

Rifampin, Ampicillin, Streptomycin, and Their Combinations in the Treatment of Enterococcal Pyelonephritis in Rats

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The activities of ampicillin, rifampin, streptomycin, and their combinations were evaluated in vitro against *Streptococcus faecalis* strain GK and in vivo in rats with an established pyelonephritis resulting from challenge with this same enterococcus. In vitro synergy was demonstrated between all combinations. Comparison of the log colony-forming units of *S. faecalis* recovered per gram of kidney tissue showed that all treated groups had significantly lower numbers than controls ($P < 0.001$). Ampicillin plus streptomycin or ampicillin alone was superior to rifampin alone or rifampin plus streptomycin at each interval ($P < 0.001$). There was no significant difference between ampicillin and rifampin plus ampicillin. The disparity between in vitro and in vivo results again raises some doubts as to the relevance of in vitro observations to clinical outcome.

The relative resistance to antibiotics of group D streptococci, commonly known as enterococci, is well known (7, 9). Since in vitro susceptibility of the enterococci to rifampin has been reported (2), we investigated the in vitro antimicrobial activity and in vivo efficacy of rifampin, ampicillin, streptomycin, and their combinations in the treatment of experimental enterococcal pyelonephritis in rats.

MATERIALS AND METHODS

Bacteria. The enterococcus used was *Streptococcus faecalis* strain GK (ATCC 23241). As in previous studies (3, 8), the inoculum was an overnight culture in brain heart infusion broth (Difco Laboratories).

Tests of antibiotic susceptibility and synergy. The minimal inhibitory concentrations of sodium ampicillin (Wyeth Laboratories), streptomycin sulfate (Pfizer Laboratories) and rifampin (CIBA Pharmaceuticals) were determined by a tube dilution method (10). The fractional inhibitory concentrations were used to determine synergy between agents (1).

Production and antibiotic treatment of pyelonephritis. Pyelonephritis was produced in randomly bred male Wistar rats, weighing 120 to 150 g, by intravenous injection of 1.4×10^9 colony-forming units of *S. faecalis* in 1 ml of broth. All animals were inoculated in one sitting. Two weeks after infection, the rats were randomly divided into groups of 20 and treated twice daily, at 7:00 a.m. and 7:00 p.m., with one of the following antibiotic regimens: (i) ampicillin, 150 mg/kg, intramuscularly; (ii) streptomycin, 25 mg/kg, intramuscularly; (iii) rifampin, 50 mg/kg, orally; (iv) ampicillin, 150 mg/kg, intramuscularly, plus streptomycin, 25 mg/kg, intramuscularly; (v) ampicillin, 150 mg/kg, intramuscularly, plus rifampin, 50 mg/kg, orally; (vi) streptomycin, 25 mg/kg, intramuscularly, plus rifampin, 50 mg/kg, orally; or (vii) no antibiotics.

The oral treatments were administered by gavage.

Ten rats from each group were sacrificed after 3 and 6 weeks of therapy. The sacrifice occurred 12 h after the last treatment. Kidneys were removed, weighed, and homogenized, and aliquots of these homogenates were cultured by methods previously described (8). Results were expressed as the mean log number of colony-forming units of *S. faecalis* per gram of kidney tissue. Comparisons between groups were made by Student *t* test. The proportion of infected kidneys were analyzed nonparametrically by chi-square analysis.

RESULTS

The minimal inhibitory concentrations of ampicillin, streptomycin, and rifampin for the GK strain were 1.56 $\mu\text{g/ml}$, 400 $\mu\text{g/ml}$, and 3.12 $\mu\text{g/ml}$, respectively. The fractional inhibitory concentration indices for ampicillin plus streptomycin and ampicillin plus rifampin were 0.5. Streptomycin plus rifampin had a fractional inhibitory concentration index of 0.25, a value representing a combination of one eighth of the minimal inhibitory concentration of each drug.

Results of the treatment experiments are shown in Table 1. At each interval and overall, the mean log number of enterococcal colony-forming units recovered per gram of kidney from treated animals was significantly less than that from any untreated group ($P < 0.001$). Ampicillin plus streptomycin and ampicillin were the most effective therapeutic regimens. Three weeks after treatment began, the mean log colony-forming units from rats that received ampicillin plus streptomycin, ampicillin, or ampicillin plus rifampin was lower than that from rats that received the other drug regimens (P

TABLE 1. Effect of therapeutic regimens on ratio of infected kidneys and renal microbial population in rats with enterococcal pyelonephritis

Treatment group (twice daily dose)	Response at 3 wk		Response at 6 wk	
	No. of kidneys culture positive/total	Mean log CFU/g of kidney \pm SEM ^a	No. of kidneys culture positive/total	Mean log CFU/g of kidney \pm SEM
Ampicillin plus streptomycin	19/20	2.05 \pm 0.23	3/20	0.22 \pm 0.12
Ampicillin	19/20	2.41 \pm 0.22	8/20	0.92 \pm 0.36
Ampicillin plus rifampin	20/20	2.45 \pm 0.16	11/18	1.04 \pm 0.21
Streptomycin plus rifampin	18/18	3.29 \pm 0.14	18/18	3.21 \pm 0.28
Rifampin	18/18	3.80 \pm 0.29	18/18	4.44 \pm 0.24
Streptomycin	18/18	4.81 \pm 0.26	20/20	4.59 \pm 0.30
Untreated controls	20/20	5.98 \pm 0.19	20/20	5.95 \pm 0.15

^a SEM, Standard error of the mean.

< 0.001). At 6 weeks after therapy began, the mean log colony-forming units from rats that received ampicillin plus streptomycin was lower than that from rats which received any other drug regimens ($P < 0.001$) except ampicillin. There was no significant difference between ampicillin and ampicillin plus rifampin at each interval. Ampicillin plus streptomycin was superior to streptomycin plus rifampin after 3 weeks ($P < 0.001$) and 6 weeks ($P < 0.001$) of treatment.

Comparison of the proportion of kidneys infected at each interval showed that after 6 weeks of therapy, the ampicillin plus streptomycin group had significantly fewer infected kidneys than any other group except ampicillin.

DISCUSSION

A report by Konopka et al. (6) demonstrated the therapeutic potential of rifampin in combination with other antibiotics against experimental enterococcal pyelonephritis. However, their study was undertaken in mice, and treatment was started 2 h after challenge and continued for only 3 days. On the basis of our data, it appears that rifampin alone or in combination with ampicillin or streptomycin is less effective in treating enterococcal pyelonephritis in the rat than ampicillin plus streptomycin or ampicillin alone. In the past, in this model, ampicillin plus streptomycin has been found to be the most active therapeutic regimen (4, 8).

The *in vivo* results are not compatible with the *in vitro* studies, in which fractional inhibitory concentration indices demonstrated synergistic activity between rifampin and ampicillin or streptomycin. The generally poor performance *in vivo* of the combination of rifampin plus streptomycin as compared to ampicillin and streptomycin is not readily explained. It may be that the lack of significant renal excretion of rifampin makes it a less efficacious agent for renal infections (5). Nevertheless, this disparity

between *in vitro* and *in vivo* results again raises some doubts as to the relevance of *in vitro* observations to clinical outcomes.

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