MICROBIOLOGICAL AND PHARMACOKINETIC STUDIES OF ACYL DEMYCINOSYLTYLOSIN AND RELATED TYLOSIN DERIVATIVES

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(Received for publication March 16, 1990)

A series of tylosins and acyl derivatives of 23-*O*-demycinosyltylosin (DMT) were initially tested for *in vitro* antibacterial activity and serum levels in squirrel monkeys (po) and mice (iv). Overall, the DMT compounds were more active *in vitro* than the tylosins. Two tetraacylated DMTs, Sch 37644 and Sch 38646, were selected from the initial studies for further evaluation and compared to erythromycin and A-56268 (6-*O*-methyl erythromycin). Sch 37644 and Sch 38646 were 2 to 8-fold less potent *in vitro* against Gram-positive bacteria than erythromycin and A-56268. In squirrel monkeys, Sch 37644 (AUC, 19.7 μg·hour/ml) and A-56268 (21.6 μg·hour/ml) had similar serum levels following po administration of 20 mg/kg, while Sch 38646 (11.8 μg·hour/ml) and erythromycin (1.5 μg·hour/ml) had lower levels. In mice administered 200 mg/kg orally, Sch 37644 (AUC, 19.4 μg·hour/ml) and Sch 38646 (15.4 μg·hour/ml) had higher serum levels than erythromycin (5.7 μg·hour/ml). A-56268 was the most active po macrolide in mouse protection studies (PD_{50}s) against Staphylococci and Streptococci, while Sch 37644 and Sch 38646 were similar to erythromycin.

Erythromycin, the most commonly prescribed macrolide antibiotic for urinary tract and respiratory infections, has the disadvantage of being poorly absorbed after po administration. Therefore, a new series of macrolide antibiotics were prepared from tylosin and tested for improved po pharmacokinetics, as well as the retention of the good efficacy of erythromycin against Gram-positive infections. The derivatives were tested for *in vitro* antimicrobial activity, and pharmacokinetics in squirrel monkeys (po) and mice (iv). On the basis of initial results, two compounds were selected for further studies, 3,23,2′-tri-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-*iso*-valeryltylosin (Sch 37644) and its 12,13-epoxy derivative (Sch 38646). These compounds were compared to erythromycin and a new orally active erythromycin derivative, A-56268 (Abbott Laboratories, Abbott Park, IL). The data were presented in part at the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy[1].

Materials and Methods

Antibiotics

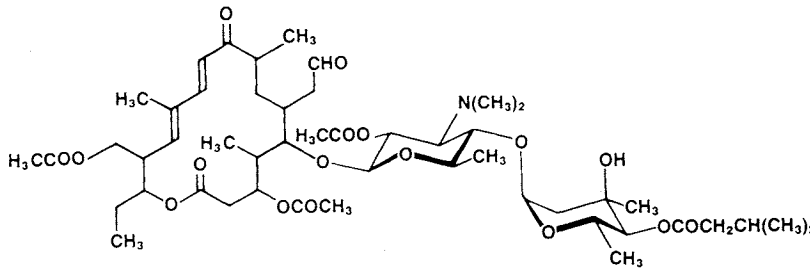
A series of tylosin derivatives were synthesized by Dr. MALLAMS in our laboratories[2,3]. These derivatives consisted of two structurally related groups of compounds: Tylosin and 23-*O*-demycinosyltylosin (DMT) and their acyl, hydrazone, and 12,13-epoxy derivatives. The structures of the two selected derivatives, Sch 37644 and Sch 38646, are shown in Fig. 1.

A-56268 (6-*O*-methyl erythromycin) was obtained from Dr. P. B. FERNANDES, Abbott Laboratories, Abbott Park, IL. Erythromycin was obtained from Sigma Chemical Co., St. Louis, MO.

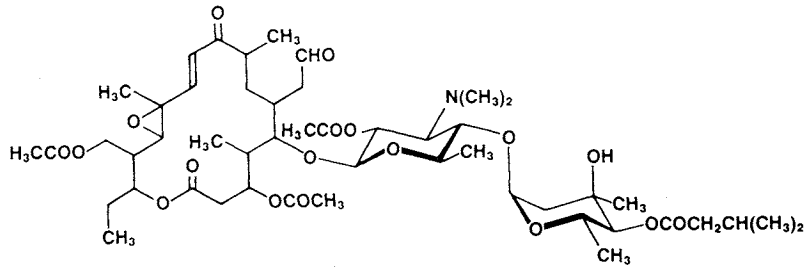
MIC Determinations

MICs (μg/ml) were determined by standard agar dilution techniques as described by MILLER *et al.*[4].

Fig. 1. Structures of Sch 37644 and Sch 38646.



Sch 37644: 3,23,2′-Tri-*O*-acetyl-4″-*O*-*iso*-valeryl-23-*O*-demycinosyltylosin (DMT)



Sch 38646: 12,13-Epoxy-3,23,2′-tri-*O*-acetyl-4″-*O*-*iso*-valeryl DMT

Serum Levels

Serum levels were determined in squirrel monkeys (1 kg) fasted 18 hours prior to receiving a single po dose (20 mg/kg) of antibiotic. Serum levels were also determined in male CF-1 mice (20 g, Harlan Sprague-Dawley, Indianapolis, IN) following either a single po (200 mg/kg) or iv (100 mg/kg) dose of antibiotic. All serum samples were tested for antibiotic activity by microbiological assay with *Micrococcus luteus* ATCC 9341 as the test bacterium.

Protection Tests (PD_{50}s)

CF-1 mice were infected intraperitoneally with an inoculum of Staphylococci or Streptococci in mucin (Sigma Chemical Co., St. Louis, MO) with 0.22% (w/v) ferric ammonium citrate. Groups of 10 mice were treated orally with a single dose of antibiotic (ranging from 0.4 to 500 mg/kg) 1 hour post-infection. PD_{50} values were estimated by probit analysis of the survivors 7 days post-infection[5].

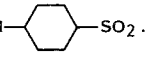
Results

In Vitro Antibacterial Activities

MIC results for the series of tylosins, DMT derivatives and other antibiotics are shown in Table 1. The geometric mean MICs (GMM) for the tylosins and acyl DMT derivatives ranged from 0.18 to 1.0 μg/ml for macrolide-susceptible Gram-positive bacteria including Staphylococci, *Bacillus subtilis*, and *M. luteus*. The GMMs for these compounds were 0.35 to 7.0 μg/ml for erythromycin-resistant Staphylococci; 0.10 to 0.71 μg/ml for Streptococci of Groups A, C and G; 0.25 to 4.0 μg/ml for Group B Streptococci; 0.03 to 1.0 μg/ml for *S. pneumoniae*; and 0.24 to 2.0 μg/ml for Group D and viridans Streptococci. The 23-*O*-DMT derivatives, Sch 37644 and Sch 38646, were 2 to 8-fold less potent than erythromycin and A-56268. However, both Sch 37644 (GMM, 1.7 μg/ml) and Sch 38646 (2.0 μg/ml), but not A-56268 ($\geq$90.5 μg/ml), were active against erythromycin-resistant Staphylococci.

Table 1. MICs of acyl DMT and related compounds.

Compounds[a]	Geometric mean MICs (μg/ml, 24 hours, Mueller-Hinton agar)						
	Staphylococcus			*Streptococcus*			
	Macrolide-susceptible[b] (17)[c]	Erythromycin-resistant (4)	Macrolide-resistant (4)	Groups A, C, G (10)	Group B (6)	*S. pneumoniae* (8)	Group D, viridans (4)
3,23,2′-Tri-OAC-4″-OIV DMT (Sch 37644)	0.41	1.7	≥128	0.16	0.35	0.25	0.42
12,13-Epoxy-3,23,2′-tri-OAC-4″-OIV DMT (Sch 38646)	0.29	2.0	≥128	0.29	1.0	0.09	0.63
6-*O*-methyl erythromycin (A-56268)	0.10	≥90.5	≥128	0.05	0.11	0.05	0.30
Erythromycin	0.10	≥90.5	≥128	0.07	0.12	0.04	0.50
Tylosin	0.56	2.0	≥128	0.33	2.0	0.33	1.6
Tylosin hydrazone	0.37	3.5	≥128	0.16	0.79	0.12	>0.89
12,13-Epoxytylosin	0.92	2.8	≥128	0.43	1.8	1.0	2.0
4″-OIV tylosin	0.57	2.6	19.0	0.57	0.79	0.50	1.2
12,13-Epoxy-4″-OIV tylosin	0.92	4.0	13.4	0.31	1.0	0.23	1.6
3-OAC-4″-OIV tylosin	0.82	4.0	42.2	0.19	1.0	0.12	0.84
3-OAC-4″-OIV tylosin hydrazone	0.88	4.0	53.8	0.33	1.0	0.14	1.0
12,13-Epoxy-3-OAC-4″-OIV tylosin	0.92	5.6	22.6	0.71	4.0	0.23	1.8
3,2′-Di-OAC-4″-OIV tylosin	0.78	4.0	26.9	0.27	1.0	0.19	0.94
12,13-Epoxy-3,2′-di-OAC-4″-OIV tylosin	1.0	5.6	26.9	0.57	4.0	0.32	1.9
23-DMT	0.22	0.87	≥128	0.23	0.50	0.03	>0.60
23-DMT hydrazone	0.79	7.0	≥128	0.54	0.63	0.10	>0.94
23-OIV DMT hydrazone	0.18	0.87	≥128	0.19	0.25	0.07	0.28
23-*O*-Benzoyl DMT hydrazone	0.25	0.50	≥128	0.29	0.50	0.12	0.50
23-*O*-Phenylacetyl DMT hydrazone	0.21	0.35	≥128	0.18	0.25	0.04	0.24
3-OAC-23-*O*-phenylacetyl-4″-OIV DMT hydrazone	0.50	2.0	≥128	0.19	0.35	0.25	0.33
4″-OIV DMT hydrazone	0.24	1.5	≥128	0.12	0.25	0.08	0.28
3-OAC-4″-OIV DMT	0.24	1.0	≥128	0.10	0.50	0.04	0.25
3-OAC-4″-OIV DMT hydrazone	0.25	2.0	≥128	0.10	0.25	0.04	0.30
12,13-Epoxy-3-OAC-4″-OIV DMT	0.31	1.7	≥128	0.20	1.0	0.07	0.67
3,2′-Di-OAC-4″-OIV DMT	0.54	4.0	≥128	0.25	1.0	0.14	0.89
3,2′-Di-OAC-4″-OIV DMT hydrazone	0.39	2.0	≥128	0.14	0.50	0.07	0.47
3,23-Di-OAC-4″-OIV DMT hydrazone	0.26	2.0	≥128	0.10	0.50	0.05	0.26
3,23,2′-Tri-OAC-4″-OIV DMT hydrazone	0.52	1.7	≥128	0.20	0.50	0.25	0.44

[a] Compound abbreviations: 4″-OIV, 4″-*O*-*iso*-valerate; OAC, *O*-acetyl; hydrazone, =N–N–  –SO_2.

[b] Contains 14 *Staphylococcus*, 2 *Bacillus subtilis*, and 1 *Micrococcus luteus* strain(s).

[c] Number of strains.

Table 2. Serum levels in squirrel monkeys (po, 20 mg/kg) and mice (iv, 100 mg/kg).

Compound[a]	AUC (μg·hour/ml)	
	Squirrel monkeys 0~6 hours	Mice 0~3 hours
3,23,2′-Tri-OAC-4″-OIV DMT (Sch 37644)	19.7[b]	69.4
12,13-Epoxy-3,23,2′-tri-OAC-4″-OIV DMT (Sch 38646)	11.8[b]	56.7
6-*O*-Methyl erythromycin (A-56268)	21.6[b]	—
Erythromycin	1.5[b]	36.1
Tylosin	2.6	23.5
Tylosin hydrazone	0.7	54.7
3-OAC-4″-OIV tylosin	7.6[b]	52.4
3-OAC-4″-OIV tylosin hydrazone	7.5	67.6
12,13-Epoxy-3-OAC-4″-OIV tylosin	9.4[b]	60.9
12,13-Epoxy-3,2′-di-OAC-4″-OIV tylosin	—	39.8
23-DMT hydrazone	—	31.7
23-OIV DMT hydrazone	0.4	49.1
23-*O*-Benzoyl OMT hydrazone	—	17.5
23-*O*-Phenylacetyl DMT hydrazone	3.1	37.6
3-OAC-23-*O*-phenylacetyl-4″-OIV DMT hydrazone	—	43.4
4″-OIV DMT hydrazone	1.2	24.9
3-OAC-4″-OIV DMT	5.5	28.3
3-OAC-4″-OIV DMT hydrazone	2.9	40.1
3,2′-Di-OAC-4″-OIV DMT	11.5	—
3,2′-Di-OAC-4″-OIV DMT hydrazone	5.8	80.5
3,23-Di-OAC-4″-OIV DMT hydrazone	4.6	61.8
3,23,2′-Tri-OAC-4″-OIV DMT hydrazone	4.8[b]	153.5

[a] See Table 1 for abbreviations.
[b] Concentration of dosing preparations used for monkeys was 3.5 mg/ml for these compounds; 20 mg/ml for all others.
—: Not done.

Fig. 2. Average serum levels of Sch 37644, Sch 38646, A-56268, and erythromycin in squirrel monkeys following administration of a single po dose (20 mg/kg).

AUC (μg·hour/ml, 0~6 hours): ○ Sch 37644 (19.7), ● Sch 38646 (11.8), △ erythromycin (1.5), ▲ A-56268 (21.6).

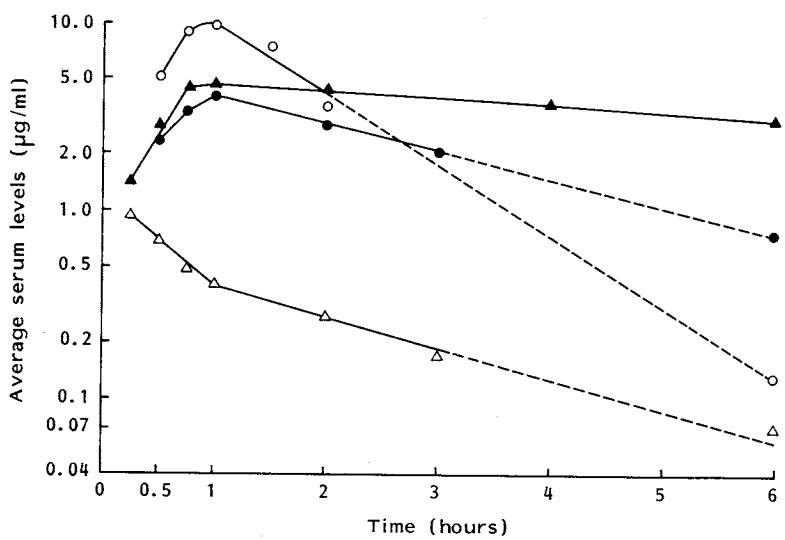


Table 3. Efficacy (PD_{50}s) of orally administered (single dose) macrolides against Gram-positive cocci.

Strains	cfus/mouse	Number of[a] LD_{100}s	PD_{50} (mg/kg)[b]			
			Sch 37644	Sch 38646	A-56268	Erythromycin
Staphylococcus aureus 78011907	2×10^8	2	85	65	8	85
S. aureus 78100502	7×10^6	10	110	70	35	125
S. aureus 83092006	9×10^8	20	330	390	65	215
S. aureus 00000835	3×10^8	2	65	45	—	50
Streptococcus agalactiae Lang	2×10^3	>50	60	50	1.8	15
S. pyogenes Y	2×10^4	10	275	265	150	265

[a] Mice were infected intraperitoneally with the indicated multiple of the inoculum which killed 100% of the animals.
[b] Estimated by probit analysis of survivors 7 days post-infection.
—: Not done.

Fig. 3. Average serum levels of Sch 37644, Sch 38646 and erythromycin in mice following administration of a single po dose (200 mg/kg).

AUC (μg·hour/ml, 0~3 hours): ○ Sch 37644 (19.4), ● Sch 38646 (15.4), △ erythromycin (5.7).

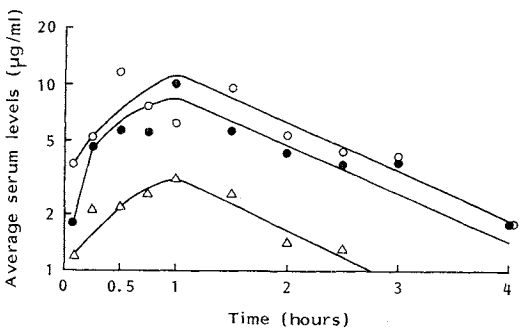
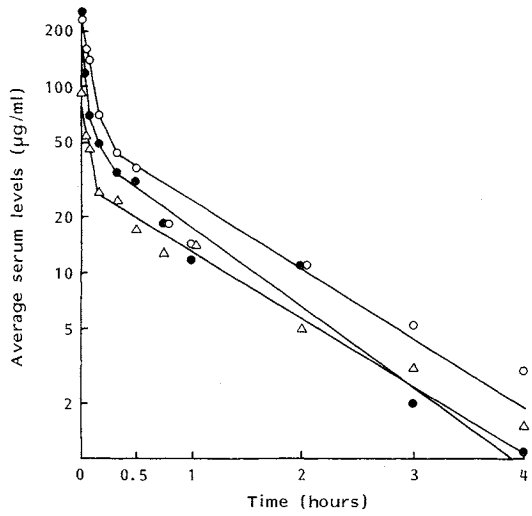


Fig. 4. Average serum levels of Sch 37644, Sch 38646 and erythromycin in mice following administration of a single iv dose (100 mg/kg).

AUC (μg·hour/ml, 0~3 hours): ○ Sch 37644 (69.4), ● Sch 38646 (56.7), △ erythromycin (36.1).



Serum Levels in Squirrel Monkeys and Mice

Serum level results following po (20 mg/kg, squirrel monkeys) or iv (100 mg/kg, mice) administration are shown in Table 2. Sch 37644 and Sch 38646 had good serum levels in both species and were selected for further evaluation. When tested in squirrel monkeys (Fig. 2), A-56268 (AUC, 21.6 μg·hour/ml) and Sch 37644 (19.7 μg·hour/ml) had the highest serum levels, followed by Sch 38646 (11.8 μg·hour/ml) and erythromycin (1.5 μg·hour/ml). In addition, following a single po dose of 200 mg/kg to mice Sch 37644 (AUC, 19.4 μg·hour/ml) and Sch 38646 (15.4 μg·hour/ml) had higher serum levels than erythromycin (5.7 μg·hour/ml) (Fig. 3). Sch 37644 and Sch 38646 also had higher serum levels than erythromycin in mice after iv administration of 100 mg/kg (Fig. 4). Serum levels for A-56268 were not determined in mice.

Mouse Protection Tests

The po efficacy of Sch 37644, Sch 38646, A-56268 and erythromycin was determined in mouse protection tests (PD_{50}s) against Staphylococci and Streptococci. The results (Table 3) indicated that

A-56268 was the most active compound against these strains. The range of PD_{50} values for A-56268 (1.8 to 150 mg/kg) was 2 to 33-fold better than those for Sch 37644 (60 to 330 mg/kg), Sch 38646 (50 to 390 mg/kg), and erythromycin (15 to 265 mg/kg). Sch 37644 and Sch 38646 were overall similar in efficacy to erythromycin.

Discussion

In vitro activity studies of a series of tylosins and DMT derivatives indicated that the DMTs were overall more potent against Streptococci and macrolide-susceptible Staphylococci than the tylosins. Two DMT derivatives, Sch 37644 and Sch 38646, slightly less potent *in vitro* than erythromycin and A-56268, were selected for further *in vivo* study.

Following po administration of 20 mg/kg to squirrel monkeys, the tetraacylated DMTs Sch 37644 and Sch 38646, had higher serum levels than the tetraacylated DMT hydrazone, diacylated tylosin, diacylated DMT, their respective hydrazones, and erythromycin. In mice administered 200 mg/kg orally, Sch 37644 and Sch 38646 again had higher serum levels than erythromycin. In addition, Sch 37644 and Sch 38646 had better po pharmacokinetics in mice than other 23-DMT derivatives reported by KIRST *et al.*[6].

Despite having superior oral absorption in monkeys, Sch 37644 and Sch 38646 showed no advantage over erythromycin against Gram-positive infections in mice (PD_{50} studies, Table 3). A-56268, the 6-*O*-methyl derivative of erythromycin, was reported by FERNANDES *et al.*[7] to have higher serum levels and better efficacy than erythromycin following po administration to mice. In our studies, A- 56268 had slightly higher serum levels than Sch 37644 and Sch 38646 in squirrel monkeys, and was more active than Sch 37644 and Sch 38646 in mouse protection studies.

The tetraacylated DMT derivatives Sch 37644 and Sch 38646, and the erythromycin derivative A-56268, represent improvements in po macrolide pharmacokinetics over that of erythromycin, with retention of efficacy at least as good as erythromycin.

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