In Vitro Killing of Erythromycin-Exposed Group A Streptococci by Polymorphonuclear Leukocytes

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After exposure to erythromycin, group A streptococci were tested for susceptibility to the antimicrobial activity of human peripheral blood neutrophils in the absence of the antibiotic. Bacterial susceptibility to phagocytic killing increased after prior exposure to supra-inhibitory levels of erythromycin for even as brief as three minutes. Extended exposure and higher concentrations of erythromycin increased phagocytic killing. Although the degree of sensitization varied in different strains of streptococci, all strains tested were significantly more susceptible to phagocytic killing after erythromycin exposure. Killing of erythromycin-treated bacteria that occurred in the absence of antibiotic was dependent upon internalization of the bacteria. Thus, the brief exposure of group A streptococci to inhibitory levels of erythromycin increases their susceptibility to phagocytic killing by peripheral blood neutrophils.

Erythromycin has been widely used for nearly 30 years as an alternative to penicillin for the treatment of infections due to group A streptococci. The goal of antibiotic prophylaxis for streptococcal infections is to maintain supra-inhibitory levels of the drug throughout the dosage interval. However, clinical studies of streptococcal infections in children have demonstrated that erythromycin regimens that maintain supra-inhibitory levels for only part of the dosage interval still eliminate the infections (1, 2). Moreover, at levels normally achieved in tissue, erythromycin is bacteriostatic (3). These observations indicate that the efficient removal of invading pathogens from infected tissues in the presence of erythromycin cannot be explained solely by its direct interaction with the pathogen.

Host factors are known to also play an important role in the response to antimicrobial agents. Experimental infections in which the efficacy of antibiotics was compared in normal and immunodeficient animals have clearly demonstrated the critical role of host factors for determining the curative dose and regimen of antibiotic therapy (4–6). These data agree with clinical observations that patients with impaired host defenses often respond poorly to antimicrobial therapy (7, 8). Previous studies in our laboratory have demonstrated that bacterial susceptibility to phagocytic killing is modulated after brief exposure of the bacteria to high levels of various antibiotics (9–11). These studies provided evidence that an antibiotic-neutrophil interaction occurs during phagocytic bactericidal activity which is independent of the continuing presence of the antibiotic.

In this study we investigated the effect that briefly exposing group A streptococci to supra-inhibitory concentrations of erythromycin had on phagocytosis and killing by human peripheral blood neutrophils in the absence of the antibiotic.

Materials and Methods

**Antibiotic Preparation.** Erythromycin was provided by Abbott Australasia Pty. Ltd., Australia. A stock solution of 1 mg erythromycin/ml ethanol was prepared from the pure powder and stored at -20°C. The antibiotic concentrate was diluted in medium-199 to the required concentration immediately before use.

**Bacteria.** Recent clinical isolates of group A streptococci were used. Streptococcal grouping was performed by latex agglutination (Streptex, Wellcome Reagents Ltd., UK). Strains were maintained in skim milk at -70°C. Prior to experiments, the bacteria were grown overnight on Columbia-base horse-blood agar. Bacteria in logarithmic phase of growth were prepared by inoculating 3–5 colonies into 10 ml of Todd-Hewitt broth. The bacteria were incubated for 3–4 h at 37°C to achieve a viable count of 5 × 10⁷–10⁸ bacteria per ml. For the preparation of antibiotic-pretreated bacteria, logarithmic-phase bacteria were mixed...
with an equal volume of the antibiotic dissolved in growth medium and incubated at 37 °C. Unless stated otherwise, the antibiotic concentrations were 4 times the minimum inhibitory concentration (MIC), and exposure time to the antibiotic was 30 min. To remove the antibiotic, the antibiotic-damaged bacteria were centrifuged for 1 min at 11,600 X g, and the bacterial pellet was resuspended in medium-199, pH 7.2 (Commonwealth Serum Laboratories, Australia). Bacteria without antibiotic exposure were treated in an identical manner. Various strains of group A streptococci were initially tested for their susceptibility to neutrophils after exposure to erythromycin. Subsequently, a single strain (strain 1) of Streptococcus pyogenes was used to investigate the parameters of antibiotic-induced modulations of phagocytic killing. This strain was isolated from pus of a patient with an infected abrasive skin lesion. Antibiotic susceptibility testing was carried out in Todd-Hewitt broth in a randomized block with factorial treatments. Independent, successive experiments provided between two and 15 replicates for each Streptococcus pyogenes strain. The four treatments analysed were the combinations of erythromycin (presence, absence) and neutrophils (presence, absence). Analyses were tabulated as means with standard deviations for each treatment. Student's t test was employed.

**Results**

**Erythromycin-Pretreatment of Streptococcus pyogenes and Susceptibility to Antibacterial Activity of Neutrophils**

The summarized results of seven experiments are shown in Figure 1. In this series of experiments the mean log10 decrease in viable bacteria, exposed for 30 min to 0.1 μg/ml (4 × MIC) of erythromycin, was 0.96 (SEM = 0.08) at 2 h compared with a log10 decrease of 0.1 (SEM = 0.07) of untreated Streptococcus pyogenes, when these bacteria were exposed to neutrophils (Figure 1A). This represents a 7-fold increased killing of erythromycin-exposed Streptococcus pyogenes compared to untreated bacteria. The decrease in viable bacteria in the presence of neutrophils could not be explained by any direct action of erythromycin, since the antibiotic was removed prior to exposure to neutrophils. In addition, the prior exposure of Streptococcus pyogenes to erythromycin had a small but significant inhibitory effect upon the regrowth of the bacteria in the absence of neutrophils, after removal of the antibiotic (Figure 1B). However, this effect was short-lived, and normal growth of the erythromycin-exposed bacteria resumed after approximately 1 h.

Heated serum failed to support phagocytic killing of control Streptococcus pyogenes. Killing of erythromycin-treated bacteria was greatly reduced in the