients did not have access to their estimates of subjective pain or stiffness from one treatment regimen to the next. In retrospect the data should have been available to make it easier for patients to make comparisons between regimens. However, the patient's assessment of discomfort is probably one of the more important indices of drug effectiveness. Paired non-parametric tests between estimates of pain and stiffness at 08.00 h were performed and the results are shown in Table 7. Patients experienced most pain and stiffness during the FFP regimen.

Tests for trends with time

Paired tests were performed between the run-in and the first, second and third fortnights of the trial, regardless of the timing of flurbiprofen administration, in order to test for trends from the beginning to the end of the trial. Table 8 shows the results for the mean grip strength and mean sum of ten joints. It is apparent that grip strength improved as the trial progressed, and that joint size decreased significantly after the run-in period.

Table 6 Group mean cosinor results for sum of ten finger joints

Study periods	*Run-in (all patients)*	*FFP*	*FPF*	*PFF*
Number in group	16	15	16	14
Amplitude (95% confidence limits)	4.2(1.2,7.3)	4.4(1.4,7.4)	4.7(1.6,7.8)	4.1(1.3,6.9)
Clock time of peak joint sizes (95% confidence limits)	$06^{49}(04^{46},09^{45})$	$06^{45}(02^{39},07^{32})$	$06^{17}(04^{44},08^{42})$	$06^{54}(04^{07},07^{24})$
Mean level (mm)	595	595	584	593

Table 7 Matrix of probabilities of significant differences in subjective pain and stiffness ratings at 08.00 h between study periods (NS, $P > 0.05$)

Study periods		*Run-in (all patients)*	*Run-in (Patients taking flurbiprofen four times a day*	*FFP*	*FPF*	*PFF*
FFP	pain	$P < 0.05$	$P < 0.05$			
	stiffness	NS	NS			
FPF	pain	NS	$P < 0.05$	NS		
	stiffness	NS	$P < 0.05$	$P < 0.01$		
PFF	pain	NS	NS	$P < 0.05$	NS	
	stiffness	$P < 0.05$	$P < 0.01$	$P < 0.01$	NS	

Discussion

Subjective assessment by the patient of pain and stiffness indicates that the regime FFP (flurbiprofen at 08.00 h and 13.00 h) was the least effective of the three twice a day regimens studied. The objective measures used do not show any differences in therapeutic effectiveness between the three twice a day flurbiprofen regimens. The least successful regimen FFP has the longest time interval between doses—19 h overnight. Symptoms are usually worse in the morning and the analysis by sine wave shows that grip strength is minimal and joint size maximal around 06.00 h. It is reasonable to suppose that an evening dose of anti-inflammatory drug is helpful in controlling the severe morning symptoms, and is missed when not provided. It is of interest that missing either of the other doses has no measurable effect, making twice daily administration with morning and evening doses an effective and practical regimen to recommend.

Pharmacokinetic data on flurbiprofen indicates a serum half-life of 3.8 h when given in the morning (Chalmers, Glass & Risdall, 1977), but as shown with indomethacin (Clench, Reinberg, Dziewanoska, Ghata & Dupont, 1977; Clench, Reiberg, Ghata & Dupont, 1975), the half-life may differ considerably at different times of administration. The half-life of the drug in the synovial fluid after morning administration is about 5 h (Chalmers *et al.*, 1977). It is possible that the drug is more effective when given twice daily because two 100 mg doses will provide higher peak serum concentrations than four smaller doses and there might then be higher synovial fluid levels and, therefore, more sustained or more effective action.

The study suggests that two 'large' doses of flurbiprofen are more effective than four 'small' doses per day. The design of the trial did not include a 'run-out' period when patients could have been studied and compared with their run-in period. The run-in period on four daily doses was not part of the crossover study. The symptom scores might have been influenced because this part of the study was not double-blind but the objective measurments are unlikely to have been so clearly different for this reason alone. It may be that the measured improvement on changing

Table 8 Matrix of probabilities of significant differences in mean grip strength and mean sum of joints, between fortnights of the trial, regardless of treatment timing (NS, $P > 0.05$)

Fortnight		*Run-in*	*First fortnight*	*Second fortnight*	*Third fortnight*
First fortnight	grip	NS			
	joints	$P < 0.01$			
Second fortnight	grip	$P < 0.05$	$P < 0.05$		
	joints	$P < 0.05$	NS		
Third fortnight	grip	$P < 0.005$	$P < 0.005$	NS	
	joints	$P < 0.01$	NS	NS	

from flurbiprofen four times a day to flurbiprofen twice a day was the result of a trend over the duration of the trial. Grip strengths improved during the trial in several patients, suggesting that there may have been a training effect with patients increasing their ability to squeeze the bag. The joint size also improved on changing from flurbiprofen four times a day to flurbiprofen twice a day, but this trend did not continue through to the end of the trial. As the method of measuring joint size was objective there could be no training effect. The improvement on changing from four times a day flurbiprofen to two doses per day is likely to be because this is a more effective way to give the drug, but confirmation from a trial designed to study the frequency of administration rather than the timing alone is needed.

This trial demonstrated that patients are able to succesfully monitor their own grip strengths and finger joint sizes at home and that using this data circadian rhythms can be characterized and analysed. This is the first description of the use of these methods for self assessment. Although diurnal variations in grip strength and joint size have previously been noted based on very few measurements, adequate documentation of the rhythmic nature of the joint changes in rheumatoid arthritis, to our knowledge, has not been attempted before. More detailed descriptions of these rhythms are being prepared for publication. The amplitudes and timing of peaks of these rhythms were similar on all the regimens studied. It is surprising that in a condition which has such marked diurnal variations in symptoms that there has not been more interest in the patterns of these fluctuations. Studies to relate these to pathogenic mechanisms and to treatment would be of interest and potential importance.

The blood tests showed that there were no toxic effects on liver function or haematology when larger doses were taken. However, three patients withdrew from the trial due to indigestion, a known side effect of propionic acid derivatives like flurbiprofen. One of these had not been intolerant when taking 50 mg four times daily. Possibly a larger dose of the drug is more irritant to the stomach than more frequent smaller doses.

Pharmacokinetics do not always predict biological effect (Scheving *et al*, 1974; Woolfson & Knapp, 1977; Cardoe, 1977; Huskisson, Scott, Boyle & Patrick, 1977). On the basis of subjective assessments, Doury & Patt (1977) found that two 150 mg doses of flurbiprofen were equally effective as three 100 mg doses in controlling the symptoms of ankylosing spondylitis and coxarthrosis. In our study two 100 mg doses of flurbiprofen appear to be more effective than four 50 mg doses. The duration of action extends beyond that expected from the pharmacokinetic data, possibly due to sustained tissue levels, to an effect persisting after disappearance of the drug or to lower blood levels than are thought to be biologically significant being useful. Cardoe (1977) found no difference in effectiveness of 75 mg, 150 mg or 300 mg flurbiprofen per day. Huskisson *et al.* (1977) showed that 150 mg or 100 mg flurbiprofen at night was superior to placebo when given in addition to other anti-inflammatory drugs. Once a day flurbiprofen alone has not been tested but might be effective if correctly timed. Our results suggest that to control morning symptoms of arthritis part of the daily dose should be given late in the evening. This would be the obvious time to test the effect of once daily doses of flurbiprofen and studies are planned. Variations in therapeutic effectiveness due to the circadian timing of treatment are best studied with single daily doses and are of particular importance when single dose regimens are to be recommended.

Patient compliance and acceptability are improved with less frequent doses, but it is obviously sensible to ascertain whether there is a best time for administration.

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