In Vivo Efficacy of 81.723 hfu, a New Pleuromutilin Derivative Against Experimentally Induced Airsacculitis in Chicks and Turkey Poults

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The efficacy of the pleuromutilin derivative 81.723 hfu was tested in chicks and turkey poults by experimentally infecting them with *Mycoplasma gallisepticum*. The data were treated to obtain the mean effective doses, and these were compared to those obtained with tylosin tartrate. The compounds were administered either by ingluvial catheter or via the drinking water. Therapy was started on the day of inoculation or 7 days thereafter, respectively. The experiments showed that the compound 81.723 hfu was significantly more active in chickens than tylosin tartrate by both methods of administration of drinking water. In turkey poults the pleuromutilin derivative and tylosin tartrate had comparable activity.

A new pleuromutilin derivative, 14-deoxy-14 (2-diethylaminoethyl)-mercaptoacidoxymutilin hydrogen fumarate, Sandoz 81.723 hfu, showed good antibacterial activity in vitro against gram-positive bacteria and mycoplasmas. The antimicrobial spectrum and the efficacy against bacterial infections in laboratory animals, including a mycoplasmal polyarthritis in rats, has been described by Drews et al. (1). The present paper deals with studies to evaluate the efficacy of 81.723 hfu in the control of an experimental mycoplasmal infection in chicks and turkey poults.

MATERIALS AND METHODS

Compounds. The physical and chemical properties of 81.723 hfu will be published elsewhere. It is readily soluble in water, and its aqueous solutions are stable in the neutral and acidic pH range. Tylan soluble (tylosin tartrate; Elanco Products, Ltd., London) was used for comparison in all experiments.

Inoculum. *Mycoplasma gallisepticum* F₈, kindly supplied by C. O. Baughn, Squibb Laboratories, Princeton, N.J., was passaged in Oxoid mycoplasma broth base, supplemented with 25% horse serum and 4% yeast extract (2) and incubated for 48 h at 37°C. A 1-ml sample of this culture contained approximately 10⁹ colony-forming units, 10 times the infective dose for 95% of the animals evaluated in preliminary tests by the Spearman-Kärber method (4).

Infection. One milliliter of this culture was inoculated into the left posterior thoracic air sac of the birds. Four days after inoculation the animals developed airsacculitis without any clinical symptoms. Infected air sacs showed diffuse turbidity and in most cases fibrinous exudate. The pathogen could be reisolated from all affected air sacs.

Animals. Three-week-old male Hubbard chicks and turkey poults weighing about 350 to 450 g were obtained from a commercial source free of *M. gallisepticum*, based on serological examinations.

Treatment. Two groups of experiments were set up. In one group the first treatment was started at the time of inoculation. In the other group the first treatment began 7 days after inoculation when airsacculitis had been established.

The compounds were dissolved in water and were administered either by individual application via an ingluvial catheter or offered ad libitum in the drinking water. The treatment by catheter was carried out three times at 24-h intervals. Eight dosages with 10 to 20 birds were used in each test. Medicated drinking water was offered for a period of 3 days to chicks and turkey poults. Three dosages in groups of four animals each were compared. These experiments were repeated three times to allow statistical analysis.

Evaluation. Additional infected untreated and noninfected birds were used as controls. The chicks and turkey poults were examined daily during the experiments and necropsied 8 days after the first treatment. Both posterior thoracic air sacs were checked for the presence of gross lesions. In addition, specimens from the air sacs were plated on supplemented PPLO agar (Oxoid) for reisolation of *M. gallisepticum*. The samples were also plated on blood agar to detect possible bacterial contaminations.

Where the drugs were administered simultaneously with inoculation, two criteria were assessed to evaluate the antimycoplasmal activity of the compounds in these experiments: (i) absence of airsac lesions at the time of necropsy; and (ii) failure to reisolate *M. gallisepticum* from the air sacs.

Necropsy revealed that treatment of an established airsacculitis, when not begun until 7 days after inoculation, did not completely reverse the lesions. In these tests, therefore, the efficacy of the compounds

was ascertained by the absence of the pathogen in the cultures prepared from affected air sacs.

The mean effective dose (ED_{50}) values in tests with application by catheter were estimated by the Spearman-Kärber method (4), and those after drinking water application were estimated by analysis of covariance with probit transformed percentages (3).

RESULTS

Figure 1 illustrates the different experimental set-up of testing 81.723 hfu in comparison with tylosin tartrate. The results in Tables 1 (test A) and 2 (test B) show the efficacy of the two compounds in chicks and turkey poulters after application by ingluvial catheter when treatment was started simultaneously with infection. The results demonstrate a distinct efficacy of 81.723 hfu in the two species. The ED_{50} values of the

pleuromutilin derivative differed significantly ($P = < 0.05$) from those obtained with tylosin tartrate.

Table 3 (tests C and D) summarizes the data from the repeated experiments in chicks and turkey poulters, in which treatment via the drinking water was commenced at the time of inoculation and continued for 3 days. The ED_{50} values are expressed in percentage of concentration of the compounds in the drinking water.

The pleuromutilin derivative proved more effective in chicks ($P = < 0.01$) than the commercial drug tested. No significant difference could be observed in tests with turkey poulters.

In the second group of studies, aimed at controlling an established airsacculitis, chicks and turkey poulters have been evaluated. Tables 4 (test E) and 5 (test F) present the results

FIG. 1. Schedule of treatment with 81.723 hfu and tylosin in poultry.

TABLE 1. ED_{50} values of 81.723 hfu and tylosin after catheter application in chicks^a

Treatment	Dosage (mg/kg body weight)	No. of animals cured/total	% Animals cured	ED_{50} (mg/kg body weight) ^b
81.723 hfu	3 × 2.5	0/20	0	3 × 5.1 = <u>15.3</u> (12.6 – 18.6)
	3 × 5	9/15	60	
	3 × 7.5	9/12	75	
	3 × 10	10/10	100	
	3 × 20	9/10	90	
	3 × 30	12/12	100	
	3 × 40	15/15	100	
	3 × 80	20/20	100	
Tylosin	3 × 3.125	0/20	0	3 × 27.7 = <u>83.1</u> (62.4 – 111)
	3 × 6.25	1/15	6.6	
	3 × 12.5	1/12	8.3	
	3 × 25	3/10	30	
	3 × 50	9/10	90	
	3 × 100	12/12	100	
	3 × 200	15/15	100	
	3 × 400	20/20	100	
Untreated infected controls		0/20	0	20/20 ^c

^a Test A: treatment started simultaneously with infection.

^b Numbers in parentheses equal 95% confidence limits.

^c Air sac lesions.

TABLE 2. ED_{50} values of 81.723 hfu and tylosin after catheter application in turkey poult^a

Treatment	Dosage (mg/kg body weight)	No. of animals cured/total	% Animals cured	ED_{50} (mg/kg body weight) ^b
81.723 hfu	3 × 2.5	0/20	0	$3 \times 11.26 = \underline{33.8}$ (27.4 – 41.7)
	3 × 5	0/15	0	
	3 × 7.5	3/12	25	
	3 × 10	4/10	40	
	3 × 20	9/10	90	
	3 × 30	12/12	100	
	3 × 40	15/15	100	
	3 × 80	19/19	100	
Tylosin	3 × 5	0/15	0	$3 \times 68.6 = \underline{205.8}$ (171 – 247.8)
	3 × 7.5	0/12	0	
	3 × 10	1/10	10	
	3 × 20	0/10	0	
	3 × 30	0/12	0	
	3 × 50	4/15	26.7	
	3 × 100	16/20	80	
	3 × 200	10/10	100	
Untreated infected controls		0/20	0	20/20 ^c

^a Test B: treatment started simultaneously with infection.^b Numbers in parentheses equal 95% confidence limits.^c Air sac lesions.TABLE 3. ED_{50} values of 81.723 hfu and tylosin after drinking water application^a

Treatment	Dosage (3 days) concn in drinking water (%)	Test groups (no. of animals cured/total)			Total	ED_{50} (% concn in drinking water)
		1	2	3		
Chicks ^b 81.723 hfu	0.012	3/4	3/4	3/4	9/12	0.007
	0.025	4/4	4/4	4/4	12/12	
	0.05	4/4	4/4	4/4	12/12	
Tylosin	0.012	0/4	1/4	3/4	4/12	0.016
	0.025	3/4	3/4	4/4	10/12	
	0.05	3/4	4/4	4/4	11/12	
Untreated infected controls		0/4	0/4	0/4	0/12	12/12 ^c
Turkey poult ^d 81.723 hfu	0.012	0/4	1/4	1/4	2/12	0.026
	0.025	2/4	4/4	0/4	6/12	
	0.05	2/4	3/4	4/4	9/12	
Tylosin	0.012	0/4	0/4	0/4	0/12	0.035
	0.025	2/4	1/4	0/4	3/12	
	0.05	2/4	3/4	4/4	9/12	
Untreated infected controls		0/4	0/4	0/4	0/12	12/12 ^c

^a Treatment started simultaneously with infection.^b Test C.^c Air sac lesions.^d Test D.

TABLE 4. ED_{50} values of 81.723 hfu and tylosin after catheter application in chicks^a

Treatment	Dosage (mg/kg body weight)	No. of animals cured/total	% Animals cured	ED_{50} (mg/kg body weight) ^b
81.723 hfu	3 × 2.5	3/20	15	3 × 21.23 = <u>63.7</u> (43.8 – 92.7)
	3 × 5	4/15	26.4	
	3 × 7.5	4/12	33	
	3 × 10	4/10	40	
	3 × 20	4/10	40	
	3 × 30	5/12	41.5	
	3 × 40	8/15	52.8	
	3 × 80	14/20	70	
Tylosin	3 × 2.5	0/20	0	3 × 38.45 = <u>115.4</u> (85.9 – 159.1)
	3 × 5	1/15	6.6	
	3 × 7.5	1/12	8.3	
	3 × 10	2/10	20	
	3 × 20	2/10	20	
	3 × 30	4/12	33	
	3 × 40	8/15	52.8	
	3 × 80	12/20	60	
Untreated infected controls		0/20	0	20/20 ^c

^a Test E: treatment started 7 days after infection.^b Numbers in parentheses equal 95% confidence limits.^c Air sac lesions.TABLE 5. ED_{50} values of 81.723 hfu and tylosin after catheter application of turkey poults^a

Treatment	Dosage (mg/kg body weight)	No. of animals cured/total	% Animals cured	ED_{50} (mg/kg body weight) ^b
81.723 hfu	3 × 10	3/19	15.8	3 × 57.6 = <u>172.8</u> (77.7 – 383.4)
	3 × 20	4/15	28.6	
	3 × 40	4/12	33.3	
	3 × 80	4/10	40	
	3 × 100	5/10	50	
	3 × 140	6/12	50	
	3 × 180	11/15	73.3	
	3 × 200	18/18	100	
Tylosin	3 × 20	0/20	0	3 × 70.3 = <u>210.9</u> (167.4 – 265.5)
	3 × 40	6/15	40	
	3 × 80	5/12	41.6	
	3 × 100	7/10	70	
	3 × 140	8/10	80	
	3 × 180	10/12	83.3	
	3 × 200	14/15	93.3	
	3 × 250	20/20	100	
Untreated infected controls		0/20	0	20/20 ^c

^a Test F: treatment started 7 days after infection.^b Numbers in parentheses equal 95% confidence limits.^c Air sac lesions.

obtained after ingluvial catheter application of the compounds.

On comparing the ED_{50} values of the drugs when tested under identical conditions, 81.723

hfu showed a significantly greater activity ($P < 0.05$) in chicks, whereas the efficacy of the two compounds in turkey poults was comparable.

TABLE 6. ED_{50} values of 81.723 hfu and tylosin after drinking water application^a

Treatment	Dosage (3 days) concn in drinking water (%)	Test groups (No. of animals cured/total)			Total	ED_{50} (% concn) in drinking water)
		1	2	3		
Chicks ^b 81.723 hfu	0.012	4/4	4/4	2/4	10/12	0.008
	0.025	4/4	4/4	4/4	12/12	
	0.05	4/4	4/4	4/4	12/12	
Tylosin	0.012	0/4	0/4	0/4	0/12	0.026
	0.025	2/4	0/4	3/4	5/12	
	0.05	4/4	4/4	4/4	12/12	
Untreated infected controls		0/4	0/4	0/4	0/12	12/12 ^c
Turkey poults ^d 81.723 hfu	0.012	3/4	3/4	2/4	8/12	<0.0125
	0.025	3/4	3/4	2/4	8/12	
	0.05	4/4	3/4	2/4	9/12	
Tylosin	0.012	0/4	0/4	0/4	0/12	>0.05
	0.025	0/4	0/4	0/4	0/12	
	0.05	1/4	0/4	0/4	1/12	
Untreated infected controls		0/4	0/4	0/4	0/12	12/12 ^c

^a Treatment started 7 days after infection.^b Test G.^c Air sac lesions.^d Test H.

Table 6 (tests G and H) summarizes the activity of 81.723 hfu and tylosin tartrate in the two species after drinking water application for 3 days. Only in chicks did the ED_{50} values differ significantly ($P = < 0.01$) in favor of the pleuromutilin derivative.

In the infected untreated group, lesions were caused regularly by the pathogen, thereby demonstrating the virulence of the inocula. In all cases *M. gallisepticum* was reisolated from affected air sacs, whereas bacterial contamination could not be demonstrated. The noninfected untreated controls showed neither clinical symptoms nor pathological lesions.

DISCUSSION

In all experiments compound 81.723 hfu proved highly effective, palatable, and safe, and was well tolerated. No side effects could be observed in the birds treated. On starting treatment at the time of infection, either by ingluvial catheter or drinking water application, 81.723 hfu showed distinct efficacy in preventing the development of airsacculitis in chicks and turkey poults. The ED_{50} values showed that 81.723

hfu was significantly more active than tylosin tartrate in the experiments with chicks. In contrast, in the experiments with turkey poults no significant difference was observed in the activity of the two compounds. The same was found on treating an established airsacculitis 7 days after inoculation.

Although necropsy showed only incomplete reversion of air sac lesions, pathogens could not be reisolated from the affected air sacs of cured birds. For control of an established airsacculitis the ED_{50} values of the two compounds were greater than when treatment was started at the time of inoculation. In the infected, untreated controls, air sac lesions were invariably found, as was the pathogen *M. gallisepticum*.

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