0022-202X/83/8105-0418$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 81:418–419, 1983

Vol. 81, No. 5
Printed in U.S.A.

Effect of Etretinate on Chemotaxis of Neutrophils from Patients with Pustular and Vulgar Psoriasis*

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The chemotactic activities of neutrophil granulocytes of patients with pustular and vulgar psoriasis were evaluated before and after treatment with etretinate. Control values before treatment were significantly different from those of vulgar psoriasis group but not from the pustular psoriasis group. The difference between the 2 groups with psoriasis was significant. Etretinate causes a significant reduction in neutrophil chemotaxis in pustular psoriasis patients and a less pronounced reduction in those with vulgar psoriasis.

The therapeutic effectiveness of etretinate for psoriasis is now known and widely accepted [1]. It is the rapid effectiveness of the drug in von Zumbusch's generalized pustular psoriasis (PP) that is of special interest to dermatologists. In vulgar psoriasis (VP), and in particular in its pustular form, there is a conspicuous infiltration of neutrophil granulocytes into the epidermis. Some authors [2–6] maintain that there is increased neutrophil chemotaxis in psoriasis, whereas others have not found this to be so.

The aim of our study was to evaluate the chemotactic activities of the neutrophil granulocytes in patients with PP and VP, before and after treatment with etretinate for 15 days.

MATERIALS AND METHODS

Patients

We studied 10 patients with von Zumbusch's generalized PP (7 men and 3 women, aged 18–68, average age 47); 10 patients with generalized VP, in patches covering more than 50% of the body surface area (8 men and 2 women, aged 28–60, average age 51). All the patients were hospitalized for treatment of psoriasis. They gave written consent to take part in the investigation.

Biopsies were taken from most of these patients to confirm the clinical diagnosis. The controls were 60 healthy normal subjects (19–54, average age 41). None of the patients had any other disease. A venous blood sample was taken from each subject at the beginning of the study and another sample was taken after 2 weeks of treatment from the patients with psoriasis.

Materials

The aromatic retinoid etretinate (Ro-10 9359), kindly supplied by Hoffmann-La Roche, was administered for 15 days, in a dose of 1 mg per kg body weight, as a single daily administration after a lipid-rich meal.

Polymorphonuclear Leukocyte Preparation

Polymorphonuclear (PMN) leukocytes were isolated by a modification of the method of Maderazo and Woronick [7]. The PMN leukocytes were adjusted to a concentration of 2×10^6 cells per ml medium. The final cell preparation contained more than 90% neutrophils, with viability greater than 95% as assessed by trypan blue exclusion.

Preparation of Chemotactic Factor

Normal human serum was incubated with 5 mg/ml Zymosan (Sigma Chemical Co.) in a 37°C water bath for 30 min. The activated serum was centrifuged (10 min, 700 *g*) and stored frozen in liquid nitrogen.

Chemotaxis Assay

This was studied in vitro by the Boyden method [8], as modified by Siccardi [9]. A two-section chamber (LP Italiana S.p.A., Milano), with the sections separated by a Millipore filter with 3-μm pores, was used. The PMN leukocytes were placed in the upper section and the culture medium, RPMI 1640 with or without chemotactic factor, in the lower section. The chambers were incubated for 1.5 h at 37°C. The filters were removed, fixed with methanol, stained with hematoxylin, and mounted on glass slides. All assays were run in duplicate.

To measure leukocyte mobility, the "leading front" technique was chosen, and the values, expressed in μm, are the differences between chemotactic and random migration in the distance covered by the 2 fastest cells in 10 microscopic fields.

Statistical Analysis

The biologic results were analyzed by the Student *t*-test for paired and unpaired data.

RESULTS

As listed in Table I, random migration did not differ from the normal in any group before or after treatment. Neutrophil granulocytes in healthy subjects have chemotactic activities which are midway between those of patients with PP and those with VP.

The control values were significantly different from the VP group ($p < 0.01$) but were not different from the PP group. Furthermore, the difference between the 2 groups with psoriasis was significant before treatment ($p < 0.0005$).

It can be observed that after 14 days of treatment with etretinate, the chemotactic values are significantly lower in PP ($p < 0.0005$) and less pronounced in VP ($p < 0.005$). The chemotactic values after treatment were rather similar.

DISCUSSION

Our data provide new insight into the intricate field of evaluation of neutrophil chemotaxis in psoriasis. Our data support the division of patients with psoriasis into 2 groups, those with PP and those with VP. In the first group, chemotactic activity appears to be slightly greater than that encountered in healthy control subjects, whereas in the VP group it is less. Although only VP chemotactic activity differs significantly from the normal, the chemotactic activities of PP and VP differ significantly from each other ($p < 0.0005$).

Treatment with etretinate brings about a dramatic reduction of chemotaxis, especially in the group of patients with PP who

Manuscript received October 12, 1982; accepted for publication March 21, 1983.

* This work was presented in part at the Symposium on Retinoids during the XVI International Congress of Dermatology, Tokyo, May 22–26, 1982.

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Abbreviations:
PMN: polymorphonuclear
PP: pustular psoriasis
VP: vulgar psoriasis

TABLE I. *Random and chemotactic activities of neutrophil granulocytes pre- and posttreatment with etretinate in patients with pustular and vulgar psoriasis*

Group	No.	Pretreatment (pre-T) Random (R)	Pretreatment (pre-T) Chemotactic (C)[b]	Posttreatment (post-T)[a] Random (R)	Posttreatment (post-T)[a] Chemotactic (C)[b]
		Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)
Controls (C)	60	18.7 ± 3.7[c] (11–26)	82.3 ± 11 (65–110)	—	—
Pustular psoriasis (PP)	10	18.2 ± 3.1[c] (15–23)	89.2 ± 6.9 (73–98)	17.9 ± 4.8[c] (8–22)	57 ± 17.9 (16–78)
Vulgar psoriasis (VP)	10	18.1 ± 2.3[c] (14–21)	74 ± 6.5 (65–88)	18.3 ± 2.3[c] (15–22)	64.6 ± 6.5 (53–76)

PP pre-T C vs C	not significant	PP pre-T C vs PP post-T C	$p < 0.0005$
VP pre-T C vs C	$p < 0.01$	VP pre-T C vs VP post-T C	$p < 0.005$
PP pre-T C vs VP pre-T C	$p < 0.0005$	PP post-T C vs VP post-T C	not significant

[a] After 15 days of etretinate, 1 mg/kg body weight per day.
[b] All the activities are in μm; chemotactic activities are the difference between total and random migration.
[c] Values of random migration do not differ from the normal in all the groups before and after treatment.

have high values to begin with, but also in those with VP. It could be hypothesized that the antigens of the stratum corneum, immunocomplexes linked to the tissues, and the physiologic chemoattractors (epidermis and proteolytic enzymes, auto-antibodies against the stratum corneum, factors of blood complement metabolites derived from arachidonic acid) [10–16] are all eliminated by exfoliation and that more of these substances are present in PP than in VP. On the other hand, it might also be that etretinate acts directly on the PMN cells to inhibit their diapedetic activity.

This inhibition is greater in PP, in which PMN leukocytes play a major role. It also agrees with the microscopically observed intravenous blocking of neutrophil granulocytes [17,18] in retinoid-treated PP, which is not perceptible in the vulgar variety.

This concurrence of theory with clinical, histologic, and laboratory data, may offer a clue to the drug's mechanism of action. The laboratory data, agree with the dramatic effects of the retinoids in therapy of PP, and explain the good but lesser activity in the vulgar form. The cellular and biochemical aspects of the decrease in chemotactic activity need further study.

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