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COMMUNICATION

Preparation and Evaluation of Erythromycin Fumarate—A New Derivative of Erythromycin

P. K. Manna[1,*] and S. K. Basu[2]

[1]*Institute of Pharmaceutical Technology, Annamalai University, Annamalainagar 608 002, India*
[2]*Department of Pharmaceutical Technology, Jadavpur University, Calcutta 700 032, India*

ABSTRACT

Erythromycin fumarate, a new water-soluble derivative of erythromycin, was prepared and its physicochemical and biological properties were evaluated. The derivative also has considerable solubility in organic solvents. Its partition coefficient data in different organic solvent–water systems may indicate that it is well-distributed in various tissues in vivo. Antimicrobial potency in vitro of the derivative, 725 μg/mg, is much higher than that of the existing derivatives and its antimicrobial spectrum is comparable to that of the parent antibiotic. The LD_{50} value of the new derivative in mice intraperitoneally is 402.7 mg/kg. Results of this and the previous investigation on pharmacokinetics and protein binding indicate that erythromycin fumarate has high potential for possible clinical application and further investigation may be undertaken.

INTRODUCTION

Erythromycin, a macrolide antibiotic, introduced in 1952 (1) is irregularly absorbed from the gastrointestinal (GI) tract (2) and is unstable under acidic conditions (3). Large intersubject variability occurs after oral administration even under standardized conditions (4,5). It has been shown that intersubject variability in pharmacokinetic parameters after intravenous erythromycin is small (6). Hence, it may be inferred that the majority of intersubject variability is due to erratic absorption and differences in absolute bioavailability. A large number of derivatives and formulations have been prepared to optimize the drug's stability and absorption. All of the derivatives of erythromycin reported earlier were found to have potency much less than that of the parent antibiotic. The

* To whom correspondence should be addressed.

authors have attempted to prepare derivatives with better potency, better availability, and less toxicity (7,8). Erythromycin fumarate is one such new water-soluble salt of erythromycin prepared by the authors. The pharmacokinetics in animal models and in vitro protein binding of the salt reported earlier (9) were encouraging, and hence the new salt, erythromycin fumarate, was subjected to further investigation. The present investigation includes preparation and evaluation of various physicochemical and biological properties of the new salt.

MATERIALS AND METHODS

Salt Preparation

Erythromycin fumarate was prepared by the method of Dutta and Basu (10,11) by reacting erythromycin base (Abbott Laboratories, North Chicago, IL) with fumaric acid (Schuchardt Munchen, Germany). The salt was recovered by lyophilization.

Physicochemical Properties

Melting point, microanalytical composition, solubility, optical rotation, partition coefficient, pH of aqueous solution, thin-layer chromatography, and infrared spectroscopic investigations were carried out for erythromycin fumarate.

The solubilities of erythromycin fumarate in H_2O, 0.1 N HCl, phosphate buffer pH 7.4, and nine organic solvents were determined by the method of Marsh and Weiss (12). Erythromycin base was used as reference standard.

Optical rotation of 1% (w/v) solution of erythromycin fumarate in 90% (v/v) ethanol was measured at 29°C in a Perkin-Elmer (Ueberlingen, Germany) polarimeter (model 241) and the specific rotation was computed.

Partition coefficients of the new antibiotic salt in two different solvent systems were determined.

pH of 1% aqueous solution of the compound was determined in an expanded scale pH meter (EC model pH 821 A, Electronic Corp. of India Ltd., Calcutta, India) and from the pH value, pK_a value was computed theoretically from the equation $pK_a = 14 - pK_b$

An infrared spectrum of the compound was recorded on a Perkin-Elmer infrared spectrophotometer (237B).

Biological Properties

The biological properties studied for the antibiotic compound include in vitro antimicrobial potency, in vitro antimicrobial spectrum, and acute toxicity. In vitro potency was determined according to the method of Grove and Randal (13) using *Sarcina lutea* ATCC 9341 as the test organism.

An in vitro antimicrobial spectrum was determined by the twofold agar dilution test using Brain Heart Infusion Agar (Difco, Detroit, MI) medium. Erythromycin base USP (952 μg/mg) was used as control during the antimicrobial spectrum study.

The acute toxicity test of Litchfield and Wilcoxon (14) was followed to determine the dose (mg/kg) which would be expected to kill one-half of an unlimited population of the same species and strain (LD_{50} value). Male albino mice of Swiss strain (20–25 mg), fasted for 18 hr with free access to water, were injected intraperitoneally with the solution of the antibiotic compound in a propylene glycol–water mixture (1:1). One vehicle control group was used at each dose level.

RESULTS AND DISCUSSION

Physicochemical Properties

Erythromycin fumarate is a white, amorphous, fluffy powder with a melting point of 157 ± 1°C.

Specific rotation computed from the optical rotation of 1% (w/v) solution in ethanol (90% v/v) was −49° for erythromycin fumarate, whereas it was −69° for erythromycin base.

Microanalytical composition of erythromycin fumarate (7.95% H, 57.98% C, and 1.67% N) was found to corroborate well with the theoretical values.

Table 1

The Solubility Data of Erythromycin Fumarate and Erythromycin Base at Room Temperature (33 ± 1°C)

	Solubility (mg/ml)	
Solvent	Erythromycin Fumarate	Erythromycin Base
Water	>20	2.1
Acetone	>20	>20
Methanol	>20	>20
Ethanol	>20	>20
Chloroform	>20	>20
Ethyl acetate	>20	>20
Benzene	>20	>20
Propylene glycol	>20	>20
Cyclohexanol	>20	>20
1:4 Dioxan	>20	>20
Phosphate buffer pH 7.4	>20	>1.8
0.1 N HCl	>20	>20

Table 2

pH, pK_a, and Partition Coefficient of Erythromycin Fumarate

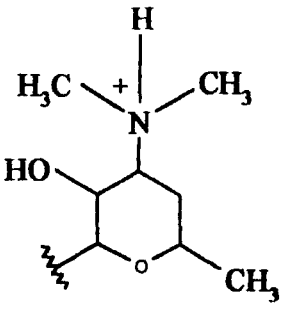
Drug	pH of 1% Aqueous Solution	pK_a	Partition Coefficient	
			Chloroform–Water	Cyclohexanol–Water
Erythromycin Fumarate	5.8	5.3	1.2	1.8

Solubility data of erythromycin fumarate (Table 1) show that it is soluble in polar as well as nonpolar solvents.

pH, pK_a, and partition coefficient of the erythromycin salt are given in Table 2. The salt partitions well both in chloroform and cyclohexanol, but it partitions better in cyclohexanol.

Thin-layer chromatographic investigation of the prepared salt performed in various solvent systems ensured homogeneity of the prepared salt. The R_f values are furnished in Table 3.

Infrared absorption spectra of erythromycin base and erythromycin fumarate showed major absorption bands at or around 3400, 1725, and 1590 cm^{-1}. The absorption band at or around 3400 cm^{-1} indicates the presence of an intact many-membered lactone ring of erythromycin in erythromycin fumarate. The absorption band at or around 1590 cm^{-1} confirms the formation of the quaternary ammonium salt of erythromycin of the type shown in the following structure:



Biological Properties

In vitro potency of erythromycin fumarate was found to be 725 μg/mg.

In vitro antimicrobial spectrum of erythromycin fumarate (Table 4) was found to be very close to that of erythromycin base.

The LD_{50} value of erythromycin fumarate was found to be 402.7 μg/mg, which is sufficiently high for safe clinical use.

CONCLUSION

The results of the present investigation show that the new derivative, erythromycin fumarate, has much higher potency than the existing derivatives and is also safe enough for clinical utilization. The partition data of the derivative indicate that it is partitioned in higher proportion in the organic phase than in the aqueous phase, which suggests the possibility of better transport and absorption characteristics of the derivative in vivo.

In a previous study, Manna, Basu, and Goswami (8) found that erythromycin fumarate had longer elimination half-life and larger overall apparent volume of distribution compared to erythromycin lactobionate (the reference standard in the study), and it was extensively bound to the plasma protein. These findings corroborate well with the physicochemical properties found in the present study and are consistent with the earlier reports (15–18) that erythromycin penetrates tissues extensively. The bet-

Table 3

R_f Values of Erythromycin Fumarate and Erythromycin Base in Different Solvent Systems Using Silica Gel G Plates

Solvent System	Erythromycin Fumarate	Erythromycin Base
Methanol:ethyl acetate:water, 1:1:2	0.25	0.23
Chloroform:methanol:acetic acid, 90:9:1	0.06	0.12
Chloroform:methanol, 1:1	0.45	0.54
Chloroform:methanol:ethyl acetate, 1:1:2	0.29	0.40

Table 4

In Vitro Antimicrobial Spectra of Erythromycin Fumarate and Erythromycin Base

Organism	Minimum Inhibitory Concentration[a] (μg/ml)	
	Erythromycin A Base	Erythromycin Fumarate
Salmonella typhi 59	30	30–35
Staphylococcus aureus 8530	<0.15	<0.15
Staphylococcus aureus 178	0.15	0.15
Staphylococcus aureus 180	>100	>100
Staphylococcus aureus 10541	0.05	0.2
Shigella flexneri 3189	12	15
Shigella sonnei 56	17	20
Bacillus pumilus 8241	0.15	0.2
Bacillus subtilis ATCC 8241	50	55
Bacillus subtilis ATCC 6633	0.15	0.30
Pseudomonas aeruginosa B-27	>100	>100
Proteus mirabilis 75	>100	>100
Klebsiella pneumoniae 77	>100	>100
Escherichia coli K12	55–60	55–60
Vibrio cholerae 564	17	20–25

[a]Values shown are base equivalents of the salt.

ter water solubility of the new derivative than the parent antibiotic may be advantageous in formulating aqueous monophasic delivery systems of the new salt.

From the data of the present and previous investigation, it may be concluded that erythromycin fumarate is potentially useful for clinical application.

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