Comparison of four antibiotics in a murine model of necrotizing cutaneous infections caused by toxigenic *Streptococcus pyogenes* and *Staphylococcus aureus*

N. Barg*

Department of Veterans Affairs, Division of Infectious Disease, Medical Center, Ann Arbor, MI 48105, USA

The ability of azithromycin, erythromycin, clarithromycin, or cefuroxime to modify the course of group A streptococcus (GAS) or *Staphylococcus aureus* soft-tissue infection was compared in a mouse model. In GAS-infected mice given azithromycin, fewer demonstrated dermonecrosis ($P = 0.0004$); the average weight gain was greater ($P < 0.05$) and the latency to sustained weight gain was shorter ($P < 0.05$) than for animals given other antibiotics. All antibiotics were effective against *S. aureus* infections, with no significant differences among treatments in parameters evaluated. The effectiveness of azithromycin in GAS-infected mice may be related to the high and sustained tissue concentrations achieved with this antibiotic.

Introduction

Infections caused by group A streptococci (Streptococcus pyogenes, GAS) are characterized by severe soft-tissue damage, including necrotizing fasciitis and marked systemic toxicity; they may induce rapid multiorgan failure, hypotension, and even death in immunocompetent hosts. Tissue invasion by *Staphylococcus aureus* may result in localized cellulitis or abscess; more serious infections have been associated with bacteremia, endocarditis, mediastinitis, osteomyelitis, septic arthritis and pneumonia.†

Penicillin and erythromycin are commonly used to treat GAS and *S. aureus* infection. However, the efficacy of penicillin is often diminished in severe infections, perhaps as a result of the lower growth rate of streptococci in large inoculum sizes. Erythromycin use is limited by gastrointestinal intolerance and the requirement for frequent dosing. Treatment of GAS and *S. aureus* infections also can be complicated by the presence of antibiotic-resistant strains.‡

The present study compared the ability of erythromycin, cefuroxime, clarithromycin and azithromycin to modify the course of serious cutaneous infections caused by Gram-positive cocc. The agents were selected on the basis of their ability to interfere with protein or cell-wall synthesis and evaluated using a published mouse model of foreign-body GAS and *S. aureus* soft-tissue infection.†

Materials and methods

Experimental animals

All experiments were conducted in 4 week old (body weight 15-20 g) outbred, immunocompetent, hairless mice (strain Crl:SKH1[hrhr][Br]) obtained from Charles River Laboratories (Wilmington, MA, USA). The antibiotic treatment group for each pathogen consisted of 15 mice. Five animals (positive controls) were infected but did not receive antibiotic treatment, and five served as uninfected (negative) controls.

Bacterial strains

The *S. pyogenes* strain used in these experiments was GAS 166, previously isolated from a patient with streptococcal toxic shock. The *S. aureus* strain was SL C3 (phage type 94/96), a nosocomial clinical strain isolated from a patient with a deep sternal wound infection and similar to the strain isolated from patients with nosocomial infections at five locations in the USA.‡

All strains were subcultured from brain-heart infusion (BHI, Difco, Detroit, MI, USA) agar plates to 10 mL of BHI broth and incubated at 37°C for 2.5-3 h until an OD$_{600}$ of 1.0 was achieved. Serial dilutions were prepared and plated to determine the actual inocula used for the experimental infections.
N. Barg

Preparation of foreign material and induction of infections

Dextran beads (Cytodex; Sigma Chemicals, St Louis, MO, USA), the foreign material, were prepared as published. Aliquots of Cytodex suspension (0.02 mL) were added to 0.5 mL of the BHI broth with or without bacteria. An additional broth was added to achieve a final volume of 1 mL, and 0.2 mL of the suspension was injected into the right flank of each mouse with a 1 mL tuberculin syringe. Each inoculated animal received an injection of $10^6$ cfu of GAS or $10^7$ cfu of S. aureus. Uninfected controls received an injection of the Cytodex suspension and BHI broth without bacteria.

Antibiotics

Azithromycin, clarithromycin, erythromycin, and cefuroxime were prepared according to the manufacturers’ instructions, and 0.2 mL of the antibiotic solutions (18 mg/kg) were injected subcutaneously approximately 30 min before inoculation with bacteria. The selected dose was the lowest dose at which significant decreases in the size and number of lesions were found in preliminary dose–response data using azithromycin in mice infected with the same bacterial strains. As the MICs of azithromycin for the inoculated strains of GAS and S. aureus are greater than those for the other antibiotics, equivalent doses of the other agents could be expected to provide protection equal to or greater than that of azithromycin.

Efficacy assessments

All animals were weighed by an observer unaware of antibiotic administrations immediately after and at 24 h (for the first 5 days) and then at 48 h intervals for a total of 14–21 days. The size of necrotic skin lesions was calculated using the formula for the area of an ellipse: $A = \pi(LW)/2$ where $L$ is length and $W$ is width. Lesion sizes were measured with a caliper, recorded each day, and plotted. The area under the curve of lesion size plotted against days served as a measure of disease severity.

Statistical analysis

Data from the treatment groups were compared by means of analyses of variance (ANOVA) as published. Post-hoc comparisons between groups were accomplished with Fisher’s protected least significant difference. The accepted level of significance for all tests was $P < 0.05$.

Results

All study drugs provided some protection from GAS infection, although azithromycin was more effective than the other antibiotics. No deaths occurred among animals that had not been infected or had received azithromycin. In contrast, one of the 15 animals that received cefuroxime, two of the 15 that received clarithromycin, and two of the 15 that were treated with erythromycin died. Three of the five infected but untreated mice died. Significant differences were noted among treatment groups in the incidence of dermonecrosis (Figure 1). None of the azithromycin-treated mice exhibited dermonecrosis, compared with 12 that received cefuroxime, ten given clarithromycin, eight treated with erythromycin, and all the infected, untreated mice ($P < 0.0004$). Differences among groups were also significant with respect to weight gain during the first 24 h of infection ($P < 0.05$; Figure 2) and the number of days until consistent daily weight gain was observed ($P < 0.05$). Azithromycin-treated mice gained an average of 0.7 g, significantly more than in the other antibiotic treatment groups ($P < 0.05$). Uninfected mice and mice that received azithromycin required a mean of 1 day until consistent daily weight gain occurred, compared with 3.2 days for animals given cefuroxime, 3.9 days for mice treated with clarithromycin, and 2.9 days for mice that received erythromycin ($P < 0.05$). Infected but untreated animals needed an average of 10.2 days to achieve consistent daily weight gain.

Figure 1. Percentage of GAS-infected mice with dermonecrosis in each antibiotic-treated group and in the infected but untreated, positive control group.

258
Antibiotics in murine cutaneous infections

The S. aureus infections were less severe than those produced by inoculation with GAS. Although dermonecrosis developed in 80% of the infected, untreated animals, this condition was not observed in any treated mice. A tibiotic treatment significantly reduced the severity of disease, as reflected by differences between positive controls and antibiotic-treated groups in the area under the lesion size-versus-day curve \((P < 0.001)\). Differences among antibiotic treatment groups, however, were not significant. A tibiotic-treated mice also did not differ significantly with respect to weight gain during the infection.

Discussion

Erythromycin, cefuroxime, clarithromycin and azithromycin can modify the course of severe cutaneous infections caused by GAS or S. aureus to varying degrees. In mice infected with GAS and treated with azithromycin, the elimination of dermonecrosis, weight gain during the 24 h after infection and latency to the onset of consistent daily weight gain after infection were significantly different from infected, untreated mice and those treated with the other antibiotics. These results are consistent with those of previous studies demonstrating the significantly greater efficacy of azithromycin relative to erythromycin and clarithromycin in models of localized infections due to S. aureus in rats and mice and an infection induced with an implanted disc inoculated with S. pyogenes.\(^8\,\!^9\)

Care should be taken in interpretation of the results of this study. A ministration of a single dose favours drugs with longer half-lives and sustained tissue concentrations. The optimal dosing regimens for antibiotics with shorter half-lives were not established, and higher or successive doses may be beneficial. A ministration of single doses, however, allows discrimination of the relative potency of the tested agents. In addition, the results reflect effects early in the course of infection. Alternative studies with increasing intervals between administration of antibiotic and infection are in progress.

Even though azithromycin in vitro had less activity against GAS than the other agents, it was more effective in preventing dermonecrosis. Several factors may explain the differences in response to the macrolides and cefuroxime in GAS-infected animals. Erythromycin, clarithromycin and azithromycin produce their antibacterial activity by binding to the 50S ribosomal subunit, thereby inhibiting natural RNA-directed polypeptide synthesis.\(^4\) It is possible that inhibition of synthesis of cytotoxic proteins prevented tissue damage in treated mice. Furthermore, azithromycin may have inhibited protein synthesis more than the other agents.

The higher efficacy of azithromycin in GAS-infected mice in the present study also may result from its ability to achieve and maintain high concentrations in infected tissues.\(^4\) Although tissue concentrations of the test agents were not measured, the results presented here show that a single dose of an antibiotic can effectively modify the course of cutaneous infections. In addition, the accumulation of azithromycin by phagocytes and potential for phagocyte-mediated delivery provide a mechanism for supplying antibiotic to the site of infection.\(^10\)

The pharmacokinetic characteristics of macrolides permit them to overcome three potential limitations of \(\beta\)-lactam antibiotics in the treatment of GAS or S. aureus soft-tissue infection: (i) inadequate penetration of the antibiotic into tissues which may be associated with high tissue pressures; (ii) slow replication of bacteria, resulting in minimal bactericidal effects; and (iii) a mechanism of action that is not directed at inhibition of bacterial protein synthesis.\(^1\) Macrolides, especially azithromycin, exhibit extensive tissue penetration and bacterial killing as a result of sustained interference with protein synthesis. These attributes as well as clinical results\(^4\) support the use of macrolides for the management of uncomplicated soft-tissue infections caused by susceptible strains of GAS or S. aureus.

References


Received 17 October 1997; returned 1 December 1997; revised 19 January 1998; accepted 6 March 1998