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Normalization of Monocyte Chemotaxis Precedes Clinical Resolution of Psoriasis Treated with Benoxaprofen

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Enhanced monocyte activity is present in psoriasis, and benoxaprofen is a drug that inhibits several aspects of monocyte function. To assess a potential pathogenic role of enhanced monocyte function in psoriasis, we determined monocyte chemotaxis and monocyte antibody-dependent cell-mediated cytotoxicity (ADCC) at week 0, 2, 4 and 8 in psoriatics being treated with benoxaprofen. In patients responding to benoxaprofen, normalization of monocyte chemotaxis occurred at week 4, before clinical resolution took place. A significant decrease of monocyte ADCC was also present at week 4, but it was only at week 8, when psoriasis had completely cleared, that monocyte ADCC was completely normalized. In patients receiving placebo or receiving benoxaprofen, but showing no or minimal clinical improvement, monocyte functions remained increased. These results are compatible with the idea that benoxaprofen may improve psoriasis by interfering with monocyte function. *Key words: Psoriasis; Monocytes; Benoxaprofen.*
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Benoxaprofen is a drug that has been shown to improve psoriasis dramatically (1, 2). The mode of action of benoxaprofen has been thought to reside in its inhibition of arachidonic acid transformation via the 5-lipoxygenase pathway (3) and/or in its capacity to inhibit migration of leukocytes, preferentially monocytes, into sites of inflammation (4). The recent reports showing benoxaprofen to be a poor inhibitor of 5-lipoxygenase activity in human leukocytes (5, 6) have put more actuality into the ability of benoxaprofen to interfere with monocyte migration. This concept that benoxaprofen may inhibit monocyte activity independent of arachidonic acid metabolism is supported by in vitro experiments showing that benoxaprofen can inhibit monocyte adherence to endothelial cells at concentrations lower than those required to inhibit arachidonic acid metabolism (7).

Increased monocyte activity has been reported in untreated psoriasis. That includes increase of chemotaxis (8) and of antibody-dependent cell-mediated cytotoxicity (ADCC) (9) in vitro, and early exocytosis of monocytes in psoriatic lesions (10). However, a potentially pathogenic role of monocytes in psoriasis is still unanswered. The present study was performed to determine, whether monocyte chemotaxis and ADCC normalized during benoxaprofen treatment, and whether a normalization of monocyte function preceeded clinical resolution. The clinical results of this study has been reported earlier (2).

MATERIAL AND METHODS

Patients. Forty patients were treated in a double-blind manner with either benoxaprofen 600 mg orally daily or placebo for a period of 8 weeks. At week 0, 2, 4 and 8 the clinical status was evaluated and simultaneously venous blood was drawn for determination of monocyte functions and benoxaprofen concentration in the serum (see below). The clinical results have been reported earlier (2). In this text

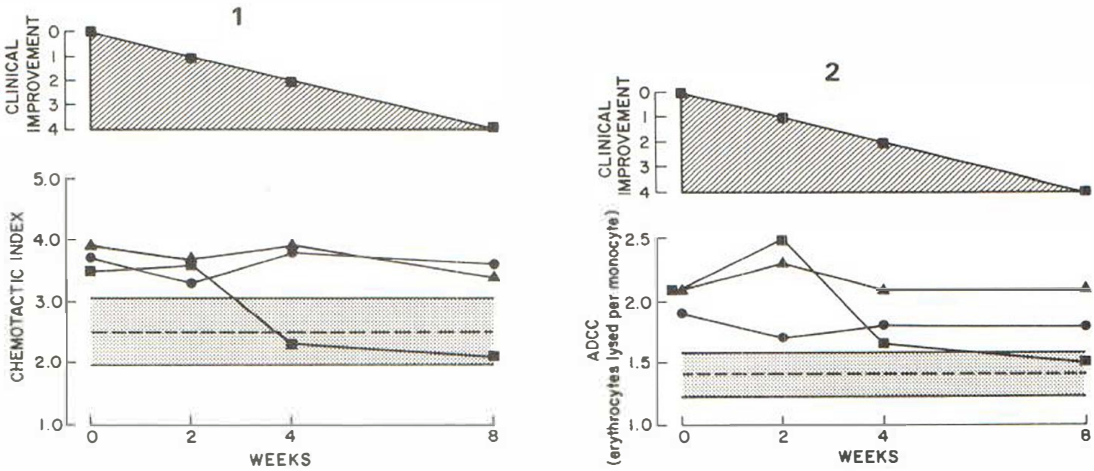

Fig. 1. Monocyte chemotaxis in psoriatics receiving placebo (●) (n=9); receiving benoxaprofen, but showing no or minimal improvement (▲) (n=3); or clearing during benoxaprofen therapy (■) (n=8). Values are means. The mean and SD for normals are indicated by a stippled line and a shaded area, respectively. For patients clearing during benoxaprofen therapy, the clinical improvement is shown as a function time. For details on the clinical grading see Material and Methods.

Fig. 2. Monocyte ADCC in psoriatics. For explanation see legend for Fig. 1.

we will only deal with the clinical results as they relate to the laboratory data. The response to therapy was graded according to the following criteria: 0, no change; 1, minimal improvement, slightly less scaling and less erythema; 2, definite improvement, partial flattening of all plaques, less scaling, and less erythema; 3, considerable improvement, nearly complete flattening of all plaques but borders of plaques still palpable; and 4, clearing, complete flattening of plaques including borders, plaques may be outlined by pigmentation.

Isolation of monocytes. Heparinized venous blood was obtained from the psoriatics and from healthy adult volunteers with a comparable age and sex distribution. Monocytes were isolated as described elsewhere by us (11). In short, mononuclear cells harvested from a Ficoll-Isopaque gradient were allowed to adhere to plastic culture flasks for 60 min at 37°C. Non-adherent lymphocytes were then removed by washings, and adherent monocytes were released by lowering the temperature to 4°C for 30 min. Judged by non-specific esterase activity, the purity of the monocyte suspensions ranged from 88% to 97% with a median of 93%, the contaminating cells being granulocytes (1–2%) and lymphocytes (3–10%). Judged by trypan blue exclusion the viability of monocytes was higher than 95%. Monocytes were resuspended in Medium RPMI 1640 (Gibco) supplemnted with 25 mM Hepes, 100 IU/ml penicillin, 100 µg/ml streptomycin and 5% (v/v) heat-inactivated fetal calf serum (Gibco), finally adjusted to pH 7.4.

Chemotaxis. In vitro chemotaxis was measured using a modification of the assay employing leukocytes prelabeled with sodium-^{51}chromate (^{51}Cr) (12). Monocytes were incubated with ^{51}Cr (Amersham, England, 1 mCi/ml, 2–10 µg Cr/ml) at 37°C for 60 min. After labeling, leukocytes were washed three times and resuspended in medium. N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) (Sigma) was used as a chemotactic stimulus at a concentration of 10^{-8} M dissolved in medium. In preliminary experiments this concentration of FMLP was found to induce maximal cell migration.

A blind well design of chemotaxis chambers was used (12). Five hundred µl of FMLP was placed in the lower compartment of the chamber and covered by a cellulose nitrate filter, pore size 0.45 µm (Nucleopore Corp., Pleasanton, CA). The cell suspension (2.5×10^6/ml) was added to the upper compartment of the chemotaxis chamber. The cells and the chemotactic agent were partitioned by a sandwich of a 5 µm pore size polycarbonate filter (upper filter = UF) placed directly on top of a 0.45 µm cellulose filter (lower filter = LF). This combination provides a mean of selectively counting cells that migrate into the lower of the 2 filters. Medium alone in the lower compartment served as control (random motility of cells). Assays were done in triplicate. Cells were incubated at 37°C in humidified air for 30 min. After incubation the cells were removed by pipetting, and the UF and the LF were

Table I. *Serum concentration of benoxaprofen*

Values are means and ranges

Patient	*n*	Week 2	Week 4	Week 8
Responders	8	118 (100–140)	116 (95–138)	135 (115–161)
Non-responders	3	133 (98–165)	125 (95–155)	130 (105–163)

separated with fine forceps. Finally, the filters were rinsed in Hank's balanced salt solution at 37°C and placed in a gamma counter.

Chemotactic activity was calculated according to the formula:

$$\frac{\text{LF cpm}}{\text{LF cpm} + \text{UF cpm}}$$

The results were expressed as the chemotactic index (CI) being the ratio of the migration in presence of FMLP and the random migration in control chambers.

Antibody-dependent cell-mediated cytotoxicity (ADCC). The cytotoxicity assay was performed with minor modifications of the procedure previously described (11). The tests were set up in duplicate. To 0.1 ml monocytes (1.0×10^6/ml) were added 0.2 ml ^{51}Cr-labelled human rhesus D-positive erythrocytes (2.0×10^6/ml or 6.0×10^6/ml) presensitized with hyperimmune human anti-D serum. This mixture was centrifuged at 150 g for 1 min and then incubated for 60 min at 37°C. After incubation, half of the supernatant (S) was withdrawn and counted together with the residue (*R*) in a gamma counter. Calculation of ^{51}Cr-release was according to the formula:

$$^{51}\text{Cr-release} = \frac{S\,\text{cpm} \times 2}{S\,\text{cpm} + R\,\text{cpm}}$$

The specific release was obtained by subtracting the ^{51}Cr-release in control tubes containing no leukocytes. Results were expressed as the number of target cells lysed per leukocyte.

Assay of benoxaprofen. The concentration of benoxaprofen was assayed according to the method described by Shatfield & Woodage (13)

Statistics. The data accumulated from the study was expressed as means and SD. The Wilcoxon rank-sum test for unpaired samples was used to assess statistical significance.

RESULTS

Before treatment with benoxaprofen monocytes expresed increased chemotaxis ($p<0.01$) (Fig. 1) and ADCC ($p<0.01$) (Fig. 2). Chemotaxis and ADCC were stimulated to the same degree in psoriatic monocytes, 43% and 46%, respectively. To interpret changes in monocyte chemotaxis and ADCC occurring during benoxaprofen therapy, psoriatic patients responding to benoxaprofen (i.e. showing complete clearance at week 8) were compared both with psoriatics receiving placebo and with psoriatics receiving benoxaprofen, but showing no or minimal clinical improvement (Figs. 1 and 2).

At week 0 there was no significant difference between these 3 groups with respect to chemotaxis and ADCC of monocytes. A gradual clinical improvement occurred in psoriatics responding to benoxaprofen, but it was only after week 8 (at the end of the study) that complete resolution of psoriasis had occurred (Figs. 1 and 2). In comparison, monocyte chemotaxis normalized already at week 4 in these patients (Fig. 1). For monocyte ADCC a significant decreae had occurred at week 4 ($p<0.01$), but it was only at week 8, when clinical resolution was present, that monocyte ADCC completely normalized (Fig. 2). In the placebo group and in the group not responding to benoxaprofen, monocyte chemotaxis and ADCC remained increased (Figs. 1 and 2).

Determination of the serum concentration of benoxaprofen showed that maximal levels were obtained already at week 2 (Table I). The concentration of benoxaprofen in serum did not differ between patients that showed a clinical improvement and patients that did not.

RESULTS

In the present study we have demonstrated that clinical resolution of psoriasis during benoxaprofen treatment is preceeded by a normalization of monocyte chemotaxis. A parallel decrease of monocyte ADCC was observed although a complete normalization of ADCC only occurred when clinical resolution was present. These results suggest that benoxaprofen may improve psoriasis by acting on monocyte chemotaxis. In support of the idea that benoxaprofen may improve psoriasis by inhibiting monocytes is the fact that benoxaprofen acts primarily on monocytes among leukocytes. These actions on monocytes include the inhibition of their binding to endothelial cells (7) and of their migration (4). In our patients a steady-state concentration of benoxaprofen in serum was obtained within 2 weeks, but it was only at week 4 that monocyte activity normalized. This delay may be explained by the high affinity of benoxaprofen for plasma proteins (14). As a consequence the equilibrium between plasma and tissues might occur slowly resulting in a delayed pharmacologic action of benoxaprofen.

Traditionally the neutrophil, rather than the monocyte, has been suggested a role in the pathogenesis of psoriasis. The presence of neutrophils is a familiar histological feature of psoriasis and from some studies it appears that neutrophil invasion of epidermis is the earliest morphologic event in psoriasis (15, 16). Furthermore, neutrophils, just as monocytes, express enhanced functional activities including chemotaxis (17, 18), phagocytosis (18), and ADCC (9). To prove that monocyte, rather than neutrophils, are primarily affected in the benoxaprofen-induced resolution of psoriasis, it would be required to measure the activities of monocytes and neutrophils in parallel. A normalization of monocyte activity preceeding that of neutrophils would substantiate the findings of the present study.

The notion that monocytes and their inflammatory mediators may be of pathogenic relevance in psoriasis does not imply the presence of an intrinsic abnormality of monocyte function in this disease. While enhanced monocyte function has been observed in psoriatics with mild disease (8), a recent and very detailed study of quiescent proriasis failed to reveal an abnormality of monocyte activities (9). In support of normal monocyte function in quiescent psoriasis is our finding of normal chemotaxis and ADCC in patients clearing in response to benoxaprofen. We, therefore, believe, that monocyte chemotaxis become stimulated during the psoriatic process. As a result of this stimulation they may release pro-inflammatory mediators that can be of importance in maintaining the skin lesions. Among the potential mediators are arachidonic acid derivatives, but other still unknown mediators, may be equally important.

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