EFFECT OF ANTIINFLAMMATORY AGENTS ON NEUTROPHIL SUPEROXIDE PRODUCTION IN RHEUMATOID ARTHRITIS

SUSANNA SPISANI, CLAUDIA MARANGONI, LIVIO DOVIGO,¹ and SERENA TRANIELLO

Istituto di Chimica Biologica
Università di Ferrara
¹Divisione di Reumatologia
Arcispedale S. Anna
44100 Ferrara, Italy

Abstract—Granulocyte superoxide production by different stimuli was studied in 14 patients suffering from rheumatoid arthritis, and in four cases defective O₂⁻ generation was shown. The effect of two chemically related drugs, such as indomethacin and oxamethacin, was also evaluated, since we have previously investigated the action of antiinflammatory agents on cell locomotion. Indomethacin did not affect O₂⁻ production, whereas oxamethacin reduced significantly superoxide generation in PMNs from all subjects tested. Moreover, the extent of the effect was dependent on the stimulant used, being larger when the activation of O₂⁻ generating system was induced by opsonized zymosan.

INTRODUCTION

Polymorphonuclear leukocytes participate in most organisms’ defenses against infectious processes. The cell microbicidal activity depends on the formation of highly reactive compounds of oxygen metabolism, including superoxide anion (O₂⁻). Superoxide mediates tissue injury occurring during acute inflammation, and the antiinflammatory activity of superoxide dismutase restricts the inflammatory response. The triggering mechanism for production of reactive oxygen species is not completely known, but there is evidence indicating the O₂⁻ generation is promoted by stimulation of granulocyte membranes by phagocytosable materials or soluble agents as well (1, 2).
Rheumatoid arthritis is characterized by the persistence of the inflammatory state and the propagation of tissue injury (3); most likely the \( \text{O}_2^- \) production plays a determinant role in the maintenance of the inflammation (4, 5), and superoxide dismutase may be unable to protect the tissue by active oxygen species damage (6).

The present objective is to study the postphagocytic superoxide anion generation in PMNs from patients suffering from rheumatoid arthritis, since in previous investigations (7, 8) we have characterized neutrophil motile function and its regulatory mechanism. The in vitro effect of two anti-inflammatory drugs on \( \text{O}_2^- \) production was studied. Very little is known about the action of therapeutic agents, particularly those with antiinflammatory activity. Since indomethacin and oxamethacin are both used in the treatment of chronic inflammatory disease, such as RA, we undertook to investigate in detail their effect on \( \text{O}_2^- \) production by two different kinds of stimulus.

**MATERIALS AND METHODS**

*Subjects.* Fourteen patients with rheumatoid arthritis were studied. All patients had classical rheumatoid arthritis (RA) as defined by the ARA criteria and active RA as defined by the presence of at least three of the following criteria: number of tender joints (6); number of swollen joints (3); and duration of morning stiffness (three quarters of an hour) and Westergren erythrocyte sedimentation rate (28 mm/h). Controls for all experiments were carried out on 37 healthy adults.

The pool of sera for zymosan opsonization was also from healthy individuals.

*Cells.* Leukocyte fractions were prepared by dextran (Pharmacia, Uppsala, Sweden) sedimentation of erythrocytes, as previously described (9), and further purified by Ficoll-Paque (Pharmacia, Uppsala, Sweden). The cells (99-100% granulocytes) were washed and resuspended in KRP (Krebs Ringer phosphate), pH 7.4 at 5-10 \( \times 10^6 \) cells/ml.

*Stimulus.* A stock solution of 1 mg/ml of phorbol myristate acetate (PMA, Sigma, St. Louis, Missouri) diluted in dimethylsulfoxide (DMSO, Sigma) was daily diluted in KRP. Zymosan A (Sigma) was opsonized by incubation for 30 min at 37°C with a pool of fresh serum (10%) from healthy individuals, then washed and resuspended in KRP at 20 mg/ml.

*Drugs.* Indomethacin and oxamethacin were from ABC, Torino, Italy. The drugs, dissolved in DMSO, were added, at concentrations varying from \( 10^{-7} \) to \( 10^{-4} \) M, in the samples compartment before the addition of PMNs. Pulsing experiments were also performed in which, before the measurement of \( \text{O}_2^- \) generation, PMNs were exposed to different concentrations of antiinflammatory agents for 15 min at 37°C, then washed and resuspended in KRP.

*Superoxide Anion Production by Zymosan-Stimulated PMNs.* \( \text{O}_2^- \) was detected by the reduction of ferricytochrome c that was inhibited by superoxide dismutase (SOD), according to the method of Gennaro and Romeo (10). Briefly, a mixture containing 1 \( \times 10^6 \) PMN in KRP, pH 7.4, and cytochrome c (type IV, Sigma) 3 mg/ml, was preincubated at 37°C for 3 min, then 2 mg/ml of opsonized zymosan was added to the reaction mixture in cuvette to obtain 1 ml final volume. The mixture was incubated at 37°C for 15 min, and the reaction was stopped by the addition of 1 mM \( N \)-ethylmaleimide (Sigma) in KRP medium. The tubes were centrifuged at maximal speed, and the absorbance of the supernatant was read at 550 nm. The amount of \( \text{O}_2^- \) produced was