

Letters to the Editor

Inhibitory Effect of Erythromycin on Superoxide Anion Production by Human Neutrophils Primed with Granulocyte-Colony Stimulating Factor

We have recently demonstrated excessive neutrophil accumulation in the airways of patients with diffuse panbronchiolitis (DPB) and have also described therapeutic benefits of low-dose, long-term administration of erythromycin which are due to antiinflammatory rather than bactericidal action (2, 4, 5, 8–10, 12). The accumulation of neutrophils in the airway might contribute to lung damage through the release of proteases, free oxygen radicals, and other degradative enzymes (1, 4, 7). In this study, therefore, we attempted to elucidate whether erythromycin has a direct inhibitory effect on superoxide anion production by *N*-formyl-methionyl-leucyl-phenylalanine (FMLP)-stimulated human neutrophils primed with granulocyte colony-stimulating factor (G-CSF).

Neutrophils were isolated from the blood of healthy volunteers with mono-poly resolving medium (M-PRM; Flow Laboratories, Irvine, Scotland) and density gradient centrifugation and were suspended in Hanks' balanced salt solution (pH 7.4) (GIBCO, Grand Island, N.Y.) with 0.5% human serum at 10^7 cells/ml. O_2^- generation by FMLP-stimulated neutrophils was measured by determining the superoxide dismutase-inhibitable reduction of cytochrome *c* by a rapid microassay method (11). Neutrophils were incubated with various concentrations of erythromycin for 30 min at 37°C in a humidified atmosphere of 5% CO_2 followed by addition of the desired dose of G-CSF (Chugai Pharmaceuticals, Tokyo, Japan) for 10 min. The cells were finally stimulated with 10^{-7} M FMLP for 10 min at 37°C, and then the absorbance changes were measured at a wave-

length of 550 nm with a Multiskan instrument (Flow Laboratories, McLean, Va.). After each incubation, cell viability was confirmed to be >95% by the trypan blue dye exclusion method.

At concentrations of 10 and 50 ng/ml, G-CSF significantly primed the O_2^- generation by human neutrophils (Fig. 1), although G-CSF alone at 10 and 50 ng/ml induced no direct superoxide production by human neutrophils during a 3-h incubation period (data not shown). Erythromycin did not significantly affect O_2^- generation by unprimed neutrophils at any dose (Fig. 1). This confirmed previous observations that erythromycin at the clinically relevant dose of 1 μ g/ml (8) had no direct inhibitory effect on unprimed neutrophils stimulated with FMLP (3, 6) and also implies that the drug neither interferes with binding of FMLP to its receptor on neutrophils nor acts as a free radical scavenger. When neutrophils were only slightly primed by 5 ng of G-CSF/ml, only a 50- μ g/ml concentration of the drug significantly inhibited FMLP-stimulated O_2^- generation ($P < 0.05$). However, when human neutrophils were significantly primed by 10 or 50 ng of G-CSF/ml prior to FMLP stimulation to partly mimic the condition of the inflammatory site, the clinically relevant dose of erythromycin markedly suppressed O_2^- generation to the baseline levels observed for unprimed neutrophils stimulated with FMLP ($P < 0.01$), as did the higher doses of 10 and 50 μ g/ml (Fig. 1). This result suggests that erythromycin acts to modulate the production of neutrophil-derived oxygen radicals at the inflammatory site from an excessive to a normal response rather than to suppress their production, ultimately reducing epithelial injury in the airways of patients with DPB.

More adequate experimental designs mimicking conditions found at the inflammatory site are necessary to evaluate the possible beneficial nonantibiotic effect of erythromycin.

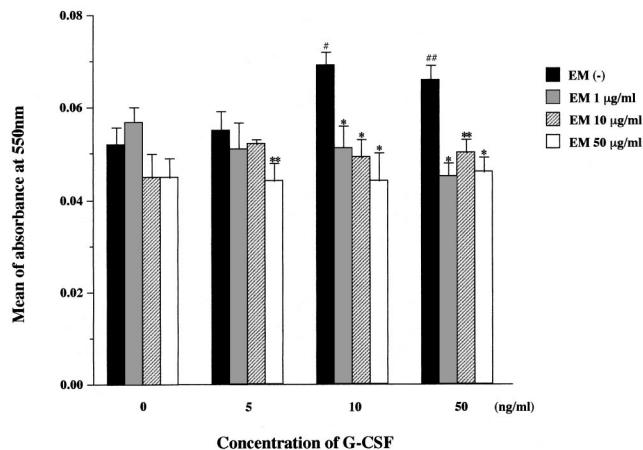


FIG. 1. Inhibitory effect of erythromycin on O_2^- generation by unprimed or G-CSF-primed human neutrophils stimulated with FMLP. Values are means (\pm standard errors of the means) of absorbance at 550 nm for three independent experiments. Statistical differences were determined by using the Student *t* test, and data were considered statistically significant when the *P* value was less than 0.05. Number signs indicate statistical significance of differences in O_2^- generation by primed versus unprimed neutrophils in the absence of erythromycin (EM) (#, $P < 0.01$; ##, $P < 0.05$). Asterisks indicate statistical significance of differences in O_2^- generation by G-CSF-primed neutrophils in the presence versus the absence of erythromycin at the indicated G-CSF concentrations (*, $P < 0.01$; **, $P < 0.05$).

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