Treatment of Experimental Staphylococcal Infection with Rifampin

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Bacteria surviving within leukocytes are protected from the lethal action of high concentrations of most antibiotics, which may explain, in part, the failure of bactericidal antibiotics to eradicate staphylococci from abscesses. Since rifampin is unique in its ability to kill intraleukocytic bacteria, the efficacy of this drug was tested in the treatment of staphylococcal infections in mice. Groups of mice infected intravenously with Staphylococcus aureus were treated with rifampin (20 mg/kg), procaine penicillin (937.5 mg/kg), or methicillin (200 mg/kg). All untreated mice died with disseminated visceral abscesses. After 10 days of therapy, survival in groups treated with penicillin and methicillin was 16 and 20%, respectively, whereas with rifampin it was 80% (P < 0.0005). Antibiotic concentrations in the serum of mice treated with penicillin, methicillin, or rifampin were bactericidal for the strain of S. aureus used. Serial bacterial counts of kidney, lung, and spleen homogenates showed that neither penicillin nor methicillin was able to eradicate staphylococci, whereas rifampin completely sterilized those organs in many mice. When abscess contents and infected peritoneal washings were incubated with high concentrations of penicillin, methicillin, or rifampin, only rifampin killed all of the bacteria. The capacity of rifampin to eradicate staphylococci from pus in vitro and from abscesses in mice appears to be related to the ability of rifampin to kill intraleukocytic bacteria.

Staphylococcus aureus can survive the intraleukocytic environment after being phagocytized by polymorphonuclear neutrophils (PMN; 12). Several investigators (1, 7, 13) have shown that bacteria surviving within phagocytes are protected from the lethal actions of high concentrations of most antibiotics. In vitro studies with 10 bactericidal antibiotics (11) showed that only rifampin was able to kill intraleukocytic staphylococci.

Rifampin is a semisynthetic antibiotic derivative of rifamycin B, with a fairly broad antibacterial spectrum (3, 8, 9) and potent antimicrobial activity (2). It has also been demonstrated in vitro that rifampin does not interfere with phagocytosis and bactericidal activity of PMN (6).

The present studies were undertaken to determine whether rifampin could eradicate staphylococci from abscesses or influence mortality in an experimental staphylococcal infection in mice.

MATERIALS AND METHODS

Animals. White, 20-g male mice (strain DUB/ICR, Dublin, Virginia, Institute for Cancer Research) were used.

Organism. The Wood 46 strain of S. aureus (coagulase-positive, hemolytic, penicillin-susceptible, mouse-virulent) was used in all experiments. This organism was passed daily on 5% sheep blood-agar plates, and 18-hr-old cultures in tryptic soy broth were used to prepare bacterial inocula. Mice were infected by injecting 10⁴ washed staphylococci in 0.1 ml of 0.9% saline into tail veins. Bacterial counts of each inoculum were determined by standard serial dilution and pour-plate techniques.

Antibiotics and antibiotic susceptibilities. Rifampin (Rimactane diagnostic powder, a gift from Ciba Pharmaceutical Co.) was dissolved in methanol and diluted with Hanks balanced salt solution (final concentration of 0.02% methanol in a 0.2-ml injection). This amount of methanol was not toxic to mice (11). Methicillin was given as sodium methicillin and penicillin was given as procaine penicillin G. Assays of minimal bactericidal concentrations (MBC) of antibiotics were performed by a twofold serial dilution technique in nutrient broth with an inoculum...
of 10^8 organisms per tube. After incubation at 37°C for 48 hr, 0.1 ml of broth from tubes showing no turbidity was spread on tryptic soy agar plates. The MBC was defined as the lowest concentration of antibiotic giving no growth in the 0.1-ml subculture.

**Treatment of infected mice.** Three days after an intravenous injection of *S. aureus*, mice were randomly divided into four groups. The first group received no antibiotic, the second group received intramuscular injections of procaine penicillin (30,000 units or 18.75 mg per mouse), the third group received intraperitoneal injections of methicillin (4.0 mg per mouse), and the fourth group received intraperitoneal injections of rifampin (0.4 mg per mouse). Antibiotics were given once daily for 10 days. The mice were observed for 20 days after completion of treatment to evaluate the effect of the antibiotics on mortality.

**Tissue bacterial counts.** Three days after an intravenous injection of *S. aureus*, mice were randomly divided into four groups and treated as described in the preceding section. Viable staphylococci were enumerated in organs of infected mice by methods previously described (10). At 1 to 20 days after infection, mice were sacrificed and organs were removed by aseptic techniques. Tissue emulsions were made in distilled water by use of a Teflon homogenizer. Organ volume was measured by determining displacement of normal saline. Bacterial counts in the homogenates were obtained by standard serial dilution and pour-plate techniques.

**Antibiotic concentrations in serum.** Groups of normal mice were studied 1, 3, 6, 18, and 24 hr after antibiotic administration to measure serum antibiotic activity. Percutaneous cardiac puncture of mice anesthetized with ether was utilized to obtain approximately 0.8 ml of blood. The cylinder-plate method was used for all assays of antibiotics (5). Standard curves were constructed from reference standards tested with *Sarcina lutea* (ATCC 9341). All sera and standards were prepared in normal mouse serum diluted 1:2 with 0.067 M potassium phosphate buffer, pH 7.35.

**Effect of antibiotics on staphylococci in pus or peritoneal exudates.** Susceptions of leukocytes with intracellular staphylococci were obtained from two sources. (i) Abscesses: 3 to 4 days after injection of staphylococci, mice usually developed renal abscesses. Stained smears of pus from these abscesses showed many PMN with intracellular and extracellular staphylococci. A 0.3-ml amount of pus was aspirated from abscesses and mixed with 2.7 ml of Hanks balanced salt solution with 20% fetal bovine serum plus 1% chick embryo extract. (ii) Peritoneal exudate: 2 hr after an intraperitoneal injection of 10^8 staphylococci, mice were killed and the peritoneum was washed with 5 ml of cold medium 199. Examination of stained smears demonstrated mononuclear phagocytes with intracellular and extracellular staphylococci. About 10% of the leukocyte population were PMN. The peritoneal washings were centrifuged at 500 × g at 5°C for 5 min. The sediment containing the leukocytes and bacteria was made up to 1.0 ml by adding cold medium 199 with 20% fetal bovine serum plus 1% chick embryo extract.

Each sample of leukocyte suspension was divided into three 1-ml portions. Rifampin (10 μg) was added to one, penicillin (100 units) was added to another, and no antibiotic was added to the third sample. The tubes were then agitated with a vortex mixer and tumbled end over end for 18 hr at 37°C. At the end of this time, the tubes were iced and centrifuged at 200 × g for 5 min. The supernatant fluid was decanted, and serial dilutions and pour-plate cultures were done to obtain viable bacterial counts. The sediment was washed with saline to remove antibiotics and was resuspended and agitated in distilled water to lyse the leukocytes. The number of viable sediment-associated bacteria was determined by serial dilution and pour-plate techniques.

**RESULTS**

As shown in Fig. 1, there were no survivors among untreated mice by 16 days after infection with staphylococci. Only 16% of mice treated with penicillin and 20% of mice treated with methicillin survived for 30 days after infection. In marked contrast, treatment with rifampin resulted in 80% survival (*P < 0.0005*).

Serial bacterial counts of kidney, lung, and spleen showed that rifampin-treated animals had significantly lower tissue bacterial counts and more sterile organs (Fig. 2–4).

The Wood 46 strain of *S. aureus* was susceptible to the antibiotics used in these experiments. The MBC for penicillin, methicillin, and rifampin was 0.098 units/ml (0.059 μg/ml), 3.13 μg/ml, and 0.195 μg/ml, respectively. Antibiotic concentrations attained in serum were bactericidal (Fig. 5) for at least part of the day with all three antibiotics.

Only rifampin sterilized pus or peritoneal washings after an overnight incubation, but rifampin, penicillin, and methicillin killed all of the staphylococci in the supernatant fluid (extracellular organisms; Fig. 6 and 7). All three antibiotics lost activity when incubated with infected peritoneal exudate. After 18 hr, 100 μg of methicillin activity/ml decreased to 6.8 μg/ml, 100 units of penicillin activity/ml dropped to 53 units/ml, and 100 μg of rifampin activity/ml fell to 60 μg/ml.

**DISCUSSION**

Rifampin treatment of mice infected with staphylococci was much more effective than treatment with penicillin or methicillin. All three antibiotics reached concentrations in serum well in excess of the MBC, and rifampin- and penicillin-treated animals had significant
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FIG. 1. Effect of antibiotics on survival of mice infected with staphylococci. A total of 270 animals were injected intravenously with $10^8$ Staphylococcus aureus (Wood 46) cells. On the third day after infection, groups of 60 mice were treated with procaine penicillin (30,000 units or 18.75 mg per mouse), methicillin (4.0 mg per mouse), or rifampin (0.4 mg per mouse) daily for 10 days; 90 mice were left untreated.

FIG. 2. Effect of treatment on staphylococcal populations in the kidneys of infected mice. Animals were injected intravenously with $10^8$ Staphylococcus aureus (Wood 46) cells. On the third day after infection, mice were treated with procaine penicillin (30,000 units or 18.75 mg per mouse), methicillin (4.0 mg per mouse), or rifampin (0.4 mg per mouse) daily for 10 days. A group of mice was left untreated. At least five mice from each group were sacrificed daily. Organ bacterial counts are expressed as the geometric mean (standard errors were less than 1 log). Animals with sterile organs/animals studied each day are shown. Days with no numerical notation represent no sterile organs.
**FIG. 3.** Effect of treatment on staphylococcal populations in the lungs of infected mice. Animals were treated and data are expressed as described in Fig. 2.

**FIG. 4.** Effect of treatment on staphylococcal populations in the spleens of infected mice. Animals were treated and data are expressed as described in Fig. 2.
antibiotic concentrations for most of the treatment period. McCune et al. (10) found that penicillin, streptomycin, and penicillin plus streptomycin failed to eradicate staphylococci from the organs of infected mice. We confirmed these findings with penicillin and also showed that methicillin did not sterilize organs. However, rifampin completely sterilized the lungs, kidneys, and spleens in most of the treated mice. Of interest are the studies of Batten (4) demonstrating that 4 months of therapy with rifampin (but not isoniazid) could sterilize the lungs and spleen of mice infected with Mycobacterium tuberculosis.

This ability of rifampin to eradicate bacteria in vivo correlates with its ability to sterilize peritoneal exudates and pus, and to kill intraleukocytic bacteria. The ability of rifampin to penetrate phagocytic membranes and kill ingested organisms may well be the explanation for its marked efficacy in vivo.

The problem of the emergence of rifampin-resistant strains has been discussed by Kunin et al. (8), but resistance to rifampin was not encountered in the present studies.

These observations suggest that rifampin has a place in the treatment of deep-seated infections associated with abscess formation when surgical
drainage is not feasible. Another area where rifampin may be useful is in the treatment of patients whose leukocytes are unable to kill ingested bacteria normally (i.e., patients with chronic granulomatous disease of childhood or Chediak-Higashi syndrome). In these situations, rifampin probably should be used with another antibiotic to minimize the development of resistant strains.

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LITERATURE CITED