Serum cytidine deaminase levels after withdrawal of non-steroidal anti-inflammatory treatment in rheumatoid arthritis

P W THOMPSON,¹ J R KIRWAN,^{1*} D D JONES,² AND H L F CURREY¹

From the ¹Bone and Joint Research Unit, The London Hospital Medical College, Turner Street, London; and ²Bronglais Hospital, Aberystwyth, Dyfed

SUMMARY Increases in joint inflammation in nine patients with rheumatoid arthritis were provoked by withdrawal of their non-steroidal anti-inflammatory drugs. Pain score, duration of morning stiffness, Ritchie articular index score, and the number of analgesic tablets consumed reached peaks after five, three, five, and five days respectively compared with values during six days of normal treatment. Changes in serum cytidine deaminase (believed to reflect polymorph turnover in inflamed joints) showed a different pattern, with a sharp peak after two days and a subsequent trough. Possible mechanisms for these differences are discussed.

Key words: disease assessment, joint inflammation, nucleoside aminohydrolase.

Many measures of joint inflammation in rheumatoid arthritis (RA) are available, but none has proved ideal. Clinical assessments such as an articular index¹ suffer from high interobserver variation, whereas conventional serological tests like the erythrocyte sedimentation rate (ESR)² are indirect measures and their relation with disease processes is poorly understood. In recent years several new laboratory measures have been reported that reflect specific inflammatory cell turnover.³ The rationale for their use derives from the principles of clinical enzymology, and they have potential both for monitoring response to treatment and for understanding the relation between synovial inflammation and joint damage.

One such measure is cytidine deaminase (CD) (EC 3.5.4.5.), a cytoplasmic enzyme released from dead and damaged polymorphs into the synovial fluid of patients with RA. It diffuses into the blood, where serum levels reflect total polymorph turnover and provide a measure of overall joint inflammation.⁴

Serum CD activity correlates moderately well ($r=0.7$) with clinical measures of joint inflammation and serum markers of the acute phase response.⁵ It is directly related to the inflammatory process and meets many of the requirements of a good measure of joint inflammation. The biochemical assay is simple and reproducible.⁶

This study was designed to evaluate the ability of serum CD to detect changes in joint inflammation when non-steroidal anti-inflammatory drug (NSAID) treatment was temporarily withdrawn from patients with RA, and to compare the pattern of response with existing clinical and laboratory measures.

Patients and methods

Ethical committee approval for the study was obtained and written informed consent received from 10 volunteers (six male, four female) with classical or definite RA⁷ (mean (SD) age 63 (14) years) who were invited to participate. All patients had been receiving one NSAID in the same daily dosage for at least one month and a variety of other drugs that were not altered during the study. Patients a and e were receiving D-penicillamine, patient j gold, and no patients were taking corticosteroids. Naproxen was taken by patients b and f, benorylate by patients c and d, and ibuprofen,

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Correspondence to Dr P W Thompson, Bone and Joint Research Unit, The London Hospital Medical College, Turner Street, London E1 2AD.

*Current address: Department of Rheumatology, Bristol Royal Infirmary, Marlborough Street, Bristol BS2 8HW.

fenbufen, ketoprofen, diclofenac, and indomethacin by patients i, g, a, h, and e respectively. Patient j withdrew after two days because of intercurrent infection requiring hospital treatment.

Patients were visited every morning at about the same time at home or at their place of work, for 12 days (day 0–day 12) except day 6 (a Sunday), by the same nurse/metrologist trained in clinical assessment and venepuncture.

The patients were studied in groups of three, three, three, and four during separate 12 day

periods. At each visit pain (10 cm visual analogue scale), duration of morning stiffness in minutes (EMS), Ritchie articular index,⁸ and drug treatment were recorded, and blood was taken.

Patients were randomly allocated to receive treatment with either six days of their normal NSAID followed by six days of no NSAID (group A, patients a, b, c, d, and e); or six days of no NSAID followed by six days of their normal NSAID (group B, patients f, g, h, i, and j), in open fashion. They were encouraged to take paracetamol tablets

Fig. 1 Individual daily CD results for nine patients. Group A (patients a–e) received their normal NSAID for six days followed by no NSAID, and group B (patients f–i) received no NSAID for six days followed by their normal NSAID.

(500 mg) up to 4 g/day in divided doses as analgesic during the period without NSAIDs and to record all drugs taken each day on forms provided.

The serum was separated within four hours of venepuncture, coded, and stored at -20°C for subsequent measurement of CD activity by the method of Jones *et al.*,^{6,9} and for C reactive protein (CRP) by the turbidometric method employing a Hyland laser nephelometer.¹⁰ The assays were performed without knowledge of the patients' treatment. The normal serum range for CD is 0.9–3.9 units.⁹

Normal serum was incubated with naproxen, ibuprofen, ketoprofen, diclofenac, and indomethacin at 1, 2, 20, and 200 times therapeutic serum levels to determine whether the assay was affected in vitro by the NSAIDs.

The difference between the groups and the differences between the peak results during the flare and baseline levels on day 5 for group A and day 12 for group B were compared using Student's *t* test.

Results

The presence of five NSAIDs in vitro, even in high concentration, had no effect on CD assay results (data not shown).

Groups A and B were similar with respect to mean (SD) age (A=65 (14), B=61 (16) years), sex

ratio (F:M, 3:2), and mean (SD) values for pain (A=42 (25), B=60 (18) mm), EMS (A=49 (64), B=65 (52) minutes), Ritchie index (A=19 (14), B=20 (10)), and CD levels (A=5.8 (2.2), B=6.3 (2.1) units), but group B had higher mean CRP levels than group A (33 mg/l v 18 mg/l, $p<0.01$).

The pattern of individual daily CD results was similar for all patients except patient b (Fig. 1). The mean daily CD levels for both patient groups during the treatment periods are shown in Fig. 2 (composite of Fig. 1). A significant peak ($p<0.05$) occurred two days after stopping NSAIDs, and a trough two days later ($p<0.05$), followed by a return to the baseline.

The flare was detected by all other measures except CRP (Fig. 3), but the pattern of response differed from that of CD as there were no troughs and the peaks occurred later.

Discussion

The results suggest that serial blood levels of CD can detect the flare produced by the withdrawal of NSAIDs from patients with RA, and support the observation that NSAIDs inhibit polymorph turnover in vivo.¹¹

The study was designed so that daily measurements could be undertaken in the patients' homes during an induced flare of joint inflammation. The 'flare' technique is a recognised method used to

Fig. 2 Mean daily CD results for group A (patients a-e who received their normal NSAID for six days followed by no NSAID), and group B (patients f-i who received no NSAID for six days followed by their normal NSAID).

Fig. 3 Mean daily scores for number of paracetamol, EMS, pain (VAS), Ritchie articular index, and CRP for group A (patients a-e who received their normal NSAID for six days followed by no NSAID), and group B (patients f-i who received no NSAID for six days followed by their normal NSAID).

select patients for trials of new NSAIDs.¹² Ideally all patients would have been monitored for 18 days and received the same NSAID during the first and last six day periods and no NSAID during the middle six day period. To minimise the numbers of daily venepunctures, however, patients were divided into the two groups. The higher mean CRP level for group B suggests a difference in the acute phase response in the two groups, but similar differences were not seen with any other clinical or serological measure of inflammation. Although this study design complicated statistical interpretation of the results, we were mainly interested in the timing and patterns of change in the clinical and serological measurements.

Differences in synovial fluid clearance rates of the NSAIDs might have been expected to affect the timing of the changes. In practice the peaks during the flare for different patients occurred within a few hours of each other when NSAIDs were withdrawn, suggesting that such considerations are not of great practical importance. The unstable baseline for all measures when NSAIDs were restarted (group B), however, might have reflected fluctuations in blood and synovial fluid levels before steady state kinetics were reached.

Serial CD levels showed similar changes for most of the patients (Fig. 1), suggesting that the mean pattern (Fig. 2) was representative of the patients as a group. Patient b showed a fall in serum CD during the flare despite changes in the clinical scores. This patient took up to 5 g/day of paracetamol during the period without NSAIDs, suggesting a possible effect of paracetamol in high doses on polymorph turnover. This point is further discussed below.

The gradual increase in EMS, pain score, and Ritchie index after treatment withdrawal is in keeping with the known inhibitory effect of NSAIDs on prostaglandin synthesis.¹³ The pattern of CD response with a sharp early peak and subsequent trough suggests a different mechanism. In vivo studies using skin window techniques have shown inhibition of polymorph migration by all NSAIDs tested, which included diclofenac, indomethacin, naproxen, ibuprofen, and piroxicam.^{11 14}

It has been estimated that the half life of a synovial fluid polymorph in RA is four hours. In a 30 ml effusion containing 25×10^9 polymorphs/litre the daily breakdown in the synovial cavity might exceed a billion cells.¹⁵ The kinetics of polymorph release from the marrow and duration in the blood are similar in normal controls and in patients with RA,¹⁶ however, therefore at least in the short term the enormous numbers of polymorphs entering inflamed joints will be balanced by the same number leaving the marrow. Sudden removal of the in-

hibitory tone of chronic NSAID treatment may allow a large influx of cells into joints. These cells would be rapidly destroyed, releasing CD into synovial fluid with the subsequent rise in serum CD activity. The peak at two days fits well with the time taken for cell migration to return to normal after NSAID removal in the skin window experiments.¹¹

The subsequent trough was not anticipated. It may result from a transient depletion of polymorphs available to migrate into the joints after the sudden influx. If so, leucopenia may be anticipated, but circulating polymorphs were not measured during this study. An alternative explanation is that paracetamol, taken as escape analgesia, has a stabilising effect on polymorph activity that has not been previously recorded. This is supported by the fact that both patients who were taking benorylate, which yields paracetamol after ingestion, showed a peak in CD levels during the flare. There is evidence that paracetamol lacks anti-inflammatory action,¹⁷ but it would be worth while checking its *in vivo* effect on polymorph migration.

Serial blood levels of CRP showed large daily variation but did not significantly change overall during the study period, supporting the belief that NSAIDs do not affect the acute phase response, at least in the short term.¹³

The open nature of the study probably introduced bias in favour of the clinical measures because the patients and the nurse/metrologist anticipated the flare. This was particularly true for the number of paracetamol taken because patients were actively encouraged to use paracetamol as analgesia during the period without NSAIDs. In contrast, the CRP and CD assays were carried out without knowledge of the patients' condition or treatment. Any comparison between the clinical and serological measures should recognise this bias and take it into account.

Serum CD is a simple, cheap, reproducible, and sensitive measure of joint inflammation in rheumatoid arthritis that reflects the turnover of a specific cell type known to be at the centre of the inflammatory process. It supplies the clinician with a serological tool for patient monitoring which is a more direct reflection of inflammation than measurements such as the ESR. Its role as an *in vivo* marker of polymorph turnover may also prove helpful in elucidating the relation between joint inflammation and destruction.

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