Short-Term Administration of Rifampin in the Prevention or Eradication of Infection Due to Foreign Bodies

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Short-term administration of rifampin was evaluated as a means of preventing or eradicating infection due to foreign bodies. Tissue cages were implanted into guinea pigs and subsequently infected with 10^3 colony-forming units of Staphylococcus aureus Wood 46. Infection developed in all tissue cages. Rifampin was administered thereafter intraperitoneally at a dosage of 7.5 mg/kg every 12 hr for 48 hr, and the tissue-cage fluid was monitored for possible development of infection by quantitative bacteriologic methods for 15 days. In all cases rifampin prevented or eradicated tissue-cage infection if treatment was initiated either 3 hr before or ≤12 hr after inoculation of microorganisms but was ineffective if initiated >12 hr after inoculation. In cases of failure of treatment, rifampin-resistant variants could be demonstrated. Rifampin seems to prevent or eradicate tissue-cage infection only if given early after bacterial inoculation.

The development of an infection in the vicinity of a foreign body or prosthetic material remains a major problem in orthopedic, cardiac, and general surgery [1–3] as well as in internal medicine [4]. Conceptually, three major groups of pathogenic factors could contribute, either singly or in combination, to the development of such infections. Altered virulence and/or surface modifications of the infecting organisms could protect them from host-dependent antibacterial systems or from antimicrobial agents; alternatively, the foreign or prosthetic surface per se could favor, either directly or indirectly, the propagation of the offending microorganism; finally, the host's natural defense mechanisms against pyogenic organisms might be altered in the vicinity of a foreign body. Preliminary experimental data that favor the latter two mechanisms have recently been presented [5, 6]. Little is known about the microbiologic determinants of prosthetic infections; however, the efficacy of preventive, short-term antibiotic thera-

py [7, 8], in contrast to the inefficacy of antibiotic therapy initiated after infection has developed, suggests a change in bacterial behavior during the establishment of foreign-body infection.

Since it is generally accepted that the consequences of infection in the vicinity of foreign material are disastrous, interest is growing in the establishment of guidelines for regimens of preventive antibiotic treatment either during insertion of prosthetic devices or during later septic manipulations. Because of the lack of an animal model, few solid data are available about optimal dosage, timing, and duration of such prevention programs and the choice of antibiotics.

In the present study we used an experimental model of foreign-body infection recently developed in our laboratory [6] to address these questions. In particular, we evaluated the efficacy of rifampin, an antibiotic with high antistaphylococcal activity and good cell membrane penetration [9–11], in preventing or eradicating foreign-body infection.

Materials and Methods

Experimental model. Sterile polytetrafluoroethylene (Teflon®) tissue cages constructed as previously described [6], with external and internal diameters of 10 mm and 8 mm, respectively, and perforated by 130 regularly spaced holes were implanted under strictly aseptic conditions into the flanks of guinea pigs weighing 500–600 g.

Animals were used for experimental infections after the incision had healed completely and the metal clips had been removed, i.e., two or more weeks after surgery.

Collection and storage of tissue-cage fluid. Samples of tissue-cage fluid were obtained by percutaneous aspiration, which has been described previously for a similar model [12]. Thereafter, samples either were used directly for bacteriologic quantitation or were stored at −70 C for further determinations.

Microbiologic characteristics of the infecting strain. Aliquots of the same initial culture of *Staphylococcus aureus* Wood 46, stored at −70 C, were used for all experiments. This strain was shown by the Kirby-Bauer method [13] to be sensitive to all common antibacterial agents tested, including penicillin, methicillin, erythromycin, tetracycline, and rifampin [14]. When submitted to a standard scheme for staphylococcal identification, the strain gave positive reactions for catalase production, plasma coagulase reaction, DNase activity, and mannitol fermentation [14].

Before inoculation into tissue cages, *S. aureus* Wood 46 was preincubated overnight in Mueller-Hinton broth, centrifuged, washed, and resuspended in 1.0 ml of 0.85% saline; 0.2 ml of this diluted suspension was injected into each tissue cage, resulting in an inoculum of ∼10^3 organisms. Clumping of microorganisms was kept at a minimum under these preincubation conditions and was monitored regularly by microscopic evaluation.

Microorganisms isolated from infected tissue cages were checked for their identity with the initial, inoculated strain by the above-mentioned biochemical tests. Appropriate antibiotic susceptibility tests also were performed for confirmation of identity between the inoculated and the isolated microorganisms.

Microbiologic techniques. Rifampin assays were performed with a microbiologic method in which *Sarcina lutea* was used as the indicator strain and DST (diagnostic sensitivity) Oxoid, (Oxoid Ltd., Beckingham, England) with 3 ml of $KH_2PO_4(1M)/100$ ml was used as the medium [15]. The assay was linear for concentrations of rifampin of 0.12 µg/ml–1.0 µg/ml. Pure rifampin (Ciba-Geigy, Basel, Switzerland) was dissolved in N, N-dimethylformamide (Sigma, St. Louis, Mo.) at a concentration of 1 mg/ml. Further dilutions were performed in PBS (Dulbecco's phosphate-

buffered saline; Gibco-Bio-Cult., Glasgow, Scotland). This solution was used for the standard curves, which were established for each assay. Identical standard curves were obtained when PBS was partially substituted with 25% serum or 75% tissue-cage fluid.

Quantitative cultures of tissue-cage fluid were performed by plating 0.1 ml on Mueller-Hinton agar, after making appropriate dilutions in sterile water, and incubating cultures for 48 hr before performing colony counts. Absence of spontaneous contamination of tissue-cage fluids was confirmed before starting each experiment by culturing undiluted fluid on Mueller-Hinton agar. Finally, in some experiments tissue cages were excised under aseptic conditions and checked for the presence of microorganisms by rolling the cages over Mueller-Hinton agar plates and culturing the plates subsequently in Mueller-Hinton broth.

Results

Choice of initial inoculum and of rifampin regimen. In a first series of experiments, the rate of development of infections was quantitated by inoculating various numbers of *S. aureus* Wood 46 into the tissue cages and by evaluating bacterial multiplication as a function of time. As shown in figure 1, infection of the tissue-cage fluid was achieved with initial inocula as small as 10^2 cfu and was readily demonstrable bacteriologically by 20 hr. With an inoculum of ⩾10^3 cfu, quantitation of bacterial counts at 22 hr showed the presence of ∼10^5–10^8 organisms/ml, most of them identified as single cocci by microscopic examination. When bacterial counts were measured after the first 24 hr, they fluctuated considerably—probably because of bacterial clumping. Finally, control curves showed that bacterial growth in Mueller-Hinton broth or under in vitro conditions in tissue-cage fluid was more rapid than in the in vivo system. These results considered together suggested that 10^3 cfu of *S. aureus* Wood 46 was an adequate initial inoculum, that an observation period of 20 hr was sufficient for the demonstration of an infection in the absence of antibiotics, and that fluid accumulating in tissue cages did not provide particularly favorable growth conditions for *S. aureus* Wood 46.

For evaluation of the efficacy of rifampin in ex-
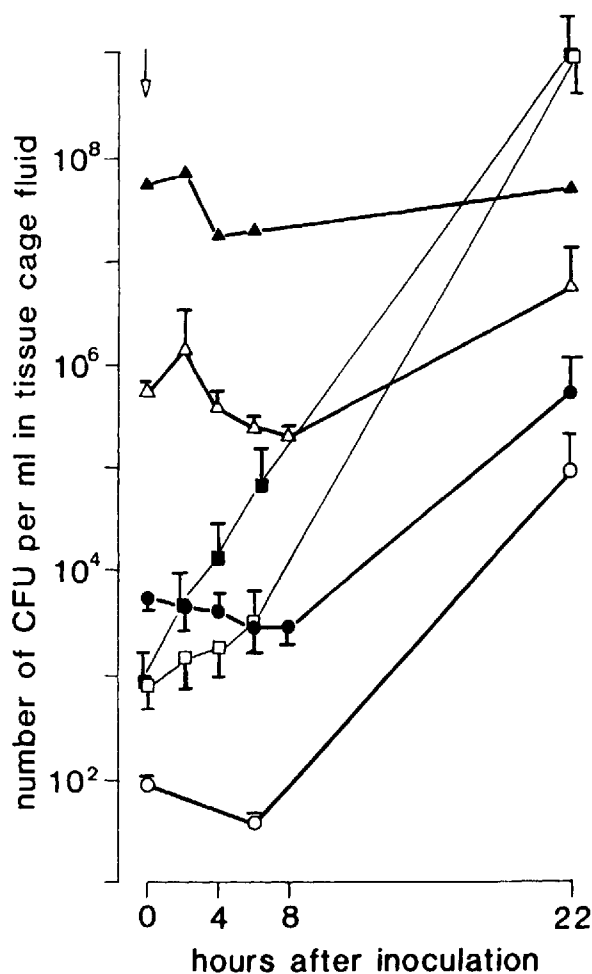
Figure 1. Growth curves of *Staphylococcus aureus* Wood 46 in tissue cages in vivo and control values of bacterial growth in either 100% tissue-cage fluid (□; n = 3) or Mueller-Hinton broth (■; n = 3) in vitro. Indicated inocula of *S. aureus* were injected into tissue cages at time zero (arrow). Values are reported as the mean ± SEM of the cfu at the indicated time intervals after experimental infection with the following initial inoculum: $\sim10^2$ cfu (O——O; n = 8), $\sim10^4$ cfu (●——●; n = 4), $\sim10^6$ cfu (△——△; n = 4), $\sim10^8$ (▲——▲); n = 2).

perimental tissue-cage infections, an antibiotic regimen had to be chosen that gave concentrations of antibiotic in tissue-cage fluid exceeding the MIC and MBC for *S. aureus* Wood 46. The MIC at 18 hr in Mueller-Hinton broth was 0.005 µg/ml with an initial inoculum of 10^5 cfu. The MBC, which was defined as the antibiotic concentration leading to a killing of 99.9% at 24 hr, was 0.046 µg/ml; no skip-tube phenomenon was observed. When tests were made in Mueller-Hinton medium supplemented with 25% pooled guinea pig serum, MIC and MBC values were 0.023 µg/ml and 0.046 µg/ml, respectively. Several antibiotic regimens were examined for the purpose of obtaining levels of antibiotic in tissue-cage fluid exceeding the MIC and MBC during the entire treatment period. Figure 2 shows that with a regimen of 7.5 mg of rifampin/kg given ip every 12 hr, trough levels of rifampin at 24 hr and 48 hr after initation of therapy were 0.115 ± 0.035 and 0.15 ± 0.027 µg/ml, respectively, values exceeding the MIC for *S. aureus* Wood 46 by factors of 5 and 6.5, respectively.

Partial prevention or eradication of infections of tissue-cage fluid with rifampin: emergence of rifampin-resistant mutants. Seven different treatment schedules (A–G; table 1) were evaluated; they differed by one parameter only—i.e., the time lag between the inoculation of the microorganisms and the initiation of therapy. This time lag varied from −3 hr (for treatment group A; antibiotic regimen started 3 hr before inoculation) to 48 hr (for group G; antibiotic regimen started 48 hr after inoculation). Tissue-cage fluids of groups A–F were aspirated and cultured 24 hr, 48 hr, six days, and 15 days after initiation of therapy.

In group G the establishment of infection was confirmed 24 hr before and at the beginning of rif-
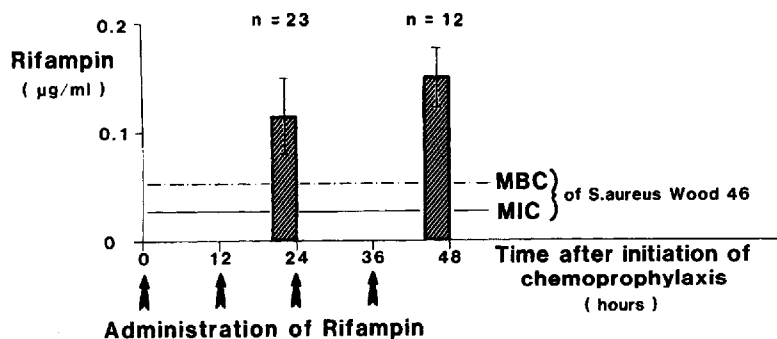
Figure 2. Trough level of rifampin (µg/ml) in tissue-cage fluid 24 hr and 48 hr after administration of 7.5 mg of rifampin/kg ip every 12 hr for 48 hr. The solid line represents the MIC and the dotted line, the MBC of the infecting strain, *Staphylococcus aureus* Wood 46.

Table 1. Treatment regimens for guinea pigs with foreign-body infections.

Prophylaxis or treatment group	Rifampin administered at indicated interval (hr) before (−) or after (+) inoculation	No. of tissue cages inoculated*
A	−3	7
B	0	8
C	+3	10
D	+6	8
E	+12	8
F	+24	8
G	+48	4

NOTE. Tissue-cage fluid was sampled for quantitative cultures at 24 hr, 48 hr, six days, and 15 days after the onset of the antibiotic prophylaxis or therapy.

* Tissue cages were inoculated with 10^3 cfu of *Staphylococcus aureus* Wood 46. Dosage of rifampin was 7.5 mg/kg ip every 12 hr for 48 hr.

ampin treatment. Further cultures of tissue-cage fluid were performed at 24 hr, six days, nine days, and 15 days after initiation of therapy. Results of these experiments are described in tables 1 and 2. When rifampin was administered either before, during, or 12 hr after inoculation of *S. aureus* Wood 46, tissue cages were invariably sterile when tested six days or 15 days after initiation of the 48-hr treatment regimen. Eradication of infection was achieved at the end of therapy, even in cases for which tissue-cage fluids were culture positive at 48 hr, i.e., contained up to 10^3 cfu/ml. If initiation of therapy was delayed beyond 12 hr after inoculation, infection could not be eradicated;

despite the fact that tissue-cage fluids were negative six days after initiation of the 48-hr therapy, eight of 11 were positive at 15 days, with bacterial counts ranging from 10^2 to 10^8 cfu/ml. It is of interest that after administration of antibiotic infection in the tissue-cage fluid recurred slowly, requiring observation periods of up to 15 days for confirmation of all relapses. These results were in striking contrast with those obtained in the absence of the antibiotic, for which bacterial growth could consistently be demonstrated in tissue-cage fluid 12 hr after inoculation.

Because of the delay in recurrence of infection, we investigated whether negative cultures actually reflected the inadequacy of the technique of aspirating tissue-cage fluid for collecting bacteria closely associated with the foreign body. Tissue cages with sterile fluid cultures were therefore excised 15 days after initiation of effective rifampin treatment and cultured according to the technique described in Materials and Methods; all cages were free of organisms. In one case of ineffective treatment—rifampin was started 48 hr after inoculation of *S. aureus* (group G)—tissue-cage fluid was negative at 15 days, whereas the excised tissue cage showed growth with both techniques (table 2 and figure 3).

The time course of the recurrence of infection is depicted in more detail in figure 3 for four tissue cages in animals in which rifampin treatment was started 48 hr after inoculation of bacteria. Bacterial counts decreased from initial values of 2.5 × 10^4–1.6 × 10^6 48 hr after inoculation to <10 cfu

Table 2. Effect of time of initiation of rifampin regimen on protection against or eradication of foreign-body infection.

Treatment group, no. of hr before (−) or after (+) inoculation that rifampin regimen initiated*	No. of tissue-cage fluids positive for infecting organism on indicated day after initiation of rifampin/no. inoculated[†]			
	1	2	6	15
A, −3	0/7	1/7 (10^1–10^2)	0/7	ND
B, 0	0/4	0/4	0/4	0/4[‡]
C, +3	0/10	0/10	0/10	ND
D, +6	4/8 (10^1–10^3)	4/8 (10^1–10^3)	0/8	ND
E, +12	6/8 (10^1–10^3)	0/16	0/16	0/8[‡]
F, +24	5/8 (10^2–10^4)	11/15 (10^1–10^8)	0/15	4/7 (10^8)
G, +48	4/4 (10^3–10^5)	ND	0/4	4/4 (10^1–10^6)[§]

* Tissue cages were inoculated with 10^3 cfu of *Staphylococcus aureus* Wood 46. Dosage of rifampin was 7.5 mg/kg ip every 12 hr for 48 hr.

[†] Numbers in parentheses are cfu/ml of tissue-cage fluid. ND = not done.

[‡] All excised tissue cages also were culture negative.

[§] In one case only the tissue cage was culture positive.
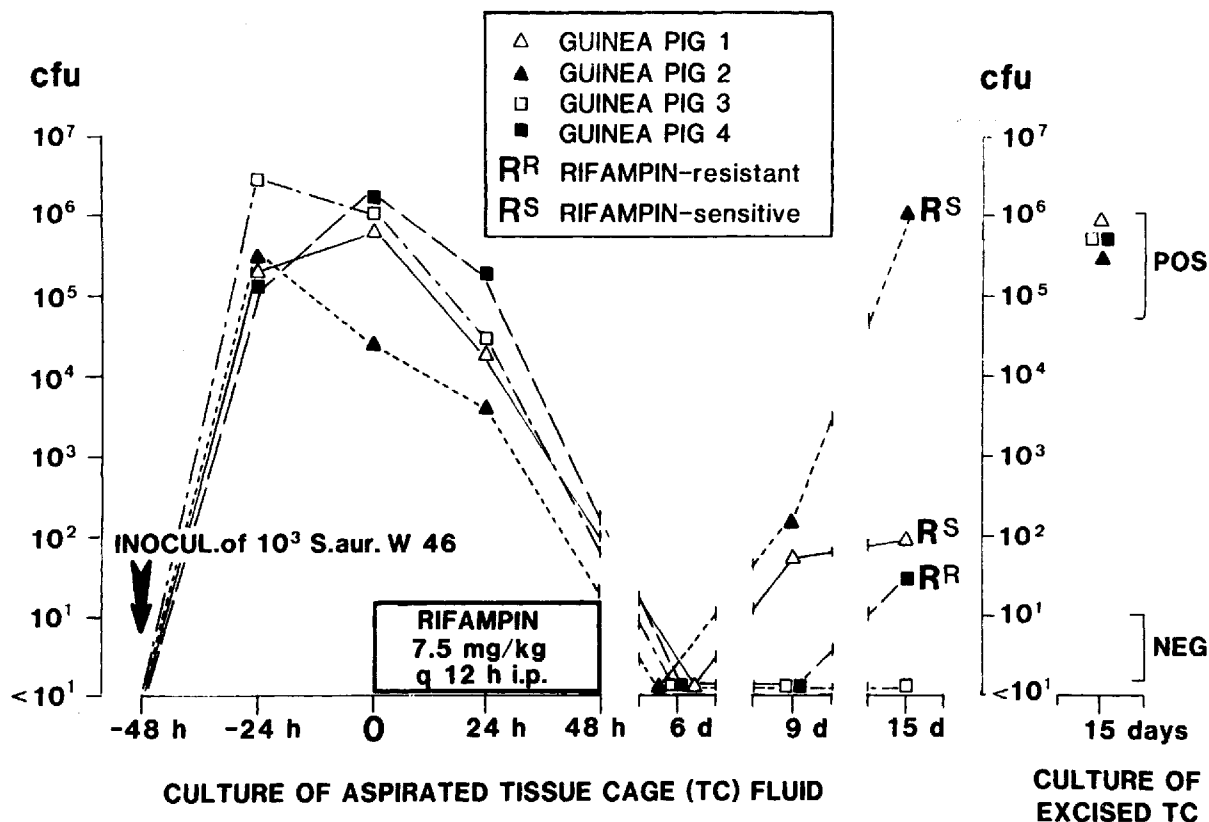
Figure 3. Number of cfu of *Staphylococcus aureus* Wood 46 in tissue-cage fluid in four guinea pigs treated according to treatment regimen G and susceptibility of isolates to rifampin at end of observation period. Time zero indicates initiation of therapy with 7.5 mg of rifampin/kg ip every 12 hr for 48 hr. Tissue cages were excised 15 days after initiation of therapy and cultured in Mueller-Hinton broth (for details see Materials and Methods); all four cages were positive for *S. aureus* Wood 46 (at right).

six days after initiation of the 48-hr therapy regimen. Bacterial counts in tissue-cage fluid gradually increased at nine days for two of four cages and at 15 days for three of four cages. The fourth tissue-cage fluid was negative throughout the observation period, whereas all excised tissue cages were positive. Finally, one of four cultures obtained at the end of the observation period showed two types of *S. aureus*, one that remained sensitive to 0.023 μg of rifampin/ml, the other that was resistant to >31 μg of rifampin/ml. Both types of *S. aureus* colonies were identical in all other biochemical characteristics and antibiotic sensitivities.

Discussion

Despite the abundant use of antibacterial agents for the prevention of postsurgical infections, there is no general consensus as to their indications, choice, timing, and duration of administration

[16]. This lack of adequate guidelines is of even greater concern for those infections that occur after insertion of prosthetic material, since such infections are associated with a particularly poor prognosis. Prosthetic valve endocarditis and osteomyelitis following reconstructive orthopedic surgery usually require the removal of the foreign material for cure [1, 17]. It therefore seems particularly desirable to establish appropriate antibiotic regimens for the prevention or early eradication of these infections, which most often are due to staphylococci.

The paucity of clinical data regarding prosthetic infections and their prevention probably reflects the difficulty in setting up adequate controlled studies, which require unambiguous criteria for the assessment of infection, accurate determinations of antibiotic concentrations in the neighborhood of the prosthesis, and well-defined end points for both duration of antibiotic therapy and

the consecutive observation periods. We believe that the present experimental model, which has previously been used for pharmacokinetic studies [12, 18] and for establishment of successful growth of fastidious microorganisms [19], can be applied to answer some of these questions.

Our results show that administration of rifampin at a dosage regimen that achieves bactericidal concentrations in tissue-cage fluid for at least 48 hr prevented the development of foreign-body infection, provided that antibacterial treatment was started ≤12 hr after inoculation of a rifampin-sensitive strain of *S. aureus*. If the time lag between the inoculation of the pathogen and the initiation of therapy was >12 hr, however, infection invariably developed despite treatment with rifampin for 48 hr, and occasionally rifampin-resistant variants emerged. Finally, whereas tissue-cage infections developed in <20 hr in the absence of rifampin administration, more than six days were required for them to be detected microbiologically after unsuccessful rifampin therapy. This observation could be explained by a prolonged postantibiotic effect, as described for other antibacterial agents [20]. Alternatively, persistent bacteria may have remained adherent to the foreign body and protected from the antibiotic effect, contaminating the tissue-cage fluid at a later stage. Finally, some rifampin-resistant mutants of *S. aureus* Wood 46 produce less catalase and are less pathogenic than the wild strain in mice [21]. In our experience, however, both the resistant and the sensitive variants were equally susceptible to the bactericidal action of peritoneal granulocytes. Present work in our laboratory is aimed at the further evaluation of these possibilities.

The experimental system presented in this communication requires two comments. First, despite others' claims to having encountered difficulty in performing bactericidal assays with *S. aureus* Wood 46 [22], we did not encounter any such problems; the MIC and MBC were in the range of those given by Mandell for the same organism [10] and by others for various strains of *S. aureus* [9, 23]. Second, we occasionally observed delayed infection of tissue-cage fluid or infection limited to the tissue cage after ineffective rifampin treatment. These observations could suggest that in the case of "effective" rifampin treatment cultures of tissue-cage fluid were falsely negative because either the observation period was too short or

microorganisms persisted on the Teflon® surface. These possibilities were ruled out by control experiments involving the excision of tissue cages with culture-negative fluid and the demonstration of the sterility of the cages by adequate culture techniques.

The incomplete protection or eradication of infection demonstrated with short-term administration of rifampin in our experimental model is in keeping with similar data obtained with experimental endocarditis [24], osteomyelitis [25], and peritonitis [26]. In all four conditions, high numbers of bacteria were exposed to rifampin, either because of a prolonged delay between inoculation and initiation of antibacterial therapy (our study and [24, 25]) or because of a high initial inoculum [26]. It is therefore not surprising that in all four experimental conditions, emergence of rifampin-resistant variants was demonstrated. This observation obtained in different experimental models might well set a limit on the use of rifampin as a single therapeutic agent—but not necessarily as a prophylactic drug—since initial inocula probably are low in clinical postsurgical infections.

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