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Evaluation of Rosaramicin Phosphate in Treatment of Experimental Syphilis in Rabbits

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The in vivo activity of rosaramicin phosphate in disseminated and localized *Treponema pallidum* infections in rabbits was compared with that of penicillin G benzathine. Rabbits were injected either intradermally or intravenously to establish infection. Groups of four animals each then received either two weekly injections of 200,000 U of penicillin G benzathine, injections of 12.5 or 25 mg of rosaramicin per kg of body weight twice a day for 10 days, or no antibiotic therapy. Treatment of the intradermal and intravenous infections was initiated on days 7 and 14 postinfection, respectively. With both infection models, striking differences were noted between the untreated control rabbits and the three groups receiving treatment; no discernible differences, however, were detected among any of the treated groups. Rabbits that had been infected intravenously did not develop disseminated lesions or orchitis after treatment, and chancres produced by intradermal infection regressed and healed rapidly after the initiation of therapy. Continued increases in treponemal and nontreponemal antibody titers posttreatment did not occur in any of the treated rabbits. Infectivity studies also suggested that the lymph nodes and testes of treated animals were free from infectious organisms. Overall, at the dosage regimens employed, both rosaramicin and penicillin G benzathine appeared to effect complete control of the experimental disease.

Rosaramicin is a macrolide antibiotic isolated from *Micromonospora rosaria* (6). Although this antibiotic is similar to erythromycin, certain in vitro studies have suggested that the activity of rosaramicin against several microorganisms is greater than that of erythromycin (7, 8). At this time, erythromycin is considered to be one of the best alternate drugs available when allergy precludes the use of penicillin in the treatment of syphilis; we reasoned that rosaramicin might prove to be equally effective in the treatment of syphilis.

Our experimental plan consisted of comparing the activity of rosaramicin with that of penicillin G benzathine in both disseminated (intravenous [i.v.] route) and localized (intradermal [i.d.] route) *Treponema pallidum* infections. Although data obtained from animal experiments can be extended only cautiously to human syphilis, the i.d. syphiloma model is widely accepted as an appropriate model for evaluation of antimicrobial regimens in *T. pallidum* infections (2, 4; B. D. Brause, J. S. Borges, and R. B. Roberts, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 15th, Washington, D.C., abstr. no. 36, 1975). In addition, we felt that inclusion of an i.v. model might provide information relevant to the disseminated (secondary) stage of human infection. Earlier studies with both erythromycin (5; Brause et al., 15th ICAAC, abstr. no. 36) and penicillin G benzathine (2) provided the framework for our choice of dosage regimens in both experiments.

MATERIALS AND METHODS

Animals. Outbred New Zealand white male rabbits weighing 2 to 3 kg were housed in individual cages at an ambient temperature of 18°C. Rabbits with a reactive Venereal Disease Research Laboratory (VDRL) test were excluded because of the possibility that this may have resulted from a subclinical infection with *Treponema cuniculi*. "Rapid hair growers" were also excluded, and all animals were shaven at least every other day throughout the course of the experiments.

Infection with *T. pallidum*. Maintenance of the Nichols strain of *T. pallidum* via intratesticular passage and preparation of suspensions of organisms for infection have been described previously (1). After centrifugation, testicular extracts were passed through 0.8-μm filters (Nuclepore Corp., Pleasanton, Calif.), and the number of treponemes was determined by dark-field microscopy. For establishment of disseminated infection, the preparation was adjusted to contain 4×10^7 treponemes per ml, and 1-ml volumes were injected i.v. into the marginal ear veins of 16 rabbits. The preparation was adjusted to contain 10^8 organisms per ml for localized infection; 0.1-ml vol-

umes were injected i.d. into four separate sites on the shaven backs of 16 animals.

Group designation and treatment. Immediately after injection, rabbits in each experiment were randomly assigned to one of four groups. With the disseminated model, treatment was begun on day 14 after i.v. infection; this point roughly corresponds to a period of 1 week before the expected onset of the disseminated rash and to a period of 2 weeks before the expected development of orchitis and disseminated papular lesions. In the localized model, treatment was begun 7 days after i.d. infection, the time at which chancres generally first become apparent for the stated inoculum size.

As stated above, the animals in both experiments were divided into four groups of four rabbits each. Group A served as untreated controls, whereas group B received two intramuscular (i.m.) injections of 200,000 U of penicillin G benzathine on days 14 and 21 or days 7 and 14 post-i.v. and post-i.d. infection, respectively. Group C (rosaramicin treated, high dose) received 25 mg of rosaramicin phosphate per kg i.m. twice daily (every 12 h) beginning on day 14 or 7 after i.v. or i.d. infection, respectively. Group D (rosaramicin treated, low dose) received 12.5 mg of rosaramicin phosphate per kg i.m. twice daily for 10 days, employing the same schedule used for group C.

Observations. Rabbits infected i.v. were examined daily for signs of overt disease, including a disseminated macular rash, generalized erythematous and indurated papules, necrosis of the papules, and the presence of an orchitis. Because individual chancres varied considerably in size, the mean diameter of induration, the degree of dissemination, and the character of lesions were recorded daily. These, in turn, were converted to a series of numerical scores, as follows: if the majority of lesions were less than 7 mm in diameter with slight erythema surrounding them, they were scored as 1+; if they were greater than 7 mm in diameter with erythema, they were scored as 2+; if they were greater than 14 mm in diameter with marked erythema and induration, they were scored as 3+; and if they were greater than 21 mm in diameter with profound erythema, induration, and various degrees of necrosis, they were scored as 4+. Notation was also made as to: (i) whether the papules progressed to a necrotic stage with dark-red to black necrotic centers; (ii) the extent of dissemination, which varied from a few sparse lesions to widespread crops; and (iii) the time at which lesions began to resolve and heal. Lesion appraisal in rabbits infected i.d. was made by using similar criteria. Photographs of each rabbit in both experiments were taken for comparative purposes; these were scheduled on a weekly basis.

Serological studies. All animals were bled on day 6 of their infection and thereafter on a weekly basis. Quantitative VDRL and fluorescent treponemal antibody absorption (FTA-Abs) tests were performed by standard procedures (3) on sera obtained from each weekly bleeding.

Necropsy and assessment of treponemes in tissues. At six weeks after infection, all rabbits were exsanguinated via cardiac puncture, and the sera were separated for final serologies. Spleens, livers, and two of the four axillary lymph nodes from each animal were prepared, fixed in 10% buffered Formalin, sectioned, and then stained with either hematoxylin and eosin or modified Warthin-Starry silver stain for histopathological examination. The remaining two axillary lymph nodes and testes were processed as follows for extraction of treponemes. Nodes were placed in 5 ml of minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.), and the testes were placed in 10 ml of minimal essential medium; these were minced and then ground in a mortar and pestle with sterile sand. The homogenate was centrifuged at 754 × *g* for 10 min to remove tissue debris, and the supernatant fluid was examined by dark-field microscopy for quantitation of treponemes. Samples of 0.1 ml of each supernatant fluid were also injected i.d. into three shaved sites on the backs of healthy, VDRL-nonreactive rabbits as a test of infectivity. These recipients were then observed for 2 months to determine whether papules developed at any of the sites of infection, and sera were obtained for routine serological studies.

RESULTS

i.v. infection. Disseminated disease developed in all untreated control animals (group A; Table 1), with a generalized rash first being noted 23 to 24 days after i.v. infection. Cutaneous lesions appeared 2 to 3 days later (day 26 or 27); by day 30, each animal had >35 individual papules ranging from 7 to 18 mm in diameter over the shaven portion of its back. These lesions continued to progress, with coalescence, until the time of sacrifice on day 42. Orchitis also appeared in all four animals during week 4 of infection. In the three treatment groups (groups B, C, and D), no clinical manifestations of disease were noted in any of the rabbits.

VDRL and FTA-Abs antibodies first appeared 2 to 3 weeks after i.v. injection of *T. pallidum* and were present in all rabbits, indicating that infection had taken place. In group A, titers steadily rose until week 5 or 6 after infection and were obviously elevated by comparison with the other three groups. Serum titers of all treated rabbits failed to increase substantially between weeks 4 and 6 of infection (Table 2) and in a number of instances actually declined.

Dark-field examination of supernatant fluids from extracted testicular and nodal tissues failed to reveal organisms morphologically compatible with *T. pallidum*. As this negative finding could have resulted from small numbers of *T. pallidum* ($<10^4$/ml) being present, material was also injected i.d. in triplicate into the backs of shaven, VDRL-nonreactive rabbits. Recipients of testicular material from group A animals developed chancres at all sites of infection between 8 and 22 days after i.d. challenge. Three of the four recipients also developed chancres at all sites injected with lymph node extracts. Development of VDRL and FTA-Abs antibodies was also de-

tected in these recipients. Lesions did not develop in any rabbit that received testicular or nodal tissue from rabbits in group B, C, or D. The recipients of testicular and nodal extracts obtained from two penicillin G benzathine-treated and one high-dose-rosaramicin-treated rabbits had weakly reactive VDRL tests at 6 weeks, although these antibodies were not detected at 5, 7, or 8 weeks; FTA-Abs titers were nonreactive in recipients of testicular or nodal tissue from all treated animals throughout the observation period (data not shown).

TABLE 1. *Experimental disease in untreated control rabbits (group A) infected i.v. with T. pallidum*[a]

Experimental parameter	Group $\bar{x}$
Onset of rash	Day 23.25 (23–24)
Duration of rash until onset of lesions	3 days (2–4)
Onset of disseminated lesions	Day 26
Severity and extent of lesions (day 30)	1–3+; >35 lesions
Severity and extent of lesions (day 37)	1–4+; necrotic centers; widespread; coalesced
Duration of lesions from onset to day of sacrifice	17 days
Onset of orchitis	Day 27.5 (27–28)
Duration of orchitis	6.75 days (6–7)

[a] Each value represents the mean of the four rabbits within the group studied. The values within parentheses represent the ranges observed.

i.d. infection. Chancres developed at all of the injection sites within 5 to 7 days after i.d. infection. The variation within or among groups of animals with respect to the time of onset of lesions was negligible (Table 3). Treatment with penicillin G benzathine (group B) or rosaramicin (groups C and D) was begun 7 days postinfection, which was actually 1 to 2 days after chancres were first noted. In group A, chancres continued to progress throughout the observation period. Although some variation was noted, the chancres enlarged and reached their maximum size (mean diameter, ~17 mm) between weeks 2 and 3 after inoculation. In groups B, C, and D, chancres began to regress shortly after initiation of treatment, irrespective of the antibiotic used or the dose. In almost all instances, the chancres in treated rabbits had healed by day 16, corresponding temporally with the last day of rosaramicin treatment. Discernible differences among these groups were not detected.

Differences were also observed between the control animals and the three treated groups with respect to titers of VDRL and FTA-Abs antibodies. The serological results at weekly intervals mimicked those obtained in the other experiment (data not shown).

Rabbits were exsanguinated on day 42 of infection. Dark-field examination of nodal and testicular tissues from all animals failed to reveal

TABLE 2. *Serum VDRL and FTA-Abs titers in rabbits infected i.v. with T. pallidum*[a]

Group	Rabbit no.	Titer at wk after infection: 2		3		4		5		6	
		VDRL	FTA-Abs	VDRL	FTA-Abs	VDRL	FTA-Abs	VDRL	FTA-Abs	VDRL	FTA-Abs
A (untreated controls)	1	WR	2	4	64	8	512	16	512	16	>2,048
	2	WR	NR	8	8	8	1,024	64	1,024	128	>2,048
	3	NR	NR	NR	8	NR	256	16	512	16	1,024
	4	NR	NR	NR	4	NR	1,024	64	>2,048	16	>2,048
B (penicillin G benzathine-treated controls)	1	WR	NR	8	8	8	32	NR	16	UD	16
	2	UD	NR	2	16	UD	16	UD	16	UD	16
	3	WR	NR	2	8	UD	16	UD	16	WR	8
	4	WR	NR	2	32	4	128	UD	32	UD	32
C (rosaramicin, high dose, 25 mg/kg, twice daily for 10 days)	1	UD	NR	UD	8	2	16	NR	16	WR	8
	2	WR	NR	2	4	2	8	UD	8	UD	8
	3	WR	NR	2	32	2	64	WR	32	UD	16
	4	WR	4	UD	8	UD	8	WR	4	WR	4
D (rosaramicin, low dose, 12.5 mg/kg, twice daily for 10 days)	1	WR	NR	2	16	2	128	UD	64	UD	64
	2	WR	NR	UD	16	UD	32	UD	32	UD	16
	3	NR	2	2	64	UD	64	UD	64	UD	32
	4	UD	NR	UD	4	UD	32	2	16	UD	16

[a] Numerical titers are expressed in terms of the reciprocal of the reactive dilution: NR, nonreactive; WR, weakly reactive; and UD, reactive undiluted.

TABLE 3. *Observations in treated and untreated rabbits after i.d. infection with T. pallidum*[a]

Group	Rabbit no.	Day of onset	Total duration of lesions (no. of days)	Duration of lesions after day 16 (last day of rosaramicin therapy)	Maximal severity of lesions		
					Score	Diam (mm)	Day first noted
A (untreated controls)	1	4.8	35.8	24.5	4+	20.8	19.5
	2	5.3	21.8	11.0	3+	18.0	17.0
	3	7.3	22.3	13.5	3+	15.0	16.5
	4	5.3	24.8	14.0	3+	14.3	10.0
B (penicillin G benzathine treated)	1	6.0	10.5	0.8	2+	7.3	7.8
	2	5.5	8.0	0.0	2+	9.3	7.8
	3	6.5	3.5	0.0	2+	7.0	7.8
	4	5.5	6.5	0.0	2+	7.5	7.0
C (rosaramicin, high dose, 25 mg/kg, twice daily for 10 days)	1	5.5	8.8	0.0	2+	8.8	7.5
	2	5.5	7.5	0.0	2+	10.8	7.3
	3	5.5	3.5	0.0	1+	5.3	6.3
	4	6.3	5.5	0.0	2+	10.3	9.5
D (rosaramicin, low dose, 12.5 mg/kg, twice daily for 10 days)	1	6.3	5.0	0.0	2+	6.5	7.5
	2	5.5	5.0	0.0	2+	7.3	6.8
	3	5.5	9.3	0.0	2+	9.5	8.3
	4	5.8	5.8	0.0	2+	10.3	7.8

[a] Each value represents the mean of four observations (one per chancre) made on each animal.

treponemes. Recipient rabbits which were injected i.d. with testicular extracts from group A animals developed chancres at all injection sites; chancres, however, did not appear at the sites of injection of lymph node extract. Recipients of extracts from the treated rabbits (all regimens) developed neither clinical nor serological signs of infection.

Complications of therapy. Penicillin G benzathine did not cause any apparent adverse reactions, locally or systemically. By day 7 or 8 of the 10-day regimens of high-dose-rosaramicin therapy, sterile abscesses were noted in the areas of antibiotic injection in three of the eight animals (i.v. and i.d. experiments combined).

DISCUSSION

The results of this study suggest that rosaramicin, at both dosage levels, effected essentially complete control of experimental syphilis in the rabbit. In the absence of elaborate preliminary studies, our choice of rosaramicin dosage regimens was strongly influenced by: (i) an earlier study with erythromycin (Brause et al., 15th ICAAC, abstr. no. 36) which had shown that 12 mg/kg administered i.m. twice a day for 7 days was capable of reducing treponeme counts in established rabbit chancres by more than 2 $\log_{10}$ units; and (ii) unpublished data supplied by Schering Corp., Bloomfield, N.J., which suggested that the trial doses were in the range of anticipated clinical doses. Selection of the i.m. route of administration was also in line with earlier studies of this activity of erythromycin in the syphiloma model (5; Brause et al., 15th ICAAC, abstr. no. 36), even though rosaramicin is not intended for i.m. use because of the tendency for macrolide antibiotics to produce sterile abscesses such as those seen in three of the eight animals receiving the high-dose regimen. As none of the rabbits receiving penicillin G benzathine weighed more than 2.6 kg at the time of treatment (data not shown), the total dose administered was almost twice the dose of 84,000 U/kg which has been shown to be effective for treatment of early experimental syphilis in rabbits.

In both infection models, differences were noted between the untreated control rabbits and the three groups receiving treatment; no differences, however, were detected among any of the treated groups. The rabbits infected i.v. did not develop disseminated lesions or orchitis after treatment, and the chancres in the i.d. model regressed and healed quite rapidly after initiation of therapy without undergoing necrosis. Substantial increases in treponemal and nontreponemal antibody titers posttreatment were not observed in either experiment. The negative infectivity studies with nodal and testicular material from treated rabbits constituted additional proof that these animals were free from infectious organisms.

As no discernible differences were noted between animals receiving low or high doses of

rosaramicin or penicillin G benzathine in either experiment, value judgments concerning the efficacy of the experimental drug over the control drug cannot be made. Further studies, in which dose effect titrations, achievable serum concentrations, and other routes of administration are examined, are necessary to determine concentrations that should be maintained for maximum therapeutic benefit. Such studies should elucidate the potential promise of this antibiotic, define any advantages which rosaramicin may have over erythromycin in the treatment of syphilis, and establish an interface between studies in animal models and in humans.

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