Drug induced impairment of polymorphonuclear cell bactericidal ability in rheumatoid arthritis

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SUMMARY Thirty three patients with rheumatoid arthritis (RA) treated only with non-steroidal anti-inflammatory drugs were divided into two groups according to the drug received (group A: diclofenac, group B: indomethacin or ketoprofen), and their polymorphonuclear (PMN) cell functions were investigated. We found that bactericidal ability was the only function significantly impaired in group A as compared with group B patients and normal controls. This modification correlated well with the reduction of control PMN bactericidal ability when this test was carried out in patient plasma. These differences between drug effects might explain some of the discrepancies between reports of PMN cell functions in RA.

Key words: non-steroidal anti-inflammatory drugs.

The role of polymorphonuclear (PMN) cells in inflammation in general,¹ and in rheumatic diseases in particular,² has been comprehensively reviewed. Studies on the functional state of PMN cells in rheumatoid arthritis (RA) have produced conflicting results, however, and a general consensus concerning the nature of the PMN defect has not yet been reached. For example, Howe *et al* reported impaired chemotaxis in some patients with RA,³ and Gale *et al* showed that PMN chemiluminescence was activated by rheumatoid serum and synovial fluid.⁴ Wilton *et al* found impaired phagocytosis,⁵ whereas Wandall found it to be normal.⁶

It seems reasonable to suggest that these discrepancies may be due to differences in drug regimens. In an attempt to test this we selected two groups of patients with RA who differed only in their drug treatment. Each group of patients was receiving only one non-steroidal anti-inflammatory drug (NSAID). In the light of the results we suggest that the differences between the PMN functions in the two groups of patients are due to differences in NSAID treatment.

Patients and methods

PATIENTS

Thirty three patients were included in the study, all

fulfilling the American Rheumatism Association criteria for classical (20 patients) or definite (13 patients) RA.⁷ There were 27 women and 6 men, 19 seropositive patients and 14 seronegative as defined by the latex and the Rose-Waaler tests. Their mean age was 52 years (range 18-79) and the mean duration of disease six years (range 1-18).

All were receiving only an NSAID, and the patients were classified into two groups according to the drug received (group A: diclofenac, group B: indomethacin or ketoprofen).

The disease activity was assessed clinically by the number of painful joints⁸ and Lee's index,⁹ radiologically by Steinbrocker's index,¹⁰ and biochemically by measuring plasma C3d¹¹ and serum C reactive protein (CRP).¹² Independent clinical assessment of disease activity correlated well with these laboratory parameters.

Extra-articular manifestations (rheumatoid nodules, splenomegaly, lymphadenopathy, pleurisy, xerophthalmia, and xerostomia) were identified from the medical records.

CONTROLS

Normal controls consisted of 33 healthy subjects who were members of the clinical or laboratory staff, or residents of an old people's home. They were matched by age and sex with the patients with RA.

CELL PREPARATION

Blood samples were collected in ethylene

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diaminetetra-acetate tubes prepared in the laboratory or in preservative free heparin tubes. Cell suspensions containing more than 90% PMN cells were obtained by Dextran T500 sedimentation (Pharmacia, Uppsala, Sweden) followed by Ficoll-Isopaque density gradient centrifugation (Eurobio, Paris, France) and hypotonic lysis of residual erythrocytes.

PMN FUNCTION STUDIES

PMN killing of *Staphylococcus aureus* was assessed by the method described by Wilkinson,¹³ and the results were expressed as percentages of bacteria killed at 60 minutes. We evaluated patient PMN bactericidal ability in control plasma and then the effect of patient plasma on control PMN bactericidal ability. This was expressed as the difference be-

Fig. 1 Bactericidal ability in controls and in two groups of patients (A: treated with diclofenac, B: treated with indomethacin or ketoprofen).

Fig. 2 Variation of control polymorphonuclear cell bactericidal ability in autologous plasma and in patient plasma (see legend of Fig. 1).

tween the result obtained in control plasma and that obtained in patient plasma.

Adherence was studied by the method described by McGregor¹⁴ using nylon fibres prewetted with 1 ml of minimal essential medium. The results were expressed as the percentage of PMN cells adherent to the nylon fibre column. Phagocytosis was assessed by the method described by Baehner and Nathan¹⁵ and modified by Windhorst *et al.*¹⁶ Phagocytic capacity was expressed as the percentage of PMN cells containing at least 10 latex particles (0.81 μ m in diameter) (Difco Laboratories, Detroit, Michigan, USA). The predominantly perinuclear arrangement of the latex beads was indicative of actual endocytosis rather than non-specific surface absorption.

SEROLOGICAL TESTS

The serum CRP was measured by radial immunodiffusion using commercially available plates (Behring, Marburg, FRG). C3d was assayed according to the technique of Perrin *et al.*¹⁷ and expressed as a percentage of maximal activation obtained by zymosan (Sigma Chemical Company, St Louis, Missouri, USA).

STATISTICAL METHODS

All figures quoted below are arithmetic means (1 standard deviation). Tests for significance were

Fig. 3 Patient polymorphonuclear cell bactericidal ability (BA) in control plasma (vertical axis) plotted against the percentage change in BA when control cells are tested in plasma from these patients (horizontal axis).

Fig. 4 Latex phagocytosis and adherence in controls and in two groups of patients (see legend of Fig. 1).

Table 1 Clinical details of patients with rheumatoid arthritis

	Group A* (n=17)	Group B* (n=16)†
Ritchie's index	10.3 (3.6)†	6.9 (7.0)
Lee's index	10.2 (8.3)	10.5 (7.2)
Steinbrocker's index	1.2 (1.0)	1.7 (1.5)
CRP (mg/ml)	40.5 (32.9)	36.8 (25.2)
C3d (% activity)	39.5 (17.1)	31.0 (23.2)
Extra-articular disease (yes/no)	4/13	6/10

Values are expressed as arithmetic means (SD).

*Group A: treated with diclofenac; group B: treated with indomethacin or ketoprofen.

†No significant differences.

made with the Mann-Whitney and the Wilcoxon tests. Correlation coefficients were determined by the Spearman rank test.

Results

As shown in Fig. 1 bactericidal ability was significantly depressed in patients treated with diclofenac, as opposed to those treated with other NSAIDs and to normal controls. Control PMN bactericidal ability was significantly reduced in group A patient plasma (Fig. 2). No significant overall impairment could be detected in group B patient plasma, although PMN cell killing of bacteria was decreased when incubated in the plasma of eight patients. These patients were receiving high doses of indomethacin (two cases) or ketoprofen (six cases). When the patients were considered as a whole (Fig. 3), however, their PMN bactericidal activity correlated well with the control PMN bactericidal activity decrease in patient plasma. This PMN function was the only one affected (Fig. 4). Adherence was found to be increased in both groups of patients. Although there tended to be more adherent PMN cells in the diclofenac treated patient group than in the indomethacin or ketoprofen treated patient groups, this difference did not reach significance. Differences in PMN cell functions appear not to be due to differences in disease activity, as shown in Table 1.

Discussion

Most experimental studies designed to investigate the effect of symptomatic drugs on PMN functions involve incubating the cells of normal controls with drugs. The results obtained, however, do not always reflect what actually happens in patients. For instance, it has been shown that aspirin, a weak in

vitro inhibitor of prostaglandin (PG) synthesis, is fully able to suppress PG synthesis at low dosage in synovial membranes, whereas indomethacin, a potent in vitro inhibitor of PG synthesis, only moderately impaired PG synthesis in the synovial tissue.¹⁸ This reinforces the need to study the effect of drugs in vivo and thus to focus attention on patient PMN cells, taking into account their drug treatment.

In the course of this prospective study we observed that the PMN bactericidal ability of patients with RA undergoing diclofenac treatment diminished. This was not the case among patients with RA treated with two other NSAIDs. It must be emphasised that the two groups of patients were comparable with regard to disease activity.

Two aspects of bactericidal activity are probably involved. The first is a series of metabolic events collectively termed the respiratory burst, since patient PMN bactericidal ability was depressed when the cells were tested in normal plasma. The second is patient plasma, since control PMN bactericidal ability was depressed when the cells were in patient plasma. It seems that the inhibition of bactericidal ability is not the consequence of an impairment of adhesiveness or bacterial engulfment, or both, because these two functions were found to be comparable in both groups of patients.

NSAIDs certainly influence the behaviour of PMN cells. The inhibition of PG synthesis is no doubt important, but there is also the impairment of lysosomal enzyme release, demonstrated by Northover in the case of indomethacin,¹⁹ and finally, the inhibition of neutral proteases.²⁰ Several mechanisms are therefore possible acting independently or collectively to reduce PMN bactericidal ability.

PMN bactericidal ability is associated with an increased passage of glucose through the hexose monophosphate shunt. It has previously been shown that phenylbutazone markedly inhibits glucose oxidation by both resting and stimulated phagocytes.²¹ Since one of the functions of the stimulated hexose monophosphate shunt is to make available a greater quantity of H₂O₂, a major component in the PMN bactericidal system, this may well explain the mechanism by which phenylbutazone inhibits the intracellular killing of bacteria. In view of the results obtained it seems reasonable to suggest that diclofenac has the same effect as phenylbutazone.

NSAIDs induce relatively rapid effects, since control PMN bactericidal ability diminishes significantly when the cells are incubated in patient plasma. It remains possible that NSAID catabolites, present at that moment in patient plasma, penetrate the cells and affect enzymes which are crucial to

cellular activation, particularly those involved in the hexose monophosphate shunt.

As folic acid levels are depressed in the serum of patients with RA²² it is possible that a decline in PMN functions is related to folic acid deficiency. We have previously reported that folic acid deficiency can reduce to a significant degree bacterial engulfment and PMN bactericidal ability.²³ In this study, however, there was no difference in the folic acid levels between the groups of patients, and phagocytosis was found to be normal.

Whatever the mechanism involved, these results point to the need to take account of drug treatment when studying cellular functions in patients with RA.²⁴ In addition to NSAIDs we have previously shown that D-penicillamine affects PMN chemotaxis. It is probable that some of the conflicting results in the literature may be explained in this way.

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