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COMMUNICATIONS

STUDIES ON ERYTHROMYCIN MELIBIONATE — A NEW WATER SOLUBLE SALT OF ERYTHROMYCIN

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ABSTRACT

A new water soluble salt of erythromycin, erythromycin melibionate was prepared and some physicochemical and biological properties of the salt was studied. The salt was found to be soluble in water as well as in various organic solvents and was partitioned well in different organic solvent - water systems.

The potency of the new salt determined by microbiological assay was found to be 560, mcg/mg. The in vitro antimicrobial spectrum of the new salt was similar to that of the erythromycin

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base. The LD_{50} values of the new salt in mice through i.p. route was found to be 632.7 mg/kg.

The results of the investigation indicate that the water soluble new salt erythromycin melibionate may be utilised for clinical application.

INTRODUCTION

Erythromycin is widely used in the treatment of various infections, caused by gram positive and some of gram negative organisms. Laboratory studies indicate that common respiratory pathogens (beta-haemolytic streptococci, pneumococci), many strains of Hasemophilus influenzae, many staphylococci, and Mycoplasma peneumoniae are inhibited by erythromycin.

Though erythromycin is widely used in therapy, its use is limited by such factors as i) poor water solubility. ii) instability in the gastric pH and iii) bitter taste. Attempts have been made to overcome these factors from time to time by several workers by preparing various salts and derivatives of the parent antibiotic. Amongst them only two water soluble salts, viz., erythromycin lactobionate and erythromycin glucoheptonate, one water insoluble salt viz. erythromycin stearate and one water insoluble ester-salt viz. erythromycin estolate have gained official recognition in U.S.P.

With a view to obtain salts with better potency, better availability and least toxicity various salts have been prepared in our laboratory from time to time. In the present investigation the authors attempted to study physico-chemical and biological properties of one such new salt.

EXPERIMENTAL

Preparation of the Salt: 1) Erythromycin melibionate was prepared by the method of Dutta and Basu (1977,1979)[1,2] by reacting erythromycin base (Abbott Laboratories, North chicago, IL 60064) with free melibionic acid liberated from calcium melibionate by passing the latter through the cation exchange resin (Amberlite IR-120).

Physicochemical Properties

Melting point, microanalytical composition, solubility optical rotation, partition co-efficient, pH in aqueous solution, thin layer chromatography and infrared spectroscopic investigations were carried out for the salt.

Solubility of the erythromycin salt in different solvents was determined by the method of Marsh & Weiss (1967)[3]. Erythromycin base was used as reference standard.

Optical rotation of 1% (w/v) solution of the erythromycin salt in 90% (v/v) ethanol was measured at 29°C in a perkin-Elmer polarimeter (Model No.241) and the specific rotation was computed.

Partition Co-efficient of the erythromycin salt was determined for two different solvent systems.

pH of 1% aqueous solution of the salt was determined in an expanded scale pH meter (EC model No pH 821 A) and from the pH value, pKa value was computed theoretically from the equation pKa = 14 - pKb (Ref.J.Am.Pharm, Assoc. 16:203 (1976).

Rf **values** of the salt ware determined by Thin Layer Chromatography using silica gel G (LOBACHEM) plates in three solvent systems.

Infrared Spectra of the salt and erythromycin base (reference standard) were recorded in a Perkin Elmer infra- red sepctrophotomater (237 B).

Biological Properties:

The following biological properties were investigated for the new salt. They are : i) Determination of Potency, ii) Evalution of antimicrobial spectrum **in vitro** and (iii) Evaluation of acute toxicity.

In-vitro Potency for the salt was determined according to the method of Grove and Randall (1955)[4] using Sarcina lutea ATCC 9341 as the test organism.

In-vitro Antimicrobial spectrum of the salt was determined by the two fold Agar Dilutin Test using Brain Heart Infusion Agar

(Difco) media. Erythromycin base U.S.P. (952 mcg/mg) was used as control during antimicrobial spectrum studies.

LD_{50} value of the salt was determined by the acute toxicity test (Litchfield and wilcoxon 1949)[5] utilizing intraperitoneal route. Male albino mice of swiss strain (20-25 g) fasted for 18 hrs with free access to water were injected intraperitonelly, the solution of the salt in propylene glycol-water mixture (1:1). At each dose level one vehicle control group was used.

RESULTS AND DISCUSSION

Physico-chemical Properties

Erythromycin melibionate was found to be white, amorphous, fluffy powder, hygroscopic in nature, bitter in taste and odourless.

Melting Point of of erythromycin melibionate was found to be $132^{\circ} \pm 2^{\circ}$.

Specific rotation of erythromycin melibionate computed from the optical rotation of 1 % ethanolic solution is -13°, and that of erythromycin base is -69°.

Microanalytical composition of erythromycin melibionate, given below, corroborates well with the molecular formula : 8.54% H, 54.3% C and 1.6% N.

Table I

The solubility Data of Erythromycin melibionate & Erythromycin base at room temperature ($33^{\circ} \pm 1^{\circ}$).

Solvent	Solubility (mg/ml)	
	Erythromycin melibionate	Erythromycin base
Water	>20	2.1
Acetone	2.3	>20
Methanol	>20	>20
Ethanol	>20	>20
Chloroform	2.35	>20
Ethyl acetate	Insoluble	>20
Benzene	5	>20
Propylene glycol	>20	>20
Cyclohexanol	3	>20
1 : 4 dioxan	4	>20
Phosphate buffer pH7.4	>20	1.8
0.1N HCl	>20	>20

Table II

pH, pKa & Partition Co-efficient of

Erythromycin melibionate

Drug	pH of 1% Aqueous Solution	pKa	Partition Co-efficient	
			Chloroform-Water	Cyclohexanol-Water
Erythromycin melibionate	6.39	5.3	0.55	0.18

Solubility data of erythromycin melibionate (Table - I) show that it is having better solubility in polar solvents than in non-polar solvents.

pH, pKa & Partition co-efficient of the rythromycin salt are given in Table-II. The salt partitionel better in chlorform than in cyclohexanol.

Thin Layer Chromatographic Investigation insured homogeneity of the prepared salt, The Rf values of the erythromycin salt in four different solvent systems are given in Table - III.

The infrared absorption spectra of erythromycin melibionate and erythromycin base showed major absorption bands in the regions of 3400 cm^{-1}, 1725 cm^{-1} and 1590 cm^{-1}. The absorption band at or around 3400 cm^{-1} is for the stretching of hydroxyl group (-OH), The absorption band at or around 1725 cm^{-1} is for

Table III

Rf Values of the erythromycin melibionate and erythromycin base in different solvent systems using silica gel G plates

Solvent system	Erythromycin melibionate	Erythromycin base
Methanol : Ethyl Acetate : Water :: 1:1:2	0.28	0.23
Chloroform : Methanol : Acetic Acid :: 90:9:1	0.04	0.12
Chloroform : Methanol :: 1:1	0.41	0.54
Chloroform: Methanol : Ethyl Acetate :: 1:1:2	0.27	0.40

carbonyl group (C = o) stretching (two carboxyl groups are present in the lactone ring of the erythromycin base molecule), indicating the presence of intact many membered lactone ring in the molecule of erythromycin melibionate. The absorption band at or around 1590 cm^{-1} is the characteristic feature of the quaternary ammonium salts of erythromycin of the type given below

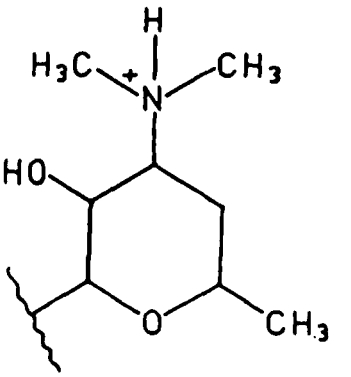

Table IV

In-Vitro Antimicrobial spectrum of erythromycin melibionate and erythromycin base.

Organism	Minimum Inhibitory Concentration mcg/mL	
	Erythromycin base	Erythromycin melibionate*
Salmonella typhimurium Ed 9	25	100
Staphylococcus aureus 8530	0.15	0.2
Staphylococcus aureus 180	>100	>100
Streptococcus faecalis 10541	0.05	0.1
Shigella sonnei 9290	12.5	50
Pseudomonas aeruginosa BMH 10	25	50
Proteus mirabilis 75	>100	>100
Klebsiella pneumoniae 10031	6.2	12.5
Escherichia coli Juhl	50	100

* Values shown are base equivalents of erythromycin melibionate

Biological Properties:

In-Vitro potency of the new erythromycin salt, erythromycin melibionate, was found to be 590 mcg/mg.

In-Vitro antimicrobial spectrum of erythromycin melibionate, (Table IV) was found to be very close to that of erythromycin base.

The LD_{50} value of erythromycin melibionate was found to be 632.7 mg/kg which is sufficienlty high for safe use clinically.

CONCLUSION

The results of the present investigation show that the new salt, erythromycin melibionate, is more potent than the existing water soluble salts; it is more soluble in polar solvents; it prtitions well between aqueous and organic phases indicating that it will be absorbed and transported in vivo; it is safe enough from the clinical point of view. Considering the above parameters it may be concluded that erythromycin melibionate is having good potentiality for clinical application and that the salt may be utilized for parenteral administration in aqueous solution form.

REFERENCES

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