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Effect of a New Fluorinated Quinolone (Ofloxacin) on the Chemiluminescence of Phagocytosing Human Neutrophils

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We measured the chemiluminescence (CL) of human neutrophils (PMNLs) exposed to different concentrations of ofloxacin (2, 4, and 6 μg/ml) readily achievable in therapy. CL reaction during zymosan phagocytosis by PMNLs obtained from human healthy volunteers was registered in a computer-linked LKB 1251 luminometer. Ofloxacin did not induce significant variations on the respiratory burst of PMNLs.

Keywords: Luminol-dependent chemiluminescence; polymorphonuclear leukocytes; ofloxacin

INTRODUCTION

Neutrophils are the most important cells in host defence against bacterial and fungal infections. In the past few years increasing interest has been given to the interactions between phagocytic cells and antimicrobial agents (Aquilini and Santini, 1987; Hauser and Remington, 1984; Yourtee and Root, 1984). Since antimicrobial agents are frequently administered, for therapy and prophylaxis, to granulocytopenic and other immunocompromised patients, the possible influence of antibiotics on phagocytic functions may be of clinical relevance. The aim of the present study was to evaluate the influence of ofloxacin on phagocytosis of human PMNLs.

Since generation of chemiluminescence (CL) during the process of phagocytosis is related to the bactericidal capacity of PMNLs (Allen *et al.*, 1972; Babior, 1978), we used luminol-dependent chemiluminescence to study the effect of ofloxacin on phagocytic function *in vitro*.

MATERIALS AND METHODS

Ofloxacin

Ofloxacin was supplied by Glaxo (Verona, Italy). The dry powder, free of buffers and preservatives, was dissolved in KRP just before use.

KRP: Krebs-Ringer phosphate (with Ca^{2+} 0.5 mmol/l and glucose 1 g/l) pH 7.4.

Preparation of PMNL

Human PMNLs were isolated from peripheral heparinized venous blood of healthy adult volunteers by one-step centrifugation of whole blood

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on Mono-Poly resolving medium (Flow Laboratories) (Ferrante and Thong, 1980). Contaminating erythrocytes were removed by osmotic lysis with hypotonic saline. Cell viability was assessed by tripan blue dye. PMNLs were washed twice and resuspended in KRP at a concentration of 10^6 cells/ml. The cells were preincubated at 37 °C for 1 h with three different concentrations of ofloxacin (2, 4, and 6 μg/ml), concentrations easily reached in serum during therapy. As controls we used cells suspended in KRP without antibiotic. The control solution was treated in the same way as the test solution.

CL assay

Chemiluminescence was measured with the method described by Siegel and Remington (1982) (modified) using 20 mg/ml Zymosan A (Sigma) opsonized for 45 min at 37 °C with a pool of human sera. Samples for CL assay contained 250 μl PMNL (10^6 cells/ml KRP), 100 μl luminol (Sigma) (5×10^6 mol/l in KRP), and 550 μl KRP. All samples were preincubated in the luminometer for 10 min at 37 °C; resting activity was then measured for 10 minutes, after which the stimulating agent (OZ, 100 μl) was added. After the addition of the stimulating agent, CL was measured every 3 minutes for 1 hour. Each specimen was tested in duplicate, and each experiment was repeated five times.

We used a computer-limited LKB 1251 luminometer which registers light emission in millivolts (mV) and allows assay of eight samples simultaneously. Photon emission was calculated based on the peak value of CL. Statistical analysis was done using Student's *t*-test.

RESULTS

Table 1 outlines the results of the various experiments. CL response was unaffected by ofloxacin. There was a statistically insignificant increase in CL values compared to those of controls at concentrations of 2 and 4 μg/ml, which correspond to average levels of the drug found in serum during therapy. Also statistically insignificant was the slight decrease at the 6 μg/ml concentration, which corresponds to the maximum concentration of the drug reached in serum 1 hour after administration of a full dose. Thus, we were unable to demonstrate any statistically significant differences between cells treated with the antibiotic and controls.

Table 1. Effect of ofloxacin on chemiluminescence of human PMNL

	Samples	CL (mV)	*P*
Control	10	46,904 ± 11,976	
OFX:			
2 μg/ml	10	62,889 ± 23,203	NS
4 μg/ml	10	52,609 ± 21,348	NS
6 μg/ml	10	34,600 ± 13,022	NS

DISCUSSION

Recently, various investigators have suggested using ofloxacin and other fluoroquinolones in the therapy and prophylaxis of infections in granulocytopenic and other immunocompromised subjects (Young, 1987). It is possible that even a slight depression of leukocyte function could be of clinical relevance in such subjects. Results obtained in our study show that ofloxacin does not induce significant effects on the respiratory burst of PMNL as measured by CL assay. Although it is difficult to establish a correlation between *in vitro* findings and any eventual effects *in vivo*, our data indicate that ofloxacin could be used in the treatment and prophylaxis of infections, in particular in patients with impaired host defences.

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