BIOLOGICAL PROPERTIES OF ER 42859, A NOVEL ERYTHROMYCIN DERIVATIVE

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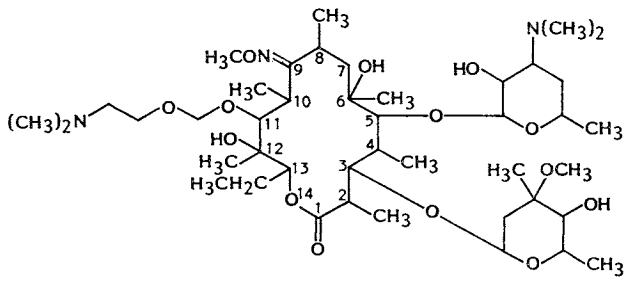
(Received for publication August 17, 1988)

The antimicrobial activity of a new semi-synthetic oral erythromycin derivative, ER 42859, was evaluated *in vitro* and *in vivo* in comparison with erythromycin, spiramycin, josamycin, oleandomycin and the newer semi-synthetic derivatives flurithromycin, roxithromycin and A-56268. MIC values of ER 42859 were superior to those of roxithromycin, oleandomycin, josamycin and spiramycin but generally 2-fold poorer than those of erythromycin. The activity equalled that of erythromycin against *Haemophilus influenzae* and was superior to that of roxithromycin and A-56268 against this organism. MIC values of the compound were greatly influenced by pH due to the dibasic nature of the molecule. ER 42859 had markedly superior activity to erythromycin, spiramycin, josamycin, oleandomycin and flurithromycin against experimental infections in mice and similar activity to roxithromycin and A-56268. Blood and tissue levels were high and prolonged in rodents. In volunteers, blood levels were prolonged but inferior to those of erythromycin.

Macrolide antibiotics are widely used for the treatment of respiratory, genital tract, skin and soft tissue infections in adults and children, predominantly by the oral route. Erythromycin, with its proven safety record and superior *in vitro* activity continues to dominate the macrolide market in Britain and the U.S.A. The recognition of *Legionella pneumophila*, *Campylobacter jejuni* and *Chlamydia trachomatis* as important human pathogens has led to an increasing number of indications for erythromycin therapy, and a resurgence of interest in the development of semi-synthetic derivatives to overcome the limitations of erythromycin. Erythromycin therapy is restricted by low blood levels and erratic oral absorption compounded by rapid degradation in gastric acid, thus frequent dosing is required and MICs vs. organisms such as *Haemophilus influenzae* are barely exceeded.

In this paper we describe the properties of ER 42859 (11-[(2-dimethylaminoethyl)oxymethyl]-erythromycin A 9-methoxime), a new semi-synthetic erythromycin which is stable to gastric acid and has a similar spectrum of antibacterial activity to that of erythromycin. The improved pharmacokinetic properties of this compound are described and related to efficacy against experimental infections.



ER 42859

ER 42859 was selected as the preferred member of a series of 11-ether 9-oximes.[1]

Materials and Methods

Antibiotics

ER 42859 (11-[(2-dimethylaminoethyl)oxymethyl]erythromycin A 9-methoxime) and ER 42859A (dihydrochloride monohydrate salt of ER 42859), were prepared by Beecham Pharmaceuticals Research Division, Brockham Park, Betchworth, U.K. Roxithromycin (erythromycin A 9-[*O*-(2-methoxyethoxy)methyloxime], Roussel) and A-56268/TE-031 (6-*O*-methyl erythromycin A, Abbott/Taisho) were also prepared by Beecham Pharmaceuticals Research Division, using published methods.[2,3] Flurithromycin (8-fluoro erythromycin A) was kindly supplied by Pierrel. Erythromycin base, erythromycin ethyl succinate, erythromycin stearate, erythromycin lactobionate, erythromycin estolate, oleandomycin phosphate, spiramycin and josamycin were obtained commercially.

Micro-organisms

The 203 bacterial strains used were clinical isolates and standard laboratory cultures. Human mycoplasmal species were either obtained from the American Type Culture Collection (ATCC) or were clinical isolates. Veterinary mycoplasmas were obtained either from the National Collection of Type Cultures (NCTC), the ATCC or were field isolates.

Determination of Acid Stability

Stability was measured in pH 2.6 citrate - phosphate buffer (0.1 M) at 37°C with a starting concentration of 200 μg/ml. Samples were removed at intervals over 2 hours, the pH adjusted to neutral and the amount of compound remaining estimated by bioassay against *Staphylococcus saprophyticus* NCTC 8340 or by reverse phase HPLC.

Determination of MICs

Bacterial MICs were determined by a standard 2-fold agar dilution method using Blood Agar Base (Difco), pH 7.4, containing 10% lysed defibrinated horse blood. 1 μl spots of overnight broth cultures were used to inoculate the plates, giving a density of approximately 10^6 cfu/spot. After 18~24 hours incubation the plates were read and the MIC recorded as the lowest dilution preventing visible growth. Antimycoplasmal MICs against human strains were determined on solidified SP4 medium[4] at pH 7.6. Veterinary antimycoplasmal MICs were obtained using FRIIS' agarose medium[5] at pH 7.4. The surfaces of the plates were inoculated with 1 μl spots containing approximately 10^4 mycoplasmas. Plates were incubated aerobically in sealed moistened plastic bags at 37°C for 6 days. Plates were read macroscopically and microscopically. The MIC was taken as the lowest concentration of drug to inhibit at least 50% of mycoplasmal growth. The MICs vs. *Ureaplasma urealyticum* were determined in FRIIS' liquid medium containing 1% urea, adjusted to pH 7.4. MICs were recorded initially when a color change first occurred in the drug-free control tube and again after 6 days incubation at 37°C.

Effect of pH on MICs

MICs against *H. influenzae*, *Staphylococcus aureus* and *Escherichia coli* were determined in BHI broth+1% Fildes extract (Oxoid) adjusted to various pHs in the range 5.0~8.0 with 0.1 M NaOH or HCl. 1 μl of a 10^{-1} dilution of an overnight broth culture was used to inoculate microtitre trays containing doubling dilutions of the test compound in 0.05 ml volumes of broth. MIC values were determined after aerobic incubation at 37°C for 18~24 hours.

Experimental Studies in Animals

The animals used were mice (Charles Rivers CD1 or OLAC MFI, male, 18~22 g) and rats (Sprague-Dawley, male, 150~250 g). Compounds were given orally as a suspension in 1% hydroxypropylmethyl cellulose or parenterally as a solution of a soluble salt in phosphate buffered saline (PBS) pH 7.4.

a) Distribution Studies in Rodents: Blood and tissue concentrations were determined in groups of five mice. Blood samples were collected from the subclavian artery in heparinised tubes and as-

sayed individually; tissue samples were pooled. For studies in rats, a minimum of four animals were used per compound. Blood samples were collected at intervals from the lateral tail vein. After 4 hours animals were killed and samples of liver, kidney, heart, lung and muscle removed. All samples were assayed individually.

Rat, mouse and human samples were assayed microbiologically using *S. saprophyticus* NCTC 8340. Tissues were homogenised in PBS to give a 20% suspension. Standards were prepared in whole heparinised blood or the appropriate tissue homogenate from undosed animals.

b) Mouse Protection Tests: *Streptococcus pneumoniae* 1629, *S. aureus* Smith and *Streptococcus pyogenes* 1580 were used to infect mice by intraperitoneal inoculation. Organisms were contained in 0.5 ml Todd-Hewitt broth (*S. pneumoniae*) or 0.5 ml of 5% hog gastric mucin (*S. aureus* and *S. pyogenes*). Two inoculum levels were used with *S. pneumoniae* 1629, a low inoculum (approx 2×10^2 cfu/mouse) for determination of the 50% median curative dose (CD_{50}) and a higher inoculum (approx 1×10^5 cfu/mouse) evaluated by prolongation of survival (TD_{50}=time to 50% mortality). CD_{50} values were calculated on the fourth day post-infection.

A respiratory tract infection with *S. pneumoniae* 1629 was produced by intranasal instillation of 50 μl of inoculum containing approximately 10^5 cfu/ml to mice under ether anaesthesia. The infection was evaluated by survival and viable counts of lung tissue on the fourth day post-infection.

S. pneumoniae models were dosed at 1, 24 and 48 hours post infection. *S. aureus* and *S. pyogenes* infections were dosed at 1 and 5 hours post-infection.

Results

Acid Stability

ER 42859 was completely stable during 2 hours incubation at 37°C in pH 2.6 citrate phosphate buffer, unlike erythromycin which was rapidly labile under similar conditions with only 15% of the compound remaining see Fig. 1.

Antimicrobial Spectrum

ER 42859 exhibited the typical antibacterial spectrum of erythromycin and other 14-membered macrolides (Table 1) with activity being predominantly against Gram-positive organisms and the more permeable Gram-negative species. Cross-resistance occurred with erythromycin-resistant organisms. The potency of this compound was markedly superior to that of oleandomycin and the 16-membered macrolides spiramycin and josamycin against the majority of aerobes and comparable to that of roxithromycin, flurithromycin and A-56268. The MICs of ER 42859 equalled those of erythromycin against the respiratory pathogens *H. influenzae*, *Branhamella catarrhalis* and *S. pneumoniae* but were 2 to 4-fold higher against other Gram-positive organisms. Activity against *Neisseria gonorrhoeae* also equalled that of erythromycin whilst the compound showed superiority against *C. jejuni* and *Campylobacter fetus*. Activity against anaerobic species was difficult to assess as the acidifying

Fig. 1. Stability of ER 42859 and erythromycin at 200 μg/ml in citrate buffer, pH 2.6.
● ER 42859, ○ erythromycin.

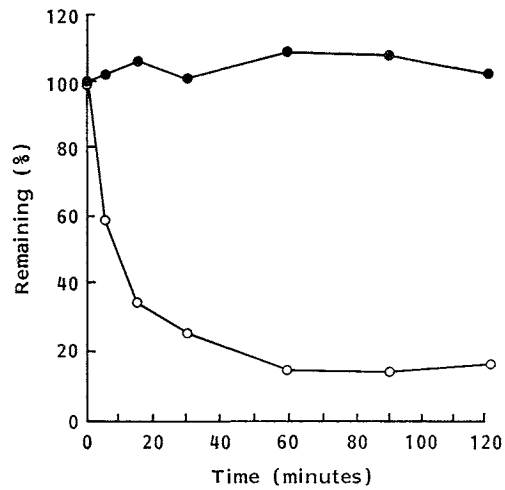


Table 1. Comparative antibacterial activity of ER 42859 and other macrolide antibiotics.

Organism	No. of strains	Geometric mean MICs (μg/ml)							
		Erythro-mycin	ER 42859	Josamycin	Oleando-mycin	Spiramycin	Roxithro-mycin	A-56268	Flurithro-mycin
Staphylococcus aureus									
a) Non-β-lactamase-producing	13	0.34	1.0	2.5	2.0	3.58	0.96	0.66	0.42
b) β-Lactamase-producing	19	0.3	1.0	2.2	1.7	4.45	1.0	0.81	0.5
c) MLS-resistant	2	>128	>128	2	4	4	>128	>128	>128
Coagulase-negative Staphylococci	8	0.39	0.84	2.0	1.4	2.0	1.0	0.77	0.39
Streptococcus sp. (Groups A, B, C, G)	23	0.026	0.041	0.3	0.43	0.21	0.076	0.042	0.015
Streptococcus pneumoniae	6	0.03	0.03	0.1	0.25	0.06	0.06	0.015	0.015
Haemophilus influenzae	20	1.8	1.74	9.2	29.4	40.8	4.14	2.64	2.0
Branhamella catarrhalis	20	0.12	0.13	1.0	0.68	2.64	0.28	0.1	0.15
Campylobacter fetus/jejuni	8~12	1.9	1.0	1.5	2.6	3.3	2.7	1.1	1.3
C. pylori	13	0.025	0.1	4	4	—	0.2	0.01	0.2
Neisseria gonorrhoeae	19	0.31	0.31	1.04	2.6	0.85	0.5	0.31	0.61
Bordetella pertussis	3	0.19	0.33	1.74	0.29	3.68	0.5	0.26	0.26
Listeria monocytogenes	12	0.2	0.24	1.9	2.8	2.74	0.47	0.13	0.24
Corynebacterium sp.	10	0.05	0.10	0.62	1.0	1	0.13	0.12	0.06
Clostridium sp.[a]	12	1.2	2.11	2	35.9	4.49	2.2	0.37	1.12
Bacteroides sp.[a]	26	4.1	27.9	0.5	8.4	32	11.9	1.74	5.79

[a] pH 6.8 after incubation.

Table 2. Effect of pH on geometric mean MICs (μg/ml) of ER 42859 and other macrolides against *Haemophilus influenzae*, *Staphylococcus aureus* and *Escherichia coli* in microtitre.

Compound	*H. influenzae* (8 strains)			*S. aureus* (8 strains)			*E. coli* (8 strains)		
	pH 6.5	pH 7.0	pH 7.5	pH 6	pH 7	pH 8	pH 6	pH 7	pH 8
ER 42859	58.7	4.0	0.59	26.9	2.8	0.15	≥166.0	69.8	7.3
Erythromycin	3.4	2.6	1.8	4.5	0.8	≤0.1	90.5	58.7	14.7
Roxithromycin	—	—	—	9.5	1.5	≤0.3	332.0	152.2	38.1
Flurithromycin	—	—	—	4.0	0.6	<0.2	138.2	41.5	8.7

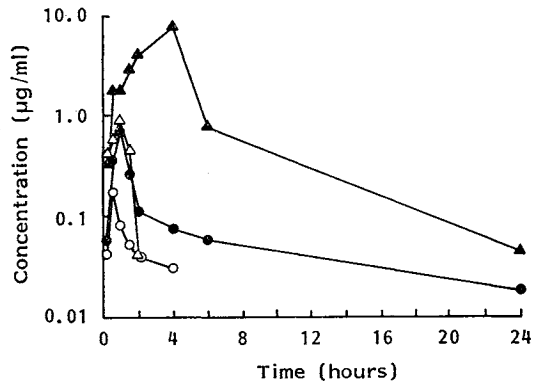
Table 3. Comparative activity of ER 42859, erythromycin and roxithromycin against human and veterinary mycoplasmas.

Organism	No. of strains	Mean MIC (μg/ml)		
		Erythromycin	ER 42859	Roxithromycin
Mycoplasma pneumoniae	7	<0.006	0.034	0.017
M. hominis	1	>10	>10	>10
Ureaplasma urealyticum	2	0.05	0.1	0.2
Mycoplasma hyopneumoniae	3	>10	0.25	10
M. bovis	2	>10	7.5	>10
M. gallisepticum	1	0.05	0.05	0.1

effect of CO_2 in the incubation atmosphere has a detrimental effect on the activity of macrolides.[6] The activity of ER 42859 is particularly susceptible to the influence of pH (Table 2).

In addition to good activity against the bacterial respiratory pathogens, ER 42859, like erythromycin, was exceptionally active against *Mycoplasma pneumoniae* (Table 3). The human genital mycoplasma *Mycoplasma hominis* was not susceptible but good activity was seen against *U. urealyticum*. Against veterinary mycoplasmas, ER 42859, erythromycin and roxithromycin were equally active against *Mycoplasma gallisepticum*. However, ER 42859 had substantial activity against strains of *Mycoplasma hyopneumoniae* which were resistant to both erythromycin and roxithromycin and also showed greater activity against *Mycoplasma bovis*.

Fig. 2. Mouse blood and lung levels of ER 42859 and erythromycin (25 mg/kg, po).
● ER 42859-blood, ○ erythromycin-blood, ▲ ER 42859-lung, △ erythromycin-lung.



Distribution Studies in Rodents

Pharmacokinetic parameters for ER 42859 and erythromycin following single doses to mice at 25 and 100 mg/kg orally or 25 mg/kg subcutaneously are given in Table 4. Peak levels (Cmax) of ER 42859 in blood and lung tissue following oral administration were enhanced 4~12-fold and 10~59-fold respectively, over those of erythromycin from the corresponding dose. Both compounds gave similar peak blood levels following subcutaneous administration, but the pneumotropic superiority of ER 42859 was clearly evident from a 24-fold difference in peak lung tissue concentration. Penetra-

Table 4. Pharmacokinetic parameters of ER 42859 and erythromycin after oral and subcutaneous administration to mice.

Compound	Dose (mg/kg)	Route	Blood			Lung tissue			Muscle		
			Cmax (μg/ml)	Tmax (minutes)	$AUC_{0\sim6\ hours}$ (μg/ml·minute)	Cmax (μg/g)	Tmax (minutes)	$AUC_{0\sim6\ hours}$ (μg/g·minute)	Cmax (μg/g)	Tmax (minutes)	$AUC_{0\sim6\ hours}$ (μg/g·minute)
ER 42859	25	po	0.70	60	53.8	8.4	240	1,554.7	1.2	60	118.9
	100	po	5.7	60	670	59.0	60	12,284.0	29.4	120	6,723.1
ER 42859A	25	sc	7.2	15	300.6	24.1	30	4,538.2	16.3	60	3,057.9
Erythromycin base	25	po	0.17	30	15.8	0.86	60	52.7	0.6	60	22.4
	100	po	0.45	90	34.4	1.0	60	120.1	1.2	60	204.5
Erythromycin lactobionate	25	sc	7.7	15	168.3	0.91	15	51.1	0.7	60	41.6

Table 5. Rat tissue levels of ER 42859 and erythromycin at 4 hours after oral administration of 100 mg/kg.

Compound	Concentration (μg/g)					
	Blood	Heart	Lung	Liver	Kidney	Muscle
ER 42859	1.3	15.0	14.6	79.4	23.5	9.2
Erythromycin	0.4	<2	<2	<2	<2	<2

Table 6. Activity of ER 42859 and other macrolides against systemic and respiratory tract infections with *Streptococcus pneumoniae* 1629 in mice.

Compound	Dose (mg/kg)[a]	Route	Systemic infection TD_{50} (days)	Respiratory tract infection		
				Average survival (%)	Average cure (%)	cfu/g lung tissue of uncured mice
Erythromycin base	25	po	<1.5	80	0	$6\times10^5\sim9\times10^7$
Erythromycin ethyl succinate	25	po	<1.5	70	0	8.1×10^7
Erythromycin estolate	25	po	<1.5	80	0	2.5×10^6
ER 42859	25	po	3.7	100	70	5.3×10^3
Roxithromycin	25	po	2.9	100	90	1.3×10^3
A-56268	25	po	4.6	100	100	N/A
Flurithromycin	25	po	2.3	100	0	2.6×10^6
Oleandomycin	25	po	1.3	90	30	1.9×10^8
Spiramycin	25	po	1.6	90	50	4.1×10^6
Josamycin	25	po	<1.0	100	40	8.2×10^7
Erythromycin lactobionate	12.5	sc	3.25	100	100	N/A
ER 42859A	12.5	sc	>6[b]	100	100	N/A
Roxithromycin lactobionate	12.5	sc	4.4	ND	ND	ND
Untreated control group	—	—	<1.0	50	0	$2\times10^7\sim2\times10^9$

[a] 10 animals/group. [b] 100% survival.
DN: Not determined, N/A: not applicable (100% cured).

tion into muscle was also greatly enhanced over that of erythromycin. Although erythromycin and ER 42859 were initially eliminated at a similar rate, ER 42859 exhibited a biphasic response with an extremely prolonged terminal $t_{\frac{1}{2}}$, resulting in compound being detectable in blood and lung at 24 hours post-dosing (Fig. 2). Cmax and AUC values for ER 42859 in all tissues were noted to increase supraproportionately with dose.

Studies in the rat (Table 5) indicated a similar marked increase in blood and tissue levels over those of erythromycin. Extensive accumulation of ER 42859 was observed in both excretory and non-excretory tissues.

Relative Potency of ER 42859 in Mouse Protection Tests

Oral ER 42859 was greatly superior to various formulations of erythromycin in its ability to prolong survival in the *S. pneumoniae* TD_{50} model, Table 6. Oleandomycin, spiramycin and josamycin showed no advantage over erythromycin while flurithromycin and roxithromycin were only marginally better. Only A-56268 had superior efficacy to ER 42859 in this model. Comparative studies on soluble salts of erythromycin, roxithromycin and ER 42859 administered subcutaneously, confirmed the superiority of ER 42859, and indicated that its enhanced activity could not be attributed solely to increased stability to gastric acid. Against a respiratory tract infection with the same strain of *S. pneumoniae* (Table 6) ER 42859 effectively eradicated the organism from 70% of the infected animals and greatly reduced the residual infection in the remainder. Erythromycin base, estolate, ethyl

Table 7. Curative effect of ER 42859 and other macrolides against intraperitoneal infections in the mouse.

Organisms	Dosage route	CD_{50} (mg/kg)				
		Erythromycin	ER 42859	Roxithromycin	A-56268	Spiramycin
Streptococcus pneumoniae 1629	po	47	35	16	13.5	—
	sc	10.25	2.7	—	—	—
Staphylococcus aureus Smith	po	150~200	95	42	27	110
Streptococcus pyogenes 1580	po	75	88	12.8	<3.1	>200

5 animals/group.

succinate and flurithromycin were ineffective against this infection. Oleandomycin and the 16-membered macrolides gave modest cure rates of 30~50% with high numbers of organisms remaining in the lungs of uncured animals. Roxithromycin was slightly more effective than ER 42859 while A-56268 was 100% curative in this model. In intraperitoneal CD_{50} tests, ER 42859 was almost 4-fold more active than erythromycin against *S. pneumoniae* subcutaneously (Table 7). The compound equalled erythromycin orally against *S. pneumoniae*, *S. aureus* and *S. pyogenes* but was somewhat less active than roxithromycin and A-56268.

Discussion

Recent developments in the macrolide field have concentrated on modifications to erythromycin with the objective of achieving pharmacokinetic improvements.[7] ER 42859 incorporates two modifications to the erythromycin molecule, a methoxime to replace the acid labile 9-keto group,[1] and an 11-ether side-chain. These modifications confer stability to gastric acid and markedly improved pharmacokinetic properties, while retaining an antibacterial spectrum and potency close to that of erythromycin. In comparative studies with other recently developed semi-synthetic erythromycins, ER 42859 was 2-fold more active than roxithromycin against many aerobic organisms and had a notable advantage over A-56268 against *H. influenzae*. Weakly basic compounds like the macrolides become increasingly ionised at acidic pH and less able to diffuse through biological membranes[8] thus accounting for the loss of antimicrobial activity at low pH. The dibasic nature of the ER 42859 molecule makes this compound particularly susceptible to fluctuations in pH and may explain the extreme pH dependence observed here, and the lack of *U. urealyticum* activity reported by RIDGWAY[9] using medium at pH 6.0.

Oral absorption of ER 42859 in rodents was superior to that of erythromycin leading to substantially higher peak blood levels. The compound was extensively accumulated in the lungs, and both blood and tissue levels were prolonged. ER 42859 showed corresponding improved efficacy over erythromycin in a range of murine infection models. Like other basic lipophilic molecules, macrolides have the ability to penetrate mammalian cells and to accumulate in tissues, thus conferring on them a potential advantage against intracellular infections which are frequently inaccessible to other antibiotics. The accumulation of erythromycin in cultured mammalian cells is highly dependent on the ratio of external to internal pH and this is thought to be partially attributable to ion trapping,[10] though active transport may also be involved.[11] A dibasic molecule may be more extensively ion-trapped, and this would correlate with the high tissue concentrations and slow release observed with ER 42859.

Recent oral studies in human volunteers using ER 42859A have shown similar prolongation to that seen in animals implying that in man also, a large depot of compound resides in the tissues. However, despite the high blood levels observed in rodents, the human blood levels obtained were inferior to those of erythromycin and do not warrant progression of this interesting compound.

Acknowledgements

The authors would like to acknowledge the contribution of Dr. E. CARTER, Mr. J. DURODIE and Mr. P. JONES to this study, and are indebted to Dr. J. H. C. NAYLER for invaluable discussion and criticism.

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