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Rifampin and Penicillin for the Elimination of Group B Streptococci in Nasally Colonized Infant Rats

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ABSTRACT. Although multiple antibiotic strategies to eradicate group B streptococci (GBS) from colonized infants and women have been utilized, no regimen has been successful in eliminating GBS carriage reliably. Because rifampin has been successful in terminating nasopharyngeal colonization with other bacteria, we tested both the *in vitro* sensitivity of GBS to rifampin and the *in vivo* efficacy of rifampin in eliminating GBS from a new animal model of nasally colonized infant rats. The minimal inhibitory concentration of rifampin for 18 clinically derived strains of type III GBS ranged from 0.1 to 0.4 μg/ml. Atraumatic nasal inoculation of infant rats with 10^6–10^7 colony forming units of GBS twice daily for 4 days resulted in heavy asymptomatic carriage for at least 10 days. Colonized animals were divided into four treatment groups: 1) saline, 2) oral rifampin, 3) intraperitoneal penicillin, or 4) oral rifampin plus intraperitoneal penicillin. Treatment was administered every 12 h for 4 days. All 78 saline-treated controls and 47 of 52 (90.4%) penicillin-treated animals had continued GBS carriage 36 h after completion of therapy. In contrast, only 18 of 52 (34.6%) rifampin-treated animals and seven of 54 (13.0%) rifampin plus penicillin-treated animals remained GBS-positive. No rifampin-resistant GBS were detected. Combination rifampin plus penicillin therapy was significantly more effective in terminating GBS carriage compared to saline or penicillin alone ($p < 0.0001$) or to rifampin ($p < 0.01$). Nasal cultures taken from a subgroup of animals 6–7 days after completion of therapy showed that 20 of 30 (66.7%) animals treated with rifampin and 43 of 54 (79.6%) animals treated with rifampin plus penicillin remained GBS-free (p = NS). These data demonstrate that, unlike penicillin alone, rifampin alone is effective, while combination rifampin plus penicillin therapy is most effective in eliminating GBS from nasally colonized infant rats. (*Pediatr Res* 19: 1183–1186, 1985)

Abbreviations

GBS, group B streptococci
MIC, minimal inhibitory concentrations
MBC, minimal bactericidal concentrations
ip, intraperitoneal
CFU, colony-forming units

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GBS remain the leading cause of sepsis and meningitis in neonates, with a mortality rate of 50–60% (1). A variety of antibiotic regimens has been utilized in recent years in efforts to eradicate GBS from colonized infants and pregnant women without consistent success (2–7). Because rifampin has been demonstrated to be effective in eradicating nasopharyngeal carriage of *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pyogenes*, (8, 9) we tested the *in vitro* sensitivity of type III GBS to rifampin and found GBS to be sensitive to this agent. Therefore, this study was designed to establish an infant rat model of asymptomatic GBS nasal colonization and to compare treatment with either rifampin or penicillin or a combination of rifampin and penicillin for their ability to terminate nasal colonization.

IN VITRO ANTIBIOTIC SENSITIVITY STUDIES

The MIC and MBC of 18 strains of type III GBS to rifampin and penicillin were determined using a broth dilution microtiter technique. Serial dilutions of antibiotic in Todd-Hewitt broth were inoculated with 10^5 CFU/ml of log-phase GBS. The lowest concentration of antibiotic that inhibited visible growth after overnight incubation in 5% CO_2 at 35° C was designated the MIC. The MBC was defined as the lowest concentration of antibiotic that prevented growth of 99.9% of the original inoculum. All organisms were clinical isolates and were grown in Todd-Hewitt broth.

The MIC of penicillin for the 18 GBS type III strains ranged from 0.01–0.04 μg/ml with MBC values from 0.02–0.08 μg/ml. The MIC of rifampin for these same 18 strains ranged from 0.1–0.4 μg/ml, while the MBC of rifampin was ≥25 μg/ml for all 18 strains. These MIC and MBC values of these drugs for GBS are similar to those determined by other investigators (10).

The MIC of penicillin for the single GBS type III strain used in the animal studies was 0.02 μg/ml with an MBC of 0.04 μg/ml. The MIC of rifampin for the GBS study strains was 0.2 μg/ml.

MATERIALS AND METHODS

Establishment of Infant Rat Model of GBS Nasal Colonization. The nares of sixty-eight 1- to 2-day-old Sprague-Dawley rats (Harlan Sprague Dawley, Madison, WI) were inoculated atraumatically twice daily for four successive days with 10^6–10^7 CFU log-phase GBS in 10 μl Todd-Hewitt broth. A single randomly selected strain of type III GBS was utilized for these animal studies. Care was taken to avoid direct contact of the inoculating pipette with the nares, and each nare was inoculated once each day. Animals were housed in standard animal care facilities with their natural mothers.

Beginning 12 h after the completion of the colonization period, nasal cultures were obtained daily for 3 days, then every other day through the 15th day after initial inocultaion. Nasal cultures were obtained by dropping 25 μl sterile normal saline on one nare and plating on blood agar 5 μl of the saline wash taken immediately from the opposite nare. Therefore, one colony represented 200 CFU/ml of nasal wash. We used a grading scale shown in Table 1. Animals whose nasal wash cultures yielded no GBS colonies were considered culture-negative.

Blood cultures were collected from the tail vein (10 λ) from all animals on the first 3 days after completion of nasal inoculation. Blood cultures were interpreted simply as positive or negative.

Antibiotic Treatment of GBS Nasally Colonized Infant Rats.

Antibiotic therapy protocol. As shown in Figure 1, 236 animals nasally colonized with GBS were randomly divided into four treatment groups: 1) saline controls ($n = 78$); 2) penicillin, 50,000 U/kg/dose ip ($n = 52$); 3) rifampin, 20 mg/kg/dose orally ($n = 52$); 4) oral rifampin plus penicillin, in the same doses above ($n = 54$). All antibiotics were given every 12 h for 4 days. Doses were calculated for the average weight per animal in each litter. All rifampin was prepared immediately prior to animal administration. Penicillin for ip injection was prepared at the beginning of the treatment period and stored at −70° C until thawed for immediate administration. Control animals received 0.2 ml saline orally or ip on the same schedule.

Rifampin powder sufficient for one set of experiments was dissolved in 0.1–0.2 ml methanol. Sterile saline was then added to provide the appropriate concentration of antibiotic in 0.2 ml. The suspension was vortexed frequently. Two-tenth-ml aliquots of the rifampin suspension were drawn into a sterile micropipette and released deep into the posterior pharynx of the infant rat.

Penicillin powder was dissolved in sufficient sterile saline to provide the desired concentration of antibiotic in 0.2 ml. The solution was drawn into a tuberculin syringe and administered ip using a 25-gauge needle. Control animals received 0.2 ml sterile saline ip or orally administered in the same manner.

Table 1. *Grading scale*

No. GBS colonies	No. organisms/ml	Grade
0	$<2 \times 10^2$	0
1–4	$<1 \times 10^3$	1+
5–49	1×10^3–9.8×10^3	2+
50–100	1×10^4–2×10^4	3+
>100	$>2 \times 10^4$	4+

Animals assigned to combination therapy with oral rifampin plus ip penicillin received both drugs within 10–15 min.

Nasal cultures were performed immediately prior to the initiation of antibiotic or saline therapy, 36 h after completion of therapy, and in some animals 6–7 days after the completion of therapy. Animals were observed for signs of illness. Blood and CSF cultures were obtained from ill-appearing animals.

Repeat MIC studies were performed on isolates of GBS from animals who continued to carry GBS in the nares after rifampin treatment.

Pharmacokinetic studies. Twelve hours after the final dose of rifampin, blood levels of rifampin were measured in three animals by an agar diffusion bioassay using *Staphylococcus aureus* as the seeding organism. The mean blood level was 4.0 μg/ml (range 3.4–5.0 μg/ml). In five other animals, the mean peak blood penicillin level at 45 min following their final dose was 15.2 μg/ml as measured by bioassay using *Bacillus subtilis.*

Statistics. The χ^2 test, using the Yates correction when appropriate, was used to determine statistical significance of data obtained.

RESULTS

Development of infant rat model of GBS nasal colonizaiton. All 68 animals were successfully colonized with $\geq 1 \times 10^4$ CFU GBS (3+ or 4+) at the end of the 4-day colonization period (Table 2). This heavy colonization persisted until at least 13 days after initial inoculation before a few animals showed decrease to 2+ colonization. One animal died on day 10 with positive blood and CSF cultures for GBS. All other animals remained well, and all other blood cultures were negative.

Table 2. *Sequential quantitative nasal cultures of 68 infant rats colonized with type III GBS*

Grade of culture	No. GBS organisms	Day after 4-day nasal colonization period						
		5	6	7	9	11	13	15
0	$<2 \times 10^2$	0	0	0	0	0	0	0
1+	$<1 \times 10^3$	0	0	0	0	0	0	0
2+	1.2×10^3–9.8×10^3	0	0	0	0	0	1	2
3+	1.0×10^4–2.0×10^4	2	2	4	3	3	5	7
4+	$>2 \times 10^4$	66	66	64	65	64*	61	58

* One animal died on day 10 with positive blood and cerebrospinal fluid cultures for GBS. All other animals remained well.

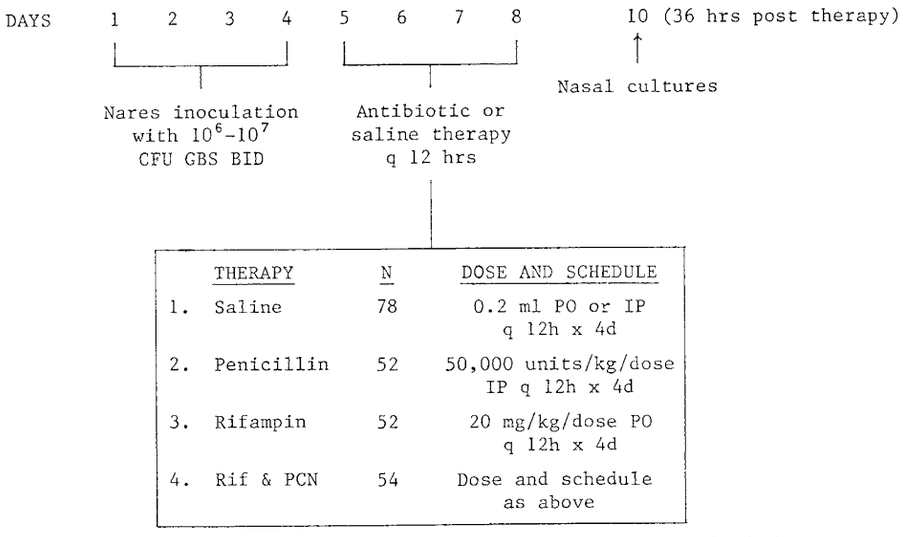

Fig. 1. Antibiotic therapy of infant rats nasally colonized with GBS.

Table 3. *Quantitative nasal cultures of infant rats with GBS nasal colonization prior to and after antibiotic treatment*

Treatment group	*n*	Prior to treatment					36 h after treatment					6–7 days after treatment					
		0	1+	2+	3+	4+	0	1+	2+	3+	4+	0	1+	2+	3+	4+	
Saline	78	0	0	0	2	76	0	0	0	3	75	26	0	0	1	5	20
Penicillin	52	0	0	0	2	50	5	16	20	3	8	26	0	3	15	4	4
Rifampin	52	0	0	0	2	50	34	13	4	1	0	30	20	6	3	1	0
Rifampin and penicillin*	54	0	0	0	1	53	47	4	2	1	0	54	43	7	3	1	0

* Rifampin and penicillin results differ from rifampin group ($p < 0.01$) and from penicillin and saline groups ($p < 0.0001$).

Efficacy of antibiotic therapy for eradicating GBS nasal colonization of infant rats. Prior to initiation of antibiotic or saline therapy, all 236 animals were heavily (3+ or 4+) colonized with GBS (Table 3). Thirty-six hours after the completion of therapy, all 78 saline-treated controls showed no reduction in the magnitude of GBS nasal colonization. In the penicillin-treated animals only five of 52 (9.6%) were culture-negative, while 16 showed 1+ colonizaiton and 20 2+ colonization. In contrast, 34/52 (65.4%) rifampin-treated animals were culture-negative and 13 were 1+ colonized. The difference in culture-negativity between these groups was statistically significant ($p < 0.0001$). Of the 54 animals treated with both rifampin and penicillin, 47 (87.0%) were culture-negative and four were 1+ colonized. This combined regimen is superior to rifampin alone ($p < 0.01$).

Six to seven days after the completion of antibiotic therapy, all saline-treated controls and all penicillin-treated animals were culture-positive. Twenty-three of the 26 (88.6%) penicillin-treated animals had $>1 \times 10^3$ CFU/ml GBS. In contrast, only 10 of 30 (33%) rifampin-treated animals and 11 of 54 (20.4%) animals treated with both rifampin and penicillin remained colonized with GBS (p = NS). Only four of 30 (13.3%) animals treated wth rifampin and four of 54 (7.4%) animals treated with rifampin plus penicillin had $>1 \times 10^3$ CFU/ml GBS in the nares when cultured 6–7 days after completion of antibiotic therapy.

The MIC of rifampin for the GBS strain used for *in vivo* studies (0.2 μg/ml) was unchanged after recovery from 17 rifampin-treated animals.

DISCUSSION

The infant rat model of nasopharyngeal carriage of GBS established in these experiments offers an opportunity to investigate colonization with GBS. Other investigators have studied the effect of intranasal inoculation on the development of disseminated infection. Ferrieri *et al.* (11) inoculated 10^1–10^8 CFU type III GBS into the anterior nares and observed only a single episode of transient bacteremia in thirty-eight 5-day-old rats. In contrast, Wennerstrom (12) studied the effect of intranasal inoculation of 10^1–10^5 CFU GBS type Ia in adult mice and found an LD_{50} 5.6 $\times 10^3$ CFU. Our infant rats, like those of Ferrieri *et al.* (11), tolerated twice daily nasal inoculation with only rare evidence of disseminated disease. The persistence of GBS organisms for at least 10 days in the nares of these infant rats makes this model valuable for investigation of GBS nasal carriage.

A variety of antibiotic regimens to eliminate GBS carriage in pregnant women has been utilized (2–5). In some instances a reduction of the colonization rate in these women and their newborns has been found, but treated mothers frequently continue to be colonized by GBS. For example, Boyer *et al.* (2) treated pregnant women colonized with GBS with parenteral ampicillin during labor. Thirty-one percent remained colonized with GBS when cultured 24 h after delivery. Gardner *et al.* (3) found that the majority of GBS-colonized women who were treated with penicillin in the late 3rd trimester remained colonized at the time of delivery.

There are several potential explanations for antibiotic failure to eradicate colonization with GBS from mucous membranes. First, the dose of antibiotic may be too low to produce an adequate concentration of antibiotic at the colonization site to eliminate the organism. Second, the timing of antibiotic prophylaxis may be incorrect. For example, many infants who develop early-onset GBS disease appear to have been infected *in utero* during labor and delivery. Intrapartum treatment of the GBS-colonized mother (2, 7) or administration of antibiotics to newborns within 1–2 h after birth (13) may be too late to eliminate invasive early-onset disease in the infant. Third, the antibiotic chosen for elimination of carriage may be ineffective despite *in vitro* sensitivity of the organism to the drug. Certainly, efforts to use ampicillin, trimethoprim/sulfamethoxazole, or cefaclor to eliminate *H. influenzae* carriage from the nasopharynx of contacts of patients with invasive *H. influenzae* disease were much less successful than treatment with rifampin (14–16). We have recently demonstrated similar results in eradicating chronic pharyngeal carriage of group A streptococci (9). Rifampin therapy was chosen because of its demonstrated activity in eliminating nasopharyngeal carriage with other bacteria.

In the animal model reported herein, we chose a 20 mg/kg dose of rifampin because a 10 mg/kg dose, more typically used for humans, failed to produce orange discoloration of the urine of treated animals and rifampin blood levels of animals treated with the lower dose were undetectable. No emergence of rifampin resistance was detected in the 17 isolates of GBS that were tested from animals treated with rifampin.

In this study, penicillin therapy was considerably less effective than rifampin in eliminating GBS nasal carriage from infant rats, in that only 9.6% of penicillin-treated animals were culture-negative 36 h after completion of therapy. In contrast, 65.4% of rifampin-treated animals were culture-negative with 92.0% showing at least a one log reduction in the numbers of GBS in nasal cultures. Therapy with rifampin plus penicillin was even more effective than rifampin alone in that 87.0% of animals treated with this combination were culture-negative 36 h after completion of therapy ($p \leq 0.01$). Furthermore, nasal cultures taken 7 days after completion of combination therapy remained negative for GBS in nearly 80% of animals. It is not completely clear why combination therapy with rifampin and penicillin is more effective than rifampin alone. Combination therapy was attempted because of concern that rifampin resistance might emerge during treatment with rifampin alone as previously reported for other bacteria (17, 18). It is possible that penicillin is synergistic with rifampin against GBS *in vivo* although our efforts as well as those of other investigators (10) failed to demonstrate consistently such an effect *in vitro*.

It would appear that a controlled clinical trial of rifampin and penicillin in combination as therapy for GBS colonized infants following systemic GBS infection is needed to determine the efficacy of this combination for eradicating nasal GBS colonization in neonates. Additional studies are needed to determine whether this antibiotic regimen can eliminate GBS from other mucosal sites in animals or humans.

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