Acta Pharm. *54* (2004) 231–242 Original research paper

Preparation and evaluation of a new erythromycin derivative – erythromycin taurate

PRABAL KUMAR MANNA*
VENUGOPAL KUMARAN
GURU PRASAD MOHANTA
RAJAPPAN MANAVALAN

Department of Pharmacy, Annamalai University, Annamalai Nagar 608002 Tamil Nadu, India

Erythromycin taurate, a new derivative of erythromycin, was prepared by reacting erythromycin base with tauric acid and its physico-chemical and biological properties were evaluated. The derivative has reasonably good solubility in organic solvents. The partition coefficient values in chloroform/water 1.17 and octanol/water 1.16 systems indicate its good distribution in various tissues *in vivo*. The *in vitro* antimicrobial potency of the derivative (833.33 μg mg^{-1}) is higher than that of the existing derivatives such as erythromycin estolate, erythromycin stearate, erythromycin ethyl succinate, erythromycin gluceptate, erythromycin lactobionate. The antimicrobial spectrum is comparable to that of the parent compound. Our results indicate that erythromycin taurate has a high potential for possible clinical application and is more efficient against *Escherichia coli* and *Klebsiella pneumoniae* than the parent base.

Received December 29, 2003
Accepted August 3, 2004

Keywords: erythromycin taurate, antimicrobial potency *in vitro*, pharmacokinetic parameters *in vivo*

Erythromycin is widely used in the treatment of various infections, caused by both Gram-positive and Gram-negative organisms. Previous studies indicate that common pathogens (streptococci, pnemuococci), many staphylococci and mycoplasma pneumoniae are inhibited by erythromycin. Although erythromycin has been widely used in therapy for nearly five decades, there are some disadvantages, such as water insolubility, instability in the gastric pH (1), bitter taste and irregular absorption from the gastro-intestinal tract. Various salts and derivatives have been prepared since it was introduced for clinical use. However, only a few of the derivatives, like erythromycin lactobionate and erythromycin glucoheptonate (water soluble salts), erythromycin stearate and erythromycin ethyl succinate (water insoluble salts) and erythromycin estolate (water insoluble ester salt), have been officially recognized by the USP. Large intersubject (2, 3) variability in serum concentration occurs after oral administration even under standardized conditions, whereas intersubject variability in pharmacokinetic parameters after intravenous administration is small (4). For this reason, the drug is listed by the

* Correspondence, e-mail: pkmanna@rediffmail.com

American Academy of Pharmaceutical Sciences as having serious bioavailability problems. With the above limitations in mind, a new erythromycin derivative, erythromycin taurate, has been prepared and its physico-chemical and biological properties have been evaluated.

Taurine is conditionally an essential amino acid, which is not utilized in protein synthesis, but is rather found free or in simple peptides. Taurine has been essential in certain aspects of mammalian development and low levels of taurine are associated with various pathological lesions, including cardiomyopathy, retinal degeneration and growth retardation (5).

Taurine has been clinically used in the treatment of a wide variety of conditions, including cardiovascular diseases (6), epilepsy and other seizure disorders (7), macular degeneration, Alzheimer disease, hepatic disorder (8) and cystic fibrosis (9). Hence, erythromycin, as a taurate salt, can be used in clinical management of the above diseases associated with bacterial infections.

EXPERIMENTAL

The materials used in the present study are: erythromycin base (Alembic, India), taurine (BDH Biochemicals, UK), culture media (Hi media, India), brain heart infusion agar (Hi media). All other materials used were of analytical grade.

Salt preparation

Erythromycin taurate was prepared by the method of Dutta and Basu (10, 11) by reacting erythromycin base with tauric acid. The salt was recovered by lyophilization.

Physicochemical properties

Melting point (DSC studies), solubility, optical rotation, partition coefficient, pH, thin layer chromatography, infrared and nuclear magnetic resonance spectroscopic investigations were carried out in order to characterize the new derivative. For all the above studies, erythromycin base according to the *Indian Pharmacopoeia* (12) was used as the reference standard.

The pH of a 1% aqueous solution of the derivative was determined using the ELICO (India) digital pH meter (model LI 612).

The solubility of erythromycin taurate in nine different pharmaceutical solvents was determined by the method of Marsh and Weiss (13). The partition coefficient was determined for the derivative in chloroform/water and octanol/water solvent systems.

The optical rotation of 1% (*m/V*) solution of the derivative in 90% (*V/V*) ethanol was measured at 27.7 °C in a P 1010 JASCO (USA) polarimeter and the specific rotation was computed.

The TLC studies were carried out using silica gel G plates (SD, Fine Chemicals, India) of 0.25 mm depth. The plates were prepared and activated at 120 °C for two hours before use. Sample of 10 µL was applied. The spots were then run in various solvent sys-

tems, like methanol, methanol/chloroform (1:1) and methanol/acetone (1:1) and identified in an iodine chamber. Erythromycin reference standard was used (Abbott Laboratories, USA).

The infrared spectrum of the derivative was recorded in a NICOLET IR spectrometer (NICOLET Inst. Corp. USA) using potassium bromide pellets. The NMR spectrum of the derivative was recorded in a JASCO GSM spectrometer (USA) using dimethyl sulphoxide as solvent. The resolution was kept at 0.49 Hz and the speed was 15 Hz. The ^{13}C NMR studies (400 MHZ, Avance, Bruker, Switzerland) were carried out using deuterated methanol as solvent for taurate and DMSO for the base. The DSC studies (Model No. DSC 821, Mettler Toledo, Switzerland) were carried out with rising of temperature at a rate of 10 °C min^{-1} in the temperature range between 40 °C and 340 °C.

Biological studies

In vitro *studies*. – The biological properties studied for the derivative include *in vitro* antimicrobial potency, *in vitro* antimicrobial spectrum (*MIC*) and *in vivo* pharmacokinetic parameters in rabbit. Erythromycin base, according to the *Indian Pharmacopoeia* (12) was used as the reference standard for the above studies.

The *in vitro* antimicrobial potency was determined following the method of Grove and Randall (14) using *Sarcina lutea* ATCC 9341 as the test organism. The concentration of erythromycin taurate was obtained by the single point assay method (12). The standard curve was prepared using erythromycin base and the zone of inhibition obtained by erythromycin taurate was plotted on the standard curve. The *in vitro* potency of the derivative was calculated as 833.3 μg mg^{-1}. The value of *in vitro* potency of erythromycin base used was 920 μg mg^{-1}.

The *in vitro* antimicrobial spectrum, *i.e.*, the minimum inhibitory concentration (*MIC*) of the derivative was determined by the two-fold agar dilution test (15). Brain heart infusion agar plates mixed with erythromycin base and the derivative were inoculated each with one loopful of 24 hour old broth culture. The plates were incubated for 18 hours at 37 °C and the *MIC* was calculated for the following organisms: *Staphylococcus aureus* NCIM 2079, *Klebsiella pneumoniae* NCIM 2957, *Bacillus pumilis* NCIM 2327, *Bacillus subtilis* NCIM 2063, *Escherichia coli* NCIM 2065 and *Pseudomonas aeruginosa* NCIM 2036**.**

In vivo *studies*. – All biological experiments performed complied with the ethical standards. *The in vivo* pharmacokinetics of the derivative was determined by using Swiss albino male rabbits, 6 months old and of around 1.5 kg body mass, as test animals. The rabbits were fed fodder feed (standard pellets, Pranav Agro Industries, India), vegetables (carrot and cabbages) and had free access to water. The feed was maintained at 120 to 150 g per day per rabbit to prevent obesity. Animals were housed separately in cages; the average temperature of the house was maintained at 18 to 21 ºC throughout their life span. There were two experimental groups (control and test), each consisting of 5 rabbits. The control group received 8 mg kg^{-1} body mass of erythromycin base and the experimental group received 8.3 mg kg^{-1} body mass of the derivative (equivalent to 8 mg kg^{-1} body mass of the base) by intravenous injection through marginal ear vein. The dose was equivalent to a single 500 mg erythromycin base dose administrated to a 60 kg human adult. Blood samples (0.5 mL) were taken immediately after the injection and at different time intervals for about three hours after injection. Serum was separated from

each blood sample and erythromycin concentration in the serum was determined by the microbiological assay (14). The concentration of erythromycin in serum was calculated graphically from the standard curve plotted with the base obtained for this purpose using the single point assay (12).

The acute toxicity studies of the derivative and the base were carried out in male albino mice (3 to 4 weeks old) following the up and down method of OECD Guideliness (16). The derivative and the base were dissolved in a 1:1 mixture of water/propylene glycol and were administered orally. The animals were kept overnight in fasting condition with water *ad libitum* before drug administration. A vehicle control group was used at each dose level.

RESULTS AND DISCUSSION

The new derivative, erythromycin taurate (Fig. 1), is a white amorphous, fluffy powder, with a very bitter taste. It was observed from the DSC thermogram (Fig. 2) that the melting point of erythromycin base and erythromycin taurate was 190.18 and 268.97 °C, respectively; there was no thermal degradation of the derivative in the higher temperature range *i.e.*, from 25 to 267 °C. The specific rotation of the derivative is –73.16°. The solubility data of the derivative (Table I) indicate that the derivative is more soluble in polar organic solvents, methanol and ethanol, than in aqueous solvents, while less soluble in low polar organic solvents. Taurine is highly soluble in methanol and ethanol (highly polar organic solvents), insoluble in water and insoluble in chloroform, ethyl acetate and acetone. The solubility of the derivative increased in water (9.9 mg mL^{-1} compared to 2.1 mg mL^{-1} of base) and in phosphate buffer pH 7.4 (9.5 mg mL^{-1} compared to 1.8 mg mL^{-1} for the base). The increase in aqueous solubility might result in improved gastro-intestinal absorption and distribution *in vivo*.

The pH of 1% (*m/V*) aqueous solution of erythromycin taurate is given in Table III. The partition coefficient values indicate that the derivative has more affinity for organic

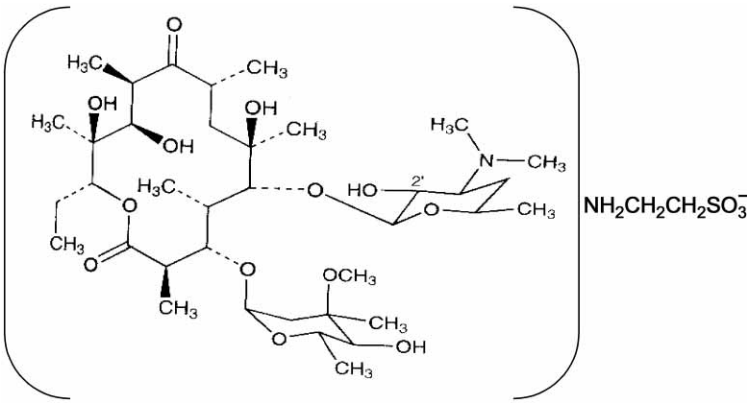

Fig. 1. Structure of erythromycin taurate ($C_{39}H_{74}N_2SO_{16}$).

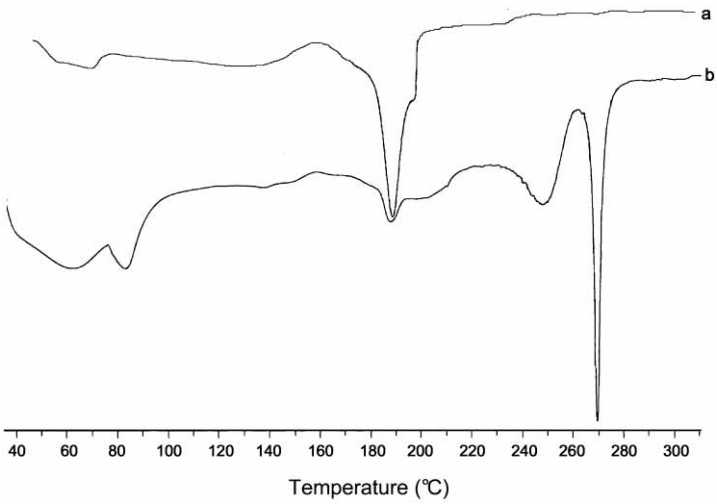

Fig. 2. DSC thermogram of: a) erythromycin base, b) DSC thermogram of erythromycin taurate.

Table I. Solubility data for erythromycin base and erythromycin taurate at 30 °C

Solvent	Base (mg mL^{-1})	Taurate (mg mL^{-1})
Water	2.1	9.93
Methanol	> 20	> 20
Ethanol	> 20	> 20
Propylene glycol	> 20	> 20
Phosphate buffer, pH 7.4	1.8	9.5
Octanol	> 20	14.75
Chloroform	> 20	15.00
Ethyl acetate	> 20	14.83
Acetone	> 20	6.37

solvents. Though the partition coefficient of the derivative (Table III) is lower compared to that of the base, its preferential distribution in octanol suggests that taurate might have comparable gastro-intestinal absorption as the base.

The single spot obtained in the thin layer chromatographic investigation for erythromycin base confirmed the homogeneity of the prepared derivative. The R_f values are given in Table II. IR spectra of the base and erythromycin taurate, the new derivative, are given in Figs. 3a and 3b, respectively. It is observed that while the OH group stretching for the base is at 3473.97 cm^{-1} for the derivative, it is shifted to 3413.36 cm^{-1} and broadened. The broadening of the absorption band in case of the derivative results from the merging of the absorption bands due to NH and OH groups. The absorption

Table II. R_f values of erythromycin taurate and erythromycin base in different solvent systems using silica gel G plate

Solvent system	Base	Taurate
Methanol	0.400	0.351
Methanol/chloroform (1:1)	0.350	0.291
Methanol/acetone (1:1)	0.380	0.349

Table III. pH and partition coefficient (ratio of concentrations) of erythromycin taurate and erythromycin base in two different solvent systems

pH 1%, *m/V*, aq. solution		Partition coefficient			
		Chloroform/water		Octanol/water	
Base	Taurate	Base	Taurate	Base	Taurate
8.20	8.47	1.45	1.17	1.60	1.16

band for C=O group stretching is at 1732.56 cm^{-1} for the base, while it is shifted to 1694.84 cm^{-1} for the derivative.

The broadening of the OH group stretching band and shifting of both OH and C=O groups stretching bands in the IR spectra of the derivative confirm the formation of the new derivative. The presence of two new bands at 1340.99 cm^{-1} and 1108.34 cm^{-1} assignable to the asymmetric and symmetric stretching due to the sulphonic acid group (present in the acid and in the derivatives) but absent in the base additionally confirms the formation of the derivative.

In the ^1H NMR spectra (Figs. 4a, b) of the derivative and of the base specific differences were observed in the region of 3 to 5 ppm; additional peaks with high intensity at 3 to 3.5 ppm due to the presence of the sulphonic acid group present in taurine appeared. In the ^{13}C NMR spectra of the base, the peak for C=O group was observed at 174 and 182 ppm, while for the derivative it was observed at 173 and 178 ppm. This significant shielding indicated the formation of the derivative. This clearly suggests that a quaternary ammonium salt (derivative) was formed. The proposed structure of the new derivative is given separately in Fig. 1. The reports of Manna *et al.* (17–19) corroborate the physico-chemical properties found in the present study.

The *in vitro* antimicrobial spectrum of erythromycin base and erythromycin taurate reveals that the *MICs* (Table IV) are significantly lower for the derivative for *Klebsiella pneumoniae* and *Escherichia coli*, while the values are even higher for *Staphylococcus aureus*, *Bacillus pumilis*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. This showed that taurate is effective against all organisms as is the base, though at different concentrations.

Individual post injection serum erythromycin concentration after administration of both erythromycin taurate and erythromycin base to the experimental animals declined in a biphasic manner. These results are consistent with those obtained in earlier studies on human volunteers (20, 21). Individual serum *versus* time data of erythromycin base and erythromycin taurate are summarized in Table V and the pharmacokinetic parame-

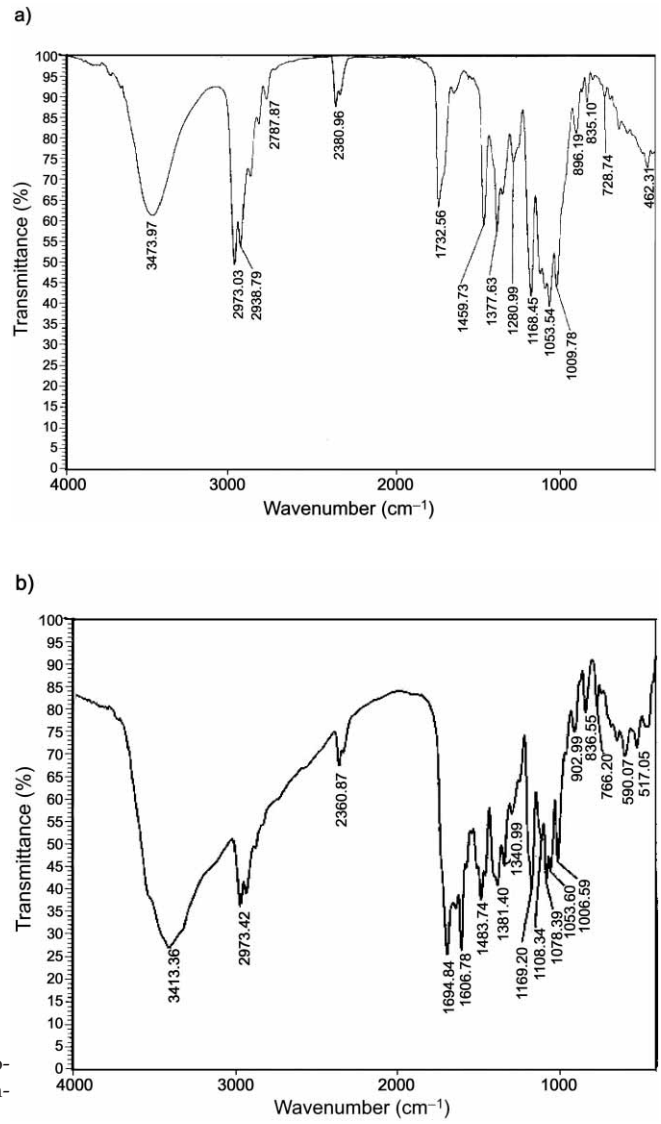

Fig. 3. IR spectra of: a) erythromycin base, b) erythromycin taurate.

ters in Table VI. This shows that the kinetics is a two-compartment model, with elimination occurring from the central compartment. The derivative's similarity in the α value, which is the »fast« intercept, indicates that the derivative's disposition phase is the same as that of the base and that it follows open two-compartmental pharmacokinetics. The β value, which is the »slow« intercept, is indicative of the short half life of the derivative (α and β are complex rate constants where $\alpha > \beta$. α is obtained from the slope of the residual line obtained by the method of residuals and β is obtained from the slope of the

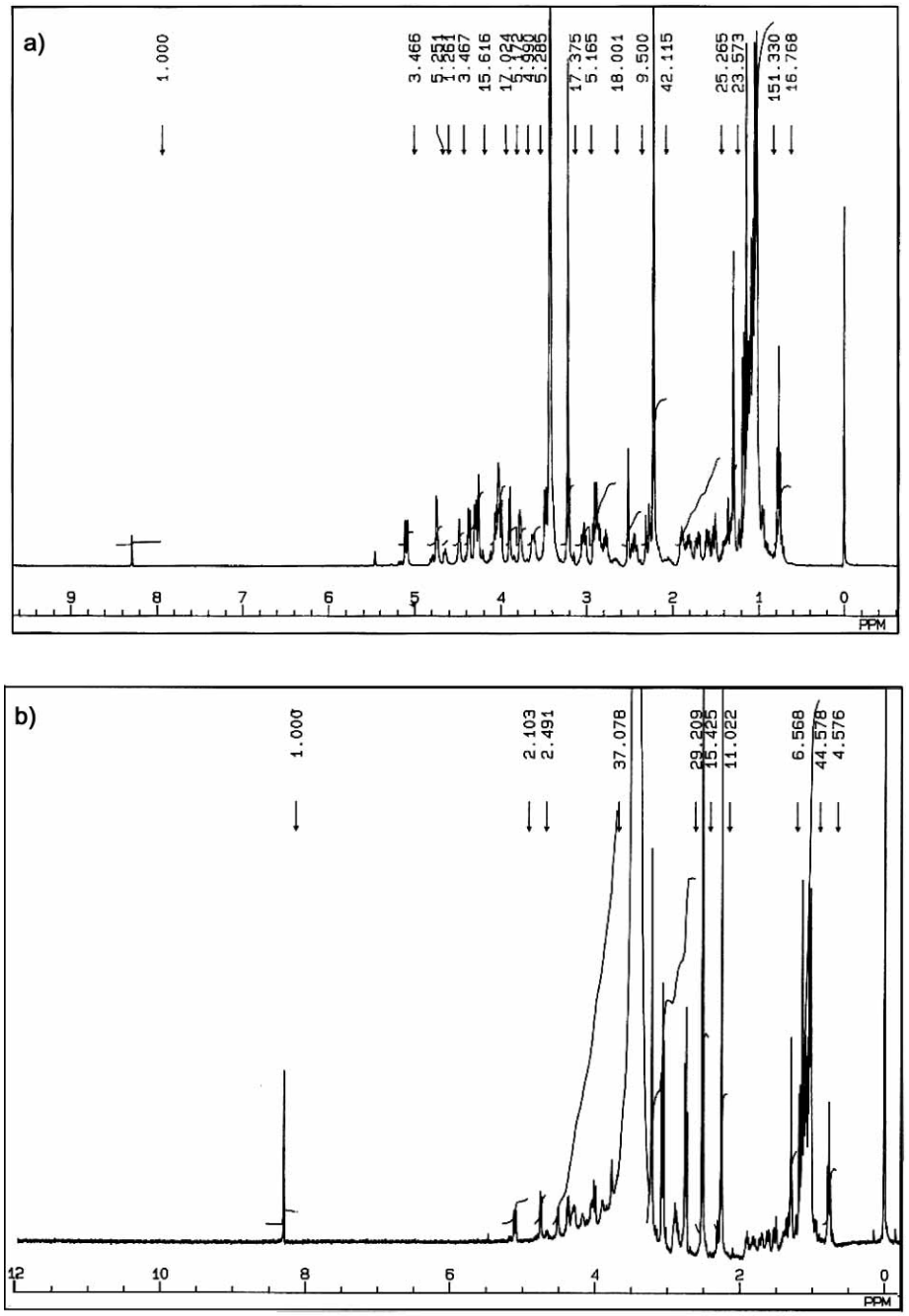

Fig. 4. ^{1}H NMR spectra of: a) erythromycin base, b) erythromycin taurate.

Table IV. In vitro *antimicrobial spectrum of erythromycin base and erythromycin taurate*

Sample No.	Microorganism	*MIC* (μg mL^{-1})	
		Base	Taurate
1	*Staphylococcus aureus* NCIM 2079	0.5	1.0
2	*Klebsiella pneumoniae* NCIM 2957	5.0	2.5
3	*Bacillus pumilis* NCIM 2327	0.3	1.0
4	*Bacillus subtilis* NCIM 2063	0.2	0.5
5	*Escherichia coli* NCIM 2065	40.0	10.0
6	*Pseudomonas aeruginosa* NCIM 2036	0.5	1.5

NCIM – National Collection of Industrial Microorganisms

Table V. Serum concentrations of erythromycin after intravenous injections of erythromycin base and erythromycin taurate

Observation No.	Time of sampling (min)	From base (μg mL^{-1})[a]	From taurate (μg mL^{-1})[a]
1	15	3.56 ± 0.04	5.22 ± 0.04
2	30	2.40 ± 0.07	4.06 ± 0.08
3	45	1.48 ± 0.04	2.14 ± 0.05
4	60	1.02 ± 0.14	1.18 ± 0.04
5	75	0.88 ± 0.04	0.87 ± 0.04
6	105	0.72 ± 0.04	0.64 ± 0.005
7	165	0.57 ± 0.01	0.54 ± 0.02
8	225	0.45 ± 0.01	0.27 ± 0.004

[a] Mean ± SD (n = 5).

slow segment of the biphasic curve in the biphasic serum drug profile). The elimination half life ($t_{1/2\,el}$), less than that of the base, is indicative of the shorter half life of the derivative. k_{12}, k_{21}, the first-order rate constants between central and peripheral compartments and k_{el}, the elimination rate constant are comparable with that of the base. The $V_{d(ss)}$ (overall apparent volume of distribution) is higher for the base and lower for the derivative, indicating that the overall distribution is less for the derivative than for the base. The V_t (apparent volume of distribution from the peripheral compartment) is lower than V_c value (apparent volume of distribution of the central compartment) for the derivative, indicating that the derivative's distribution is higher from the central compartment than from the peripheral tissue or compartment. This is reflected by the shorter half-life and higher serum concentrations attained by the derivative than by the erythromycin base.

Though the derivative equivalent to the base was injected variations in the pharmacokinetic values (Table VI) were observed for the erythromycin derivative and the base. This variation indicates that the derivative is not likely to have the same clinical efficacy as that of the base. The calculated distribution volumes may provide a basis for

Table VI. Pharmacokinetic parameters of erythromycin base and erythromycin taurate

Parameter	Erythromycin base	Erythromycin taurate
Cpo (μg mL^{-1})	7.0	10.2
α (min^{-1})	0.0479	0.0477
β (min^{-1})	4.606×10^{-3}	6.52×10^{-3}
$t_{1/2el}$ (min)	150.45	106.28
k_{12} (min^{-1})	0.0213	0.0157
k_{21} (min^{-1})	0.0108	0.0115
k_{el} (min^{-1})	0.0204	0.027
V_c (%)	118.50	81.89
$V_{d(ss)}$ (%)	352.20	141.69
V_t (%)	233.70	59.80

Cpo – initial serum concentration
α and β - "fast" and "slow" slopes of the biphasic serum drug decay profile
$t_{1/2el}$ – elimination half life
k_{12}, k_{21}, k_{el} – first–order rate constants of drug distribution for central and peripheral compartments and drug distribution, respectively
V_c (%) – apparent volume of distribution in the central compartment
$V_{d(ss)}$ (%) – overall apparent volume in the steady state
V_t (%) – apparent volume of the peripheral compartment

calculating absolute absorption efficiencies from the oral dose of the erythromycin derivative.

The acute toxicity studies in mice have already revealed that the LD_{50} value of erythromycin taurate is equal to that of the parent base (1090 mg kg^{-1}). This value being much higher than the usual therapeutic dose of the base (30–40 mg kg^{-1}) suggests the safety of the derivative.

CONCLUSIONS

It is evident from the findings of the present investigations that physico-chemical and biological properties of the derivative are satisfactory for its use in therapy. The current results indicate its higher efficacy against *Escherichia coli* and *Klebsiella pneumoniae* than that of the parent base. Thus, the new derivative may be exploited clinically and further research may be carried out in this direction. Further studies such as subcutaneous and chronic toxicity studies in animals, formulation development and development of new delivery systems should be undertaken.

Acknowledgement. – The authors thank the University Grants Commission (UGC), New Delhi, India, for funding this project.

REFERENCES

1. L. P. Garrod, H. P. Lambert and F. O. Grady, *Antibiotics and Chemotherapy*, Churchill Livingstone Publishers, Edinburgh 1973.
2. A. H. C. Chun and J. A. Seitz, Drug bioavailability information and its utility, *J. Am. Pharm. Assoc.* **14** (1974) 407–414.
3. A. H. C. Chun and J. A. Seitz, Pharmacokinetics and biological availability of erythromycin, *Infection* **5** (1977) 14–22.
4. C. E. Mathur, K. C. Austin, C. R. Philpot and P. J. McDonald, Absorption and oral bioavailability of erythromycin, *Br. J. Clin. Pharmacol.* **12** (1981) 131–140.
5. J. A. Shuraman, Taurine in development, *Physiol. Rev.* **73** (1993) 119–147.
6. R. J. Huxtable and C. A. Sebring, *Cardiovascular actions of taurine*, in *Sulfur Amino Acids – Biochemical and Clinical Aspects* (Eds. K. Kuriyama, R. J. Huxtable and I. Wata), Alan R. Liss Publications, New York 1983, pp. 5–37.
7. E. M. Airaksinen, S. S. Oja and K. M. Marnela, Effect of taurine treatments on epileptic patients, *Prog. Clin. Biol. Res.* **39** (1980) 157–166.
8. Y. Matsuyama, T. Marita, M. Higuchi and T. Sujii, The effect of taurine administration on patients with acute hepatitis, *Prog. Clin. Biol. Res.* **125** (1983) 461–468.
9. S. Carasco, R. Codoceo and G. Prieto, Effect of taurine supplements on growth, fat absorption and bile acid on cystic fibrosis, *Acta Univ. Carol.* **36** (1990) 152–156.
10. S. K. Dutta and S. K. Basu, *Preparation of Erythromycin Aldobionate*, Indian Pat. No. 142584, 30 July 1977; ref. *Chem. Abstr.* **85** (1978) 121653a.
11. S. K. Dutta and S. K. Basu, *Preparation of Erythromycin Aldobionate*, US Pat. 4137397, 30 Jan 1979; ref. *Chem. Abstr.* **92** (1979) 111271h.
12. *Indian Pharmacopoeia*, Vol. I, The Controller of Publications, New Delhi, pp. 292–293.
13. J. R. Marsh and P. J Weiss, Solubility of antibiotics in 26 solvents, *J. Assoc. Offic. Anal. Chem.* **50** (1977) 457–462.
14. D. C. Grove and U. A. Randall, *Assay Methods of Antibiotics – A Laboratory Manual*, Marcel Dekker, New York (1955).
15. D. Greenwood, R. C. B. Slock and J. F. Peutherer, *Medical Microbiology – a Guide to Microbial Infection: Pathogenesis, Immunity Laboratory Diagnosis and Control*, 16th ed., Churchill Livingstone, Edinburgh 2002, pp. 58–59.
16. OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects, Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure (adapted December 17, 2001), Organisation for Economic Co-operation and Development, 2001.
17. P. K. Manna, S. K. Basu, and B. B. Goswami, Studies on erythromycin melibionate – a new water soluble salt of erythromycin, *Drug. Dev. Ind. Pharm.* **21** (1995) 2097–2107.
18. P. K. Manna and S. K. Basu, Preparation and evaluation of erythromycin fumarate – a new derivative of erythromycin, *Drug. Dev. Ind. Pharm.* **24** (1998) 879–882.
19. P. K. Manna and S. K. Basu, Studies of erythromycin maltobionate, a new derivative of erythromycin, *Drug. Dev. Ind. Pharm.* **25** (1999) 391–395.
20. P. G. Welling and W. A. Craig, Pharmacokinetics of intravenous erythromycin, *Am. J. Pharm. Sci.* **67** (1978) 1057–1058.
21. M. Gibaldi and D. Perrier, *Pharmacokinetics*, Churchill Livingstone Publications, New York 1983, Vol I, Appendix – 3, pp. 281–284.

S A Ž E T A K

Priprava i evaluacija novog derivata eritromicina – eritromicin taurat

PRABAL KUMAR MANNA, VENUGOPAL KUMARAN, GURU PRASAD MOHANTA i RAJAPPAN MANAVALAN

Eritromicin taurat pripravljen je reakcijom eritromicin baze s taurinskom kiselinom. U radu su evaluirana fizičko-kemijska i biološka svojstva ovog novog derivata eritromicina. Eritromicin taurat je razmjerno dobro topljiv u organskim otapalima. Koeficijent razdjeljenja u sustavu kloroform/voda bio je 1,17 a u sustavu oktanol/voda 1,16, što ukazuje da se ova ljekovita tvar može dobro raspodijeliti u različitim tkivima *in vivo*. Antimikrobna aktivnost *in vitro* (833,33 μg mg^{-1}) veća je od aktivnosti postojećih derivata eritromicina: estolata, stearata, etil sukcinata, gluceptata, laktobionata. Spektar antimikrobnog djelovanja sličan je spektru samog eritromicina. Zbog svega toga moguća je klinička primjena eritromicin taurata.

Ključne riječi: eritromicin taurat, djelovanje *in vitro*, farmakokinetički parametri *in vivo*

Department of Pharmacy, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India