

Chemotherapy of experimental streptococcal endocarditis

Part III Failure of a bacteriostatic agent (tetracycline) in prophylaxis¹

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SYNOPSIS Bacteriostatic agents are frequently recommended as alternatives to penicillin for prophylaxis of bacterial endocarditis. To test the efficacy of this group of antimicrobials, prophylaxis of experimental streptococcal endocarditis was attempted with tetracycline. The number of streptococci colonizing the aortic valves of rabbits was not affected by inhibitory levels of tetracycline, but multiplication was checked. Streptococcus survived in vegetations for seven days despite the continuous presence of tetracycline, and multiplied when the drug was withdrawn. It is therefore suggested that bacteriostatic agents may be valueless for prophylaxis of bacterial endocarditis.

Clinical experience has shown that the bacteriostatic antimicrobial agents are only rarely successful in the treatment of bacterial endocarditis. Nevertheless, the same agents are often recommended as alternatives to penicillin for prophylaxis during procedures which may induce transient bacteraemia (Roth, Montano, Piccolo, Cavallaro, Sharkey, and Celentano, 1953; Friedberg, 1966a; Khairat, 1966; Dorney, 1970; Cluff and Fekety, 1970; Beeson, 1971). In such situations drugs are given in single doses or short courses; to be effective they would have to exert a rapid and complete antibacterial action. On theoretical grounds alone, therefore, bacteriostatic drugs would appear to be unsuited to this purpose.

Controlled clinical trials cannot be used to test the efficacy of antibacterial drugs for prophylaxis, because the incidence of bacterial endocarditis in susceptible patients after any single bacteraemic episode is very low (Hook and Kaye, 1967). Reduction of the proportion of positive blood cultures after tooth extraction has been cited as evidence favouring the use of tetracycline as a prophylactic agent (Roth *et al*, 1953; Khairat, 1966). However, this evidence may not relate to the ability of an antibiotic to prevent implantation and survival of bacteria on an endocardial focus, and may in fact be misleading due to the presence of antibiotic in the cultured blood. We have therefore carried out a

direct examination of the effect of tetracycline on colonization of valves in an experimental model for endocarditis.

The model employed was a modification of that described in 1971 by Perlman and Freedman. A polyethylene catheter is placed in the left side of the heart; this procedure causes the formation of small vegetations, which may resemble the original nidus on which human bacterial endocarditis develops (Grant, Wood, and Jones, 1928; Angrist, 1950). Bacterial endocarditis can then be reliably produced by a single intravenous injection of streptococci (Durack, Beeson, and Petersdorf, 1973). This model has been used to study the value of penicillins and other agents in preventing bacterial endocarditis. Preliminary investigations, using single intravenous injections, suggested that bacteriostatic drugs were ineffective (Durack and Petersdorf, 1973). To assess the prophylactic value of a bacteriostatic antimicrobial in detail, several regimens employing repeated doses of tetracycline were studied.

Methods

PRODUCTION OF BACTERIAL ENDOCARDITIS

New Zealand White rabbits, 1-2kg, of both sexes, were anaesthetized with 40-60 mg of pentobarbitone given intravenously. The right internal carotid artery was exposed and opened between ligatures. The lower ligature was loosened and a polyethylene catheter of external diameter 0.8 mm and internal

¹Parts I and II have been published in other journals (see references).

diameter 0.4 mm containing sterile saline was passed toward the heart. When pulsation, resistance, and reflux of arterial blood indicated that it had reached the aortic valve or passed into the left ventricle, the catheter was secured in place by tightening the ligatures; any excess was cut off and the upper end sealed with a heated spatula. The skin was then closed over the catheter with silk sutures. Rabbits were left undisturbed for one to three days, after which approximately 10^8 colony-forming units of the test organism was injected intravenously.

TEST ORGANISM

The viridans streptococcus used throughout was a strain of *Streptococcus sanguis* serotype 2 (NCTC 7864), originally obtained from the blood of a patient with bacterial endocarditis. The minimal inhibitory concentration (MIC) of tetracycline hydrochloride for this organism was 0.20 µg/ml.

ADMINISTRATION OF TETRACYCLINE

Tetracycline hydrochloride (Achromycin, Lederle) was given intramuscularly in a dose of 15 mg/kg, according to one of the following schedules: (1) one dose given 0.5 hours before bacteraemia; (2) four doses at six-hour intervals, starting 0.5 hour before bacteraemia; (3) 21 doses at eight-hour intervals (seven days), starting 0.5 hour before bacteraemia.

SERUM LEVELS OF TETRACYCLINE

Antimicrobial activity due to tetracycline in serum was measured by drawing blood from an ear vein at intervals after intramuscular injection of the drug. Serial two-fold dilutions of serum were made in glucose broth, and approximately 10^4 units of the test strain of *Streptococcus sanguis* was added to 1 ml of each dilution. The highest dilution showing no visible growth after 18 hours at 37°C was taken as the maximum inhibitory (bacteriostatic) dilution of serum. To test for bactericidal activity, a standard loopful from each tube was plated on blood agar and incubated at 37°C for 48 hours.

EVALUATION OF INFECTION

Rabbits were killed by intravenous injections of pentobarbitone 1.5 hours, or one, seven, or nine days after infection. The hearts were removed with sterile instruments and dipped briefly in boiling water to eliminate surface contaminants. The aorta and left ventricle were opened; vegetations were excised, weighed, homogenized in glass tissue grinders, and suspended in 1 ml of glucose broth.

The number of colony-forming units of strepto-

cocci per gram vegetation was determined by incorporating 0.5 ml of serial 10-fold dilutions of the homogenate into blood agar pour plates, incubating at 37°C, and counting colonies. The final dilution of homogenized vegetation in agar was never less than 1 in 500, so that any antibiotic contained in the vegetation could be presumed diluted below an effective concentration.

Results

Mean inhibitory activity of rabbit serum following 15 mg/kg tetracycline intramuscularly is depicted in the figure. Undiluted serum was not bactericidal for this streptococcus, even at the time when tetracycline levels were highest. The MIC was exceeded for eight hours and the highest level found was 0.5 hour after injection of the drug, which was the time bacteria were injected intravenously into the test animals.

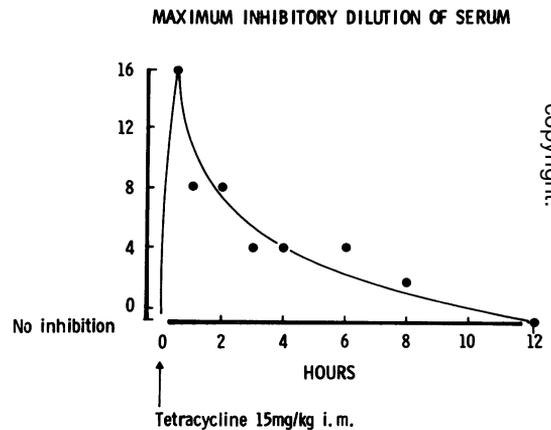


Fig. Reciprocal of maximum inhibitory dilution of serum for this strain of *Streptococcus sanguis* after intramuscular tetracycline (mean of results from six rabbits).

The results of attempted prophylaxis in 51 rabbits are summarized in the table. The number of organisms on vegetations 1.5 hours after bacteraemia was the same in animals receiving a single intramuscular injection of tetracycline 0.5 hour before bacteraemia as in controls. Similarly, the number of streptococci per gram vegetation 24 hours after bacteraemia was the same in animals receiving one dose of tetracycline as in controls.

After four doses at six-hour intervals all of eight animals were infected, but the number of streptococci present was the same as in untreated controls

| Tetracycline | Interval between Doses (hr) | Interval between Infection and Sacrifice | Approximate Drug-free Interval before Sacrifice | No. Infected/No. Animals | Log ₁₀ Streptococci per g Vegetation (Mean ± SD) |
|---------------------|-----------------------------|--|---|--------------------------|---|
| None | - | 1.5 hours | - | 8/8 | 5.5 ± 0.6 |
| Single dose | - | 1.5 hours | No interval | 6/6 | 5.0 ± 0.6 |
| None | - | 1 day | - | 5/5 | 9.4 ± 0.8 |
| Single dose | - | 1 day | 16 hours | 6/6 | 9.2 ± 0.7 |
| One-day treatment | 6 | 1 day | No interval | 8/8 | 5.5 ± 0.8 |
| Seven-day treatment | 8 | 7 days | No interval | 2/4 | 2.6 ± 3.4 |
| Seven-day treatment | 8 | 9 days | 2 days | 13/14 | 6.8 ± 3.3 |

Table Number of streptococci in vegetations from untreated rabbits and from rabbits given one, four, or 21 doses of tetracycline

1.5 hours after bacteraemia. In other words, the antimicrobial had not prevented colonization of vegetations, but had served to check multiplication during the period of treatment.

When 21 doses of tetracycline were given at eight-hour intervals, two of four animals examined eight hours after the last dose had sterile vegetations, and the other two had low counts of streptococci. It therefore appeared that tetracycline given for a week might prevent the disease in some animals. However, when the same regimen was used but the animals were not killed until two days after the last dose, 13 of 14 were found to be infected, with moderately high counts of streptococci.

Discussion

The goals of attempted prophylaxis of bacterial endocarditis have been summarized by the American Heart Association (1965): (1) to reduce the magnitude and duration of bacteraemia; (2) to eradicate bacteria which may become implanted on the endocardium.

Bacteriostatic agents may partly achieve the first of these goals (Roth *et al*, 1953; Khairat, 1966). However, two objections may be raised. The evidence that tetracycline decreased the incidence of positive blood cultures after tooth extraction is not decisive because the samples were not diluted enough to eliminate residual antibiotic effect (Roth *et al*, 1953; Khairat, 1966). Even if the incidence of positive blood cultures is reduced, this may not necessarily prevent seeding of valves. The results of the present study show that the presence of good inhibitory levels of tetracycline did not lower the number of bacteria reaching the valve.

In this experimental study, bacteria implanted on vegetations survived even when tetracycline was given three times daily for seven days. This regimen was chosen to determine whether prolonged administration of a bacteriostatic agent might allow host defence mechanisms to sterilize the vegetations.

That this failed may be due to paucity of phagocytes in rabbit vegetations (Durack and Beeson, 1972) as in human lesions (Gross and Fried, 1937). It is possible that administration of bacteriostatic drugs for even longer than seven days would have allowed eventual sterilization of vegetations, but such regimens are impractical for clinical use.

Prolonged survival of tetracycline-sensitive organisms under these conditions emphasizes that the vegetation offers bacteria 'privileged sanctuary' in which host defences are ineffective, because in this model the natural defences were unable to dispose of even a few hundred 'non-pathogenic' streptococci over seven days. Although there was an apparent decrease in the number of streptococci immediately after seven days' continuous treatment, 13 of 14 vegetations were moderately heavily infected when two days were allowed to pass before the animals were killed. Although the number of bacteria in the vegetation after a two-day, drug-free interval had increased, it had not reached the level found in untreated animals or in those which had received only a single dose of tetracycline. This suggests that prolonged exposure to tetracycline had some inhibitory effect on the survivors, but it must be assumed that they had the potential, by continued growth, to cause the clinical manifestations of bacterial endocarditis. Regrowth from a few survivors probably also occurs when patients treated for bacterial endocarditis with tetracycline relapse promptly when the drug is withdrawn despite an excellent clinical and subjective response during treatment (Friedberg, 1966b).

Admittedly, the experimental model employed in this work, with a large bacterial inoculum in the presence of a foreign body, poses a severe test for any chemotherapeutic agent; but it can be said that in the same circumstances some bactericidal drugs were effective (Durack and Petersdorf, 1973).

Because tetracycline had no effect on colonization of vegetations by streptococci, and did not prevent subsequent prolonged survival of the bacteria, we

conclude that bacteriostatic agents should not be recommended for prophylaxis of bacterial endocarditis in man.

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