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INTRAVENOUS PHARMACOKINETICS AND IN VITRO PROTEIN-BINDING STUDIES OF TWO NEW SALTS OF ERYTHROMYCIN

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ABSTRACT

Erythromycin pharmacokinetics were examined for two new salts of erythromycin, erythromycin melibionate and erythromycin penicillanate using erythromycin lactobionate for comparison, following intravenous injection to rabbits. The biological half-lives of erythromycin in serum were 99 minutes for erythromycin melibionate, 121 minutes for erythromycin penicillanate and 103 minutes for erythromycin lactobionate. Post intravenous administration serum erythromycin levels were adequately described by two compartment model kinetics, and values for the distribution volume of the central compartment

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and the overall distribution have been given. Estimated erythromycin distribution volumes may facilitate calculation of absorption efficiencies of erythromycin and its salts after oral doses. Serum protein binding study using horse serum showed that erythromycin melibionate, erythromycin penicillanate and erythromycin base were bound to the serum protein to the extent of 87.16%, 89.41% and 88.83% respectively; and released from the drug-protein complex to the extent of 11.25%, 8.66% and 10.66% respectively.

INTRODUCTION

Pharmacokinetics investigation as well as protein binding studies of drugs are significant clinically[1,2]. With a view to this consideration detailed pharmacokinetics and serum protein binding studies of the two new salts of erythromycin, erythromycin melibionate and erythromycin penicillanate have been undertaken by the authors. Erythromycin lactobionate has been used for comparative evaluation during pharmacokinetic investigation and erythromycin base during protein binding studies.

EXPERIMENTAL

A. PHARMACOKINETICS

Experimental animals: Fresh healthy rabbits weighing around 1.5 kg were used in the investigation. The experiment was carried out in triplicate with each of the salts.

Materials: 1. Drugs - a) Erythromycin melibionate[3,4] (prepared in our laboratory). b. Erythromycin penicillanate (Prepared in our laboratory). c. Erythromycin lactobionate (obtained from Abbott Laboratories,, North Chicago, IL60064). 2. Components for assay media - i) Peptone (Difco), ii) Pancreatic digest of casein (Difco), iii) Yeast extract (Difco), iv) Beef extract (BDH), v) Dextrose (BDH), vi) Bacto Agar (Difco). 3. Components of phosphatee buffer - i) Dibasic potassium phosphate K_2HPO_4 (BDH), ii) Monobasic potassium phosphate KH_2PO_4 (BDH).

Methods: Each of the three rabbits received 8 mg/kg of erythromycin base equivalent as the melibionate salt, by intravenous injection through marginal ear vein. The dose is equivalent to single human adult dose of 500 mg erythromycin.

Blood samples (0.5 ml) were taken immediately before giving injection and at different time intervals for about three hours after injection.

Erythromycin activity in serum was measured by the method of Grove and Randall[5], using Sarcina lutea ATCC 9341 as the test organism.

The same procedure was followed using erythromycin penicillanate and erythromycin lactobionate.

B. PROTEIN BINDING

Materials: 1) Drugs - a. Erythromycin melibionate[3,4] b. Erythromycin penicillanate. Erythromycin base. 2) Horse serum (obtained from Bengal Chemical and Pharmaceuticals works,

Calcutta). 3) Dialysis tubings (20"/30" wide-flat), purchased from CSIR Centre for Biochemicals, New Delhi - 110 007.

Method: The protein binding of the drugs were measured by equilibrium dialysis method[6]. Erythromycin activity was measured by the method of Grove & Randall[5] using Sarcina lutea ATCC 9341 as the test organism. The reversibility in binding of the erythromycin salts to serum protein was evaluated by immersing the dialysis sacks to fresh dialysis fluid after equilibrium was established and repeating the method[6].

RESULTS AND DISCUSSION

Individual post injection serum erythromycin levels of the two new salts as well as the reference standard (Erythromycin lactobionate) declined in a biphasic manner, as reported previously[7,8]. Individual serum erythromycin levels, for erythromycin melibionate, erythromycin penicillanate and erythromycin lactobionate are summarized in Table I, Table II, and Table III respectively. The comparison of the serum profiles of mean serum erythromycin levels of the new salts with the lactobionate salt are shown in Figure 1 & Figure II respectively. This type of profiles are in consistent with two-compartment model kinetics, with elimination occuring from the central compartment. Serum erythromycin levels were interpreted in terms of this model. The model is depicted in Scheme I, serum erythromycin levels, Cpt, are described by[9]

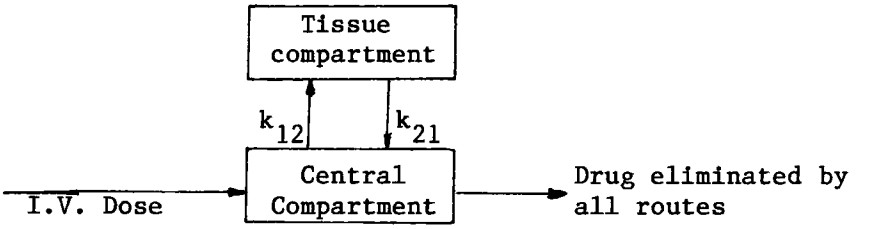
$$Cpt = A\, e^{-\alpha t} + B\, e^{-\beta t} \text{ and}$$

$$A = \frac{X_0\,(\alpha - k_{21})}{Vc\,(\alpha - \beta)} \quad \text{and} \quad B = \frac{X_0\,(k_{21} - \beta)}{V_c\,(\alpha - \beta)}$$

$$\alpha = \frac{(k_{el} + k_{21} + k_{12})}{2} + \frac{\sqrt{(k_{el} + k_{21} + k_{12})^2 - 4k_{21}\,k_{el}}}{2}$$

$$\beta = \frac{(k_{el} + k_{21} + k_{12})}{2} - \frac{\sqrt{(k_{el} + k_{21} + k_{12})^2 - 4k_{21}\,k_{el}}}{2}$$

Where k_{12}, k_{21}, & k_{el} are first order rate constants for drug distribution between central and peripheral compartments and also drug elimination, respectively; X_0 is the dose, Vc is the



Scheme I

apparent volume of distribution of the central compartment, t is the total time elapsed since the time of injection; α & β are complex rate constants, where α > β and the elimination half-life is given by ln 2/β.

Table I

Serum Concentration Data Following Intravenous Injection of Erythromycin melibionate

No. of obs. (n)	Time of sampling (minutes)	Serum erythromycin concentration* Cpt. mcg/ml
1	04	5.2 ± 0.2
2	10	3.9 ± 0.3
3	15	3.03 ± 0.115
4	20	2.53 ± 0.115
5	30	1.633 ± 0.152
6	50	1.4 ± 0.1
7	80	1.1 ± 0.1
8	120	0.846 ± 0.070
9	155	0.66 ± 0.06
10	190	0.516 ± 0.045

* Values shown are means ± S.D. for N = 3.

The pharmacokinetic parameters[9] as described below were calculated graphically:

The constant α was obtained from the slope of the residual line, obtained by the method of residuals[10], which is equal to $-\alpha/2.303$.

A and B are the intercepts on the y ordinate of the fast (α) and slow (β) segments of the biphasic plasma drug decay profile. Cpo, the theoretical concentration of erythromycin in plasma at zero time = A+B.

Table II

Serum Concentration Data Following Intravenous Injection of Erythromycin Penicillanate

No. of Observation (n)	Time of Sampling (minutes)	Serum Erythromycin Concentration* Cpt. mcg/ml
1	5	4.833 ± 0.351
2	10	3.8 ± 0.346
3	15	3.0 ± 0.173
4	20	2.4 ± 0.173
5	30	1.8 ± 0.1
6	45	1.233 ± 0.057
7	65	1.083 ± 0.104
8	85	0.973 ± 0.046
9	110	0.866 ± 0.046
10	135	0.76 ± 0.069
11	185	0.5466 ± 0.046

* Values shown are means ± S.D. for N = 3.

$$Vc\% = \frac{\text{Dose} \times 100}{\text{Cpo} \times \text{Body weight}} ; \quad kel = \frac{A + B}{A/\alpha + B/\beta} ;$$

$$k_{21} = \frac{\alpha \cdot \beta}{k_{el}} ; \quad k_{12} = \alpha + \beta - k_{21} - k_{el}.$$

Vd(ss)%, the overall apparent distribution volume (apparent distribution volume at steady state) expressed as percent of

Table III

Serum Concentration Data Following Intravenous Injection of Erythromycin Lactobionate

No. of Observation (n)	Time of Sampling (minutes)	Serum Erythromycin Concentration* Cpt. mcg/ml
1	03	4.2 ± 0.30
2	10	3.13 ± 0.251
3	15	2.45 ± 0.229
4	25	1.73 ± 0.189
5	45	1.06 ± 0.076
6	90	0.786 ± 0.061
7	120	0.64 ± 0.034
8	150	0.52 ± 0.040

* Values shown are means ± S.D. for N = 3.

total body weight = $\frac{k_{12} + k_{21}}{k_{21}}$ x Vc%; Vt%, the apparent volume of the peripheral compartment expressed as percent of the total body weight = Vd(ss)% - Vc%.

The values of the pharmacokinetic parameters of the present investigation are summarized in Table IV.

The results of serum protein binding and reversibility studies are furnished in Table Va and Table Vb respectively.

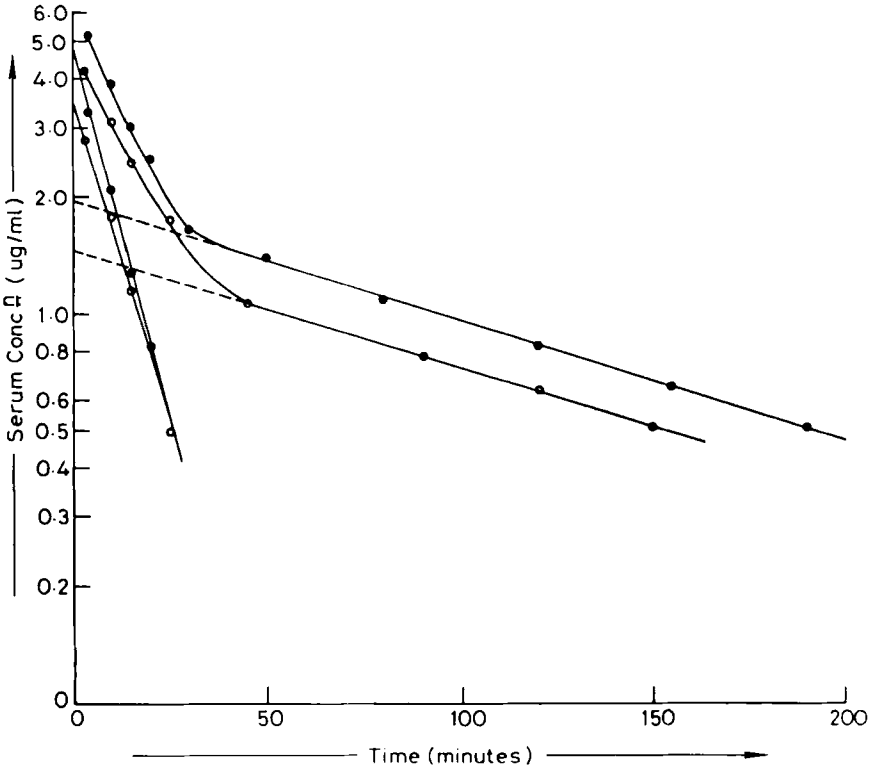


FIGURE I

Semilog plot of observed average serum concentrations and residuals versus time after intravenous injection of 500 mg/ 60 kg Erythromycin base equivalents as Erythromycin melibionate (●) and Erythromycin lactobionate (o) to rabbit.

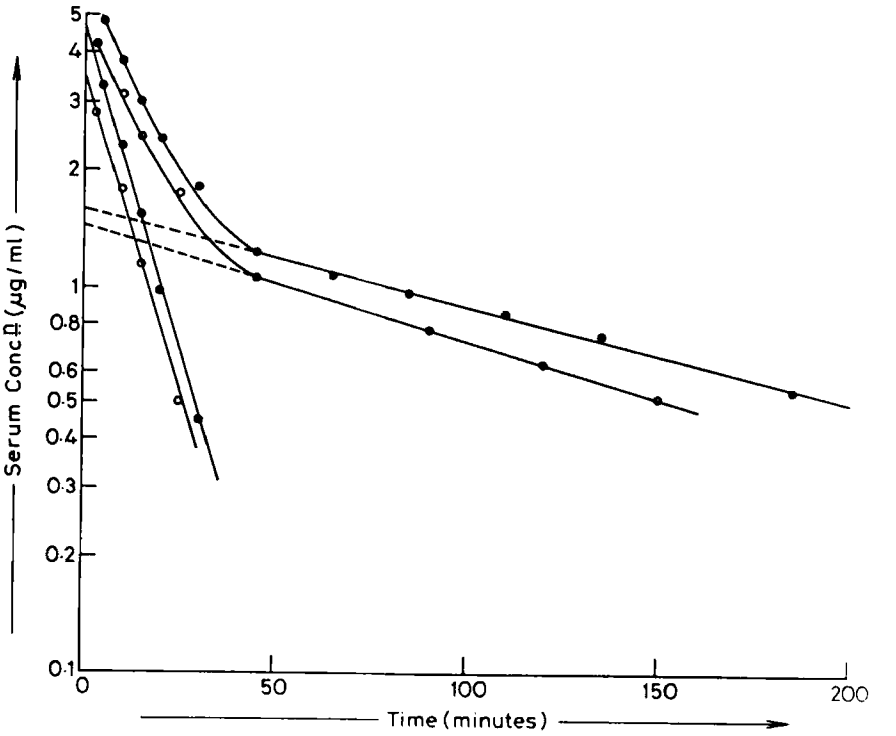


FIGURE II

Semilog plot of observed average serum concentrations and residuals versus time after intravenous injection of 500 mg/ 60 kg Erythromycin base equivalents as Erythromycin penicillanate (•) and Erythromycin lactobionate (o) to rabbit.

Table IV

Values of Pharmacokinetic Parameters of the Erythromycin salts.

Parameters	Erythromycin melibionate	Erythromycin penicillanate	Erythromycin lactobionate
Cpo, mcg/ml	6.75	6.2	4.95
α, min^{-1}	0.087	0.077	0.075
β, min^{-1}	7.0×10^{-3}	5.7×10^{-3}	6.7×10^{-3}
$t_{1/2}$(elim), min	99	121	103
k_{12}, min^{-1}	0.044	0.040	0.036
k_{21}, min^{-1}	0.030	0.024	0.026
kel, min^{-1}	0.0202	0.0183	0.0189
Vc%	123	134	168
Vd(ss)%	302	357	395
Vt%	179	223	227

Though same base equivalent dose of each salt was injected, variation in the pharmacokinetic values (Table IV) are observed for the erythromycin salts. This variation indicates that individual salts are not likely to have the same clinical efficacy. Biological half-life of Erythromycin penicillanate is found greater than that of Erythromycin lactobionate, the reference standard and that for the Erythromycin melibionate, though not greater, is quite comparable with the reference standard.

Table V

Data of Protein Binding (a) and Reversibility (b) Studies.

Drug	Extent of protein binding*
Erythromycin melibionate	87.16% ± 0.288
Erythromycin penicillanate	89.41% ± 0.520
Erythromycin base	88.83% ± 0.763

(a)

Drug	Percent released from Drug-Protein complex*
Erythromycin melibionate	11.25% ± 0.25
Erythromycin penicillanate	8.66% ± 0.288
Erythromycin base	10.66% ± 0.381

(b)

*Values shown are means ± S.D. for N = 3 and are base equivalents of the respective salts.

The large distribution volumes (Table IV) are in consistent with reported extensive tissue penetration of erythromycin[11-14]. Application of this concept is verified by high protein binding of the new salts of erythromycin (Table Va). The data of

reversibility study (Table Vb) showed that the drugs are reversibly bound to serum-protein which is expected to retard elimination from the body and is reflected in the elimination half-lives of the salts.

The calculated distribution volumes in Table IV may thus provide a basis for calculating absolute absorption efficiencies from oral doses of respective erythromycin salts.

REFERENCES

1. "Remington's Pharmaceutical Sciences" 15th Edn., 1975,p.707.

2. Lachman, L., Liberman, H.A. & Kanig, J.L. "Theory and Practice of Industrial Pharmacy" 2nd Edn. 1976, p-119.

3. S.K. Dutta and S.K. Basu, Indian Patent 142584, July 30, 1977.

4. S.K. Dutta, and S.K. Basu, U.S. Patent, 4137379, 1979.

5. D.C. Grove and W.A. Randall; "Assay methods of antibiotics - a Laboratory mannual (1955)", Medical Encyclopedia, New York

6. G.W. Houghton et al., "Eur J. Clin. Pharmacol." 9, 73 (1975)

7. R.S. Griffith, M.D. Johnstone and W.J. Smith, Antibiotic Annual, 496 (1953-1954).

8. P.G. Welling; A.W. Craig, Am.J. of Pharm. Sci. 67, 1057-8, (1978).

9. Stephen H. Curry, "Drug Disposition and Pharmacokinetics", Blackwell Scientific Publications/ Oxford London Edingburgh Melbourne, 2nd Edn. 1977, p-158.

10. M. Gibaldi and D. Perrier, "Pharmacokinetics" Vol. I, Appendix-3, p-281-284.

11. C.C. Lee, R.C. Anderson and K.K. Chen, Antibiot. Chemother., 3, 920 (1953).

12. C.C. Lee and R.O. Forman, ibid., 11. 107 (1960).

13. R.H. Parker, Mod. Treatment. 6, 1071 (1969).

14. D.G. Winningham, N.J. Nemoy and T.A. Stamey, Nature, 219, 139 (1968).