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Research Article

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These studies demonstrate that diuresis resulting from administration of dextrose in water plus ampicillin starting 4 or 21 days after intravenous injection of enterococci reduces renal titers more than ampicillin or diuresis alone.

Influence of Water Diuresis on Antimicrobial Treatment of Enterococcal Pyelonephritis

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ABSTRACT In the present studies, the effect of ampicillin (40 mg intramuscularly twice a day) in combination with water diuresis, produced by the ingestion of 5% dextrose in water, was determined on renal titers of enterococci after intravenous inoculation of 4×10^8–2×10^9 enterococci into rats.

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INTRODUCTION

Intravenous injection of large numbers of enterococci in healthy rats without urinary tract obstruction or prior trauma to the kidney produces chronic pyelonephritis (1). Therapy with penicillin or ampicillin can alter the course of the infection. Guze, Hubert, and Kalmanson (2) showed that intramuscular injection of 100,000 units of penicillin daily when started 24 hr after infection with enterococci resulted in sterile kidneys by 15 days. When treatment was delayed for 2 wk, although titers of enterococci decreased in the kidneys, the kidneys remained infected despite 3 wk of penicillin therapy. Kaye and Rocha (3) injected 80 mg of ampicillin intramuscularly daily for 2 wk in rats with enterococcal pyelonephritis. Although the titers of enterococci decreased, most kidneys remained infected whether treatment was started 1 wk or 6 wk after the intravenous inoculation of enterococci.

Water diuresis produced by offering rats 5% dextrose in water has also been reported to alter the course of enterococcal pyelonephritis in rats. Andriole (4) found that production of diuresis starting 1 day before intravenous inoculation of enterococci resulted in bilateral sterile kidneys in 11% of rats diuresing for 1 wk and in 29% of rats diuresing for 2 wk. Although most of the remaining kidneys contained fewer enterococci than kidneys from nondiuresing rats with enterococcal pyelonephritis, the median titers were over 10^4 per kidney. Similar results wer obtained when dextrose in water was offered to the rats 8 days after intravenous injection of enterococci. However, Rutsky, Clapp, and Robinson (5) were not able to demonstrate as marked or consistent an effect of water diuresis in enterococcal pyelonephritis in rats. These investigators found that after injection of enterococci into rats drinking dextrose in water, neither the incidence of infection nor the average bacterial counts from infected kidneys were significantly different from those in infected rats drinking water.

The present study was undertaken to evaluate the effectiveness of the combination of water diuresis and ampicillin therapy in enterococcal pyelonephritis when

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therapy was started 4 days or 3 wk after initiation of infection.

METHODS

Animals. White male Sprague-Dawley Rats (Blue Spruce Farms, Altamont, N. Y.) weighing between 150 and 250 g were used for all experiments and were given Purina Laboratory Rat Chow (Ralston Purina Co., St. Louis, Mo.). The food was free of antimicrobial drugs.

Bacteria. A strain of enterococcus (3) which was inhibited by 1.56 μg/ml of ampicillin was used in all experiments.

Stock cultures were maintained by storing samples of an 18 hr culture in Trypticase soy broth (Baltimore Biological Laboratories, Baltimore, Md.) at −20°C. Inocula for each experiment were prepared by subculturing a sample of the stock culture in Trypticase soy broth and incubating at 37°C for 18 hr.

Bacterial enumeration. The numbers of bacteria in broth were determined by plating 0.1 ml of each specimen in Trypticase soy agar pour plates and making serial 100-fold dilutions in saline solution and plating 1.0- and 0.1-ml samples of each dilution.

Numbers of bacteria in a kidney were determined in the same manner, after homogenizing the kidney in 1 ml Trypticase soy broth using Teflon tissue grinders (Tri-R Instruments, Inc., Rockville Centre, N. Y.). The total number of viable bacteria in a tissue or in broth was calculated from colony counts after incubation of the plates for 24 hr at 37°C. As this technique will not detect less than 10 bacteria per kidney, all "sterile" kidneys were recorded as containing 10 (log number 1) bacteria. In each experiment representative colonies were identified as enterococci.

Method of producing diuresis. Diuresis was produced by offering rats 5% dextrose in tap water. Rats having access to 5% dextrose in tap water drink more than rats offered tap water and undergo water diuresis (6). Combistix reagent (Ames Co., Elkhart, Ind.) was used to detect the presence of glycosuria in the rats drinking dextrose in water.

Experiments. Table I outlines the experimental design. An inoculum of 4×10^8–2×10^9 enterococci in 1 ml of Trypticase soy broth was injected intravenously in the tail veins of rats. In some experiments enterococci were injected into rats that had been drinking tap water or 5% dextrose in tap water for 4 days. The 5% dextrose in water was continued after infection. Starting 4 days after inoculation of enterococci, one-half of the animals drinking dextrose in water and one-half drinking tap water were started on therapy with 40 mg (0.2 ml) sodium ampicillin intramuscularly twice a day at 8 a.m. and 5 p.m. The rest of the animals received 0.2 ml of saline solution intramuscularly at the same times. The combined regimens of dextrose in water or tap water plus injections of ampicillin or saline solution were continued for 7 or 14 days in different groups of rats.

In other experiments dextrose in water was not offered until 21 days after inoculation of enterococci. These rats then received dextrose in water or tap water plus injections of ampicillin or saline solution for 7 or 14 days.

The rats were permitted free access to food and tap water or 5% dextrose in tap water. At least 2–3 urine specimens obtained more than 72 hr apart were collected from each rat for determination of osmolality by placing individual rats in metabolic cages the sides of which had been siliconized. The urine collection for osmolality was made into iced containers over a 2 hr period during which the rats received neither food nor water (to prevent dilution of the urine sample). The osmolality was determined in an osmometer (Fiske Osmometer, model G-12, Fiske Associates, Inc., Uxbridge, Mass.).

TABLE I

Experimental Design

Day of injection of bacteria	Days of administration of 5% dextrose/water* (experimental day number)	Days of injection of ampicillin or saline solution (experimental day number)
5	1–15	9–15
5	1–22	9–22
1	21–27	21–27
1	21–34	21–34

* Controls received tap water.

Ampicillin and saline injections and dextrose in water were discontinued 1 wk before determination of numbers of enterococci in the kidneys. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium. The kidneys were exposed through a midline abdominal incision using sterile technique. The rats were killed by exsanguination into the chest. The kidneys were removed with sterile instruments. Each kidney was homogenized and the number of organisms in each kidney was determined.

Some rats did not diurese when offered 5% dextrose in water. Therefore, rats drinking dextrose in water that did not have at least one urine osmolality below 400 mOsm/kg water were eliminated from the study. Experience in this laboratory indicates that rats with enterococcal pyelonephritis drinking tap water rarely have urine osmolalities below 400, and rats drinking 5% dextrose in water usually have osmolalities below 400.

RESULTS

The effect of drinking 5% dextrose in water on the kidney, urine, and water consumption have been described by Andriole and Epstein (6). In the present study as in previous experiments (4–7) the urine was free of dextrose. Table II demonstrates mean urine osmolalities in rats diuresing from drinking 5% dextrose in water and in rats drinking tap water. The di-

TABLE II

*Mean Urine Osmolalities in Rats Receiving Different Therapies**

	D/W‡ plus ampicillin	Tap water plus ampicillin	D/W plus saline	Tap water plus saline
Mean osmolality (*mOsm*) ±SE	302±42	876±71	322±66	798±64

* In calculating the means for each group, the average osmolality for each rat was used. The animals are pooled regardless of when therapy was started or duration of therapy.

‡ 5% dextrose in water.

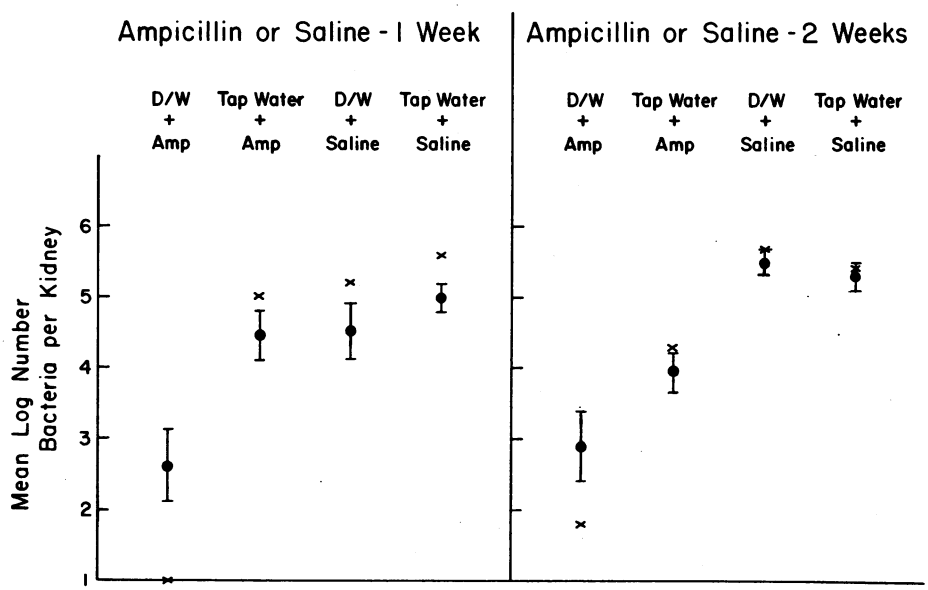

FIGURE 1 Median (×) and mean (●) log bacterial numbers with standard errors of the mean in kidneys of rats after intravenous inoculation of 4×10^8–2×10^9 enterococci. The rats received injections of ampicillin or saline solution starting 4 days after initiation of infection and continuing for 1 or 2 wk. The rats were drinking tap water or 5% dextrose in tap water (D/W) starting 4 days before infection and continuing as long as the injections of ampicillin or saline solution. There were 8–25 rats in each group.

uresing rats had significantly lower urine osmolalities ($P < 0.01$) than the nondiuresing rats.

Fig. 1 demonstrates experiments in which diuresis was started 4 days before challenge with enterococci, and injections of ampicillin or saline solution were added 4 days after inoculation of enterococci. Diuresing rats receiving ampicillin had lower renal titers of enterococci than diuresing rats receiving saline injections

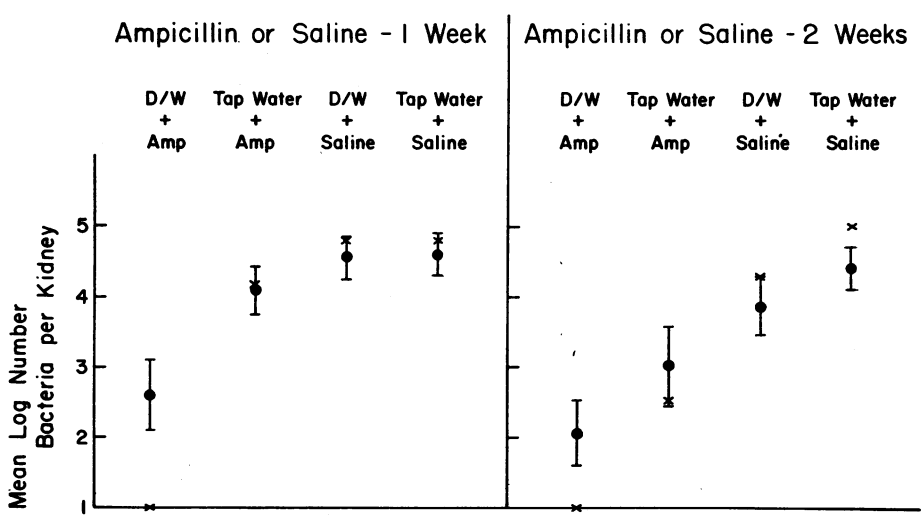

FIGURE 2 Median (×) and mean (●) log bacterial numbers with standard errors of the mean in kidneys of rats after intravenous inoculation of 4×10^8–2×10^9 enterococci. The rats received injections of ampicillin or saline solution starting 3 wk after initiation of infection and continuing for 1 or 2 wk. The rats were drinking tap water or 5% dextrose in tap water (D/W) simultaneously with the injections of ampicillin or saline solution. There were 7–19 rats in each group.

or than nondiuresing rats receiving ampicillin or saline injections. In animals treated for 1 wk (left side of Fig. 1), neither ampicillin therapy in nondiuresing rats nor water diuresis in rats receiving saline injections (mean log bacterial numbers per kidney 4.4 and 4.5, respectively, with median log numbers 5.0 and 5.2) resulted in a significant reduction in renal titers of enterococci ($P > 0.05$) as compared with nondiuresing rats receiving saline injections (mean log bacterial number 5.0 with a median of 5.6). However, diuresis combined with treatment with ampicillin for 1 wk significantly reduced renal titers of bacteria (mean log 2.6 and median log 1.0) as compared with each of the other groups ($P < 0.01$ for each comparison). The numbers of rat kidneys that were sterile (< 10 bacteria per kidney) or contained log number 4.0 or more enterococci per kidney are shown in Table III.

In animals treated for 2 wk (right side of Fig. 1), water diuresis plus saline injections did not significantly ($P > 0.05$) affect renal titers of enterococci (mean log 5.5 and median log 5.7) as compared with nondiuresing rats receiving saline injections (mean log 5.3 with median log 5.4). Ampicillin therapy for 2 wk in nondiuresing rats significantly reduced renal titers (mean log 3.9 with a median of 4.3) as compared with diuresing and nondiuresing rats receiving saline injections ($P < 0.01$ for each comparison). Combining diuresis with ampicillin therapy for 2 wk (mean log number 2.9 and median log 1.8) resulted in a further significant reduction in renal titers of enterococci when compared with nondiuresing rats receiving ampicillin ($P < 0.05$) and with diuresing or nondiuresing rats receiving saline injections ($P < 0.01$) for each comparison). The numbers of rat kidneys that were sterile (< 10 bacteria per kidney) or contained log number 4.0 or more enterococci per kidney are shown in Table III.

Similar results were obtained in experiments in which therapy was delayed until 3 wk after the inoculation of enterococci (Fig. 2). In animals treated for 1 wk (left side of Fig. 2), neither ampicillin in nondiuresing rats nor saline injections in diuresing rats (mean log numbers 4.1 and 4.6, respectively, with median log titers 4.2 and 4.8) resulted in a significant reduction of renal titers ($P > 0.05$) as compared with nondiuresing rats receiving saline injections (mean log number 4.6 with median log 4.8). However, diuresis combined with ampicillin for 1 wk (mean log 2.6 and median 1.0) reduced renal titers significantly as compared with each of the other three groups ($P < 0.01$ for each comparison). The numbers of rat kidneys that were sterile (< 10 bacteria per kidney) or contained log number 4.0 or more enterococci per kidney are shown in Table III.

TABLE III

Numbers of Sterile or Infected Kidneys

	Number of kidneys		
	Sterile (<10 bacteria per kidney)	Log number 4.0 or more enterococci per kidney	Total kidneys
Diuresis started 4 days before and ampicillin 4 days after initiation of infection			
Therapy for 1 wk			
D/W* + ampicillin	14(58)‡	7(29)‡	24
Tap water + ampicillin	7(21)	23(68)	34
D/W + saline	1(5)	15(68)	22
Tap water + saline	3(6)	40(80)	50
Therapy for 2 wk			
D/W + ampicillin	7(44)	5(31)	16
Tap water + ampicillin	5(13)	12(32)	38
D/W + saline	0	12(100)	12
Tap water + saline	2(5)	37(84)	44
Diuresis and ampicillin started 3 wk after initiation of infection			
Therapy for 1 wk			
D/W + ampicillin	8(50)	3(19)	16
Tap water + ampicillin	6(16)	23(61)	38
D/W + saline	4(11)	27(77)	35
Tap water + saline	2(7)	19(68)	28
Therapy for 2 wk			
D/W + ampicillin	9(64)	3(21)	14
Tap water + ampicillin	6(43)	6(43)	14
D/W + saline	2(15)	7(54)	13
Tap water + saline	5(16)	23(72)	32

* 5% dextrose in water.

‡ All numbers in parentheses refer to percentages.

In animals treated for 2 wk (right side of Fig. 2), water diuresis plus saline injections did not significantly ($P > 0.05$) affect renal titers of enterococci (mean log number 3.9 and median log 4.3) as compared with nondiuresing rats receiving saline injections (mean log 4.4 with median 5.0). Ampicillin therapy for 2 wk (mean log 3.0 and median 2.5) reduced renal titers significantly ($P < 0.05$) as compared with nondiuresing rats receiving saline injections. The reduction was not significant ($P > 0.05$) when compared to diuresing rats receiving saline injections. Therapy with ampicillin for 2 wk plus diuresis (mean log number 2.1 and median 1.0) reduced renal titers significantly

as compared with diuresing rats receiving saline injections ($P < 0.01$) and nondiuresing rats receiving saline injections ($P < 0.01$) but not as compared with nondiuresing rats receiving ampicillin injections ($P > 0.05$). The numbers of rat kidneys that were sterile (< 10 bacteria per kidney) or contained log number 4.0 or more enterococci per kidney are shown in Table III.

In an attempt to determine whether the renal titers of enterococci were lower at the end of therapy and then subsequently increased during the week before killing the animals, experiments were performed in which rats were killed 24 hr after the last injection of ampicillin or saline (and therefore also 24 hr after termination of dextrose in water). In these experiments penicillinase (Difco Laboratories, Detroit, Mich.) was added to the Trypticase soy agar pour plates used for determining renal titers (50,000 units of penicillinase/10 ml of media). There were no differences in renal titers between animals killed 1 or 7 days after the last exposure to ampicillin, saline, or dextrose in water.

Further experiments were carried out in order to determine if intramuscular injection of 0.2 ml saline solution twice daily influenced renal titers of enterococci in rats drinking tap water. Injection of saline solution for 1 or 2 wk starting 4 or 21 days after inoculation of enterococci did not significantly affect renal titers ($P > 0.05$) as compared with renal titers in infected rats receiving no injections.

DISCUSSION

These studies demonstrate that 1 wk of therapy with ampicillin (80 mg daily) had little effect on renal titers of enterococci in rats with enterococcal pyelonephritis. However, 2 wk of ampicillin therapy resulted in a decrease in renal titers that was significant.

The combination of diuresis from dextrose in water and ampicillin therapy produced a much greater lowering of bacterial titers which was equally impressive after one or two weeks of therapy. There were no differences in results obtained when therapy with ampicillin was started four or 21 days after the inoculation of enterococci.

Andriole (4) reported that water diuresis alone protected rats against initiation of enterococcal pyelonephritis and lowered renal bacterial titers in established enterococcal pyelonephritis. Rutsky et al. (5) studied the effect of water diuresis in preventing the establishment of enterococcal pyelonephritis. Although they noted lower renal titers of enterococci in diuresing rats, the decreases were not significant.

In contrast to these studies, water diuresis had no effect on renal titers of enterococci in the present study. The reasons for the differences in the therapeutic effects of water diuresis between the present study and the previous two studies (4, 5) are not known, but several possible explanations can be offered: (*a*) The strain of enterococcus used in the present studies was different from the strain used by the other investigators. (*b*) Although Sprague-Dawley rats were employed by all of the investigators, the animals came from different farms. And (*c*) in the present studies, animals with spontaneous antidiuresis (i.e., drinking tap water) had much lower mean urine osmolalities than in the studies of Andriole (4) and Rutsky et al. (5). Therefore the relative differences in urinary osmolality between diuresing and nondiuresing animals was much greater in the studies of Andriole (4) and Rutsky et al. (5) than in the present studies.

There are several possibilities to explain the therapeutic effect of diuresis in combination with ampicillin therapy observed in the present studies. These relate to the effect of diuresis on reducing the normally hypertonic renal medullary interstitium. It has previously been shown by Rocha and Fekety (8) that there is impaired leukocyte migration in the renal medulla and that this is associated with enhanced medullary susceptibility to infection. Andriole (4, 9) has demonstrated that there is earlier polymorphonuclear leukocytic migration in the inflamed renal medullae of animals during diuresis than with antidiuresis. Studies by Chernew and Braude (10) and Cohn (11) showed that the concentration of solutes found in the normal medulla inhibits phagocytosis. Cohn showed that progressive lowering of osmolality resulted in decreased phagocytic inhibition, particularly when the osmolality approached isotonicity. Therefore, reduction of medullary hypertonicity favors leukocytic mobilization and phagocytosis which would enhance the antibacterial effect of ampicillin.

The hypertonic medullary interstitium theoretically provides a favorable milieu for the maintenance of cell-wall-deficient bacteria, and enterococci are known to become cell-wall-deficient on exposure to penicillin (12). If these forms contribute to the persistence of enterococci in the renal medulla, the decrease in medullary tonicity produced by water diuresis would render these forms less likely to survive.

Water diuresis results in increased medullary blood flow. This may result in the better delivery of antibacterial substances such as complement, antibody, and antibiotics to the site of infection. Furthermore complement is inactivated by hypertonicity (13, 14) and diuresis eliminates medullary hypertonicity.

Although, in the present studies, water diuresis had no effect in the prevention or therapy of enterococcal pyelonephritis, the addition of water diuresis to ampicillin treatment markedly potentiated the effect of

ampicillin alone in decreasing renal titers of enterococci. The exact mechanism of this potentiation is unknown.

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