STUDIES ON THE BIOAVAILABILITY OF SOME NEW ERYTHROMYCIN ESTERS

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Erythromycin has acquired renewed significance because of its activity against chlamydia[1,2], chancroid[3], *Mycoplasma pneumoniae*[4], whooping cough[5,6] and legionnaires' disease[7].

To avoid inactivation of erythromycin by the low gastric pH after oral intake, entero-soluble formulations have been designed and several esters or salts of erythromycin have been prepared. Commonly used oral forms of erythromycin are erythromycin base as entero-soluble tablets, erythromycin stearate, ethylsuccinate and estolate.

In this paper we report the synthesis and bioavailability in rats of nine erythromycin esters in comparison with some established erythromycin derivatives.

The esters were prepared by treatment of erythromycin A in acetone with 1.25 equivalent of pivaloyloxymethylsuccinoyl chloride, 2-, or 3-, or 4-fluorobenzoyl chloride, 3,4-dimethoxybenzoyl chloride, 3,4-methylenedioxybenzoyl chloride, 3,4,5-trimethoxybenzoyl chloride, 2-thiophencarbonyl chloride or 1-adamantanecarbonyl chloride respectively, in the presence of an excess of $NaHCO_3$. After dilution with buffer solution (pH 7), the esters were obtained as white crystals.

The bioavailabilities in rats of the nine esters were compared to those of three standard derivatives by measuring the plasma levels and the urinary excretions after oral administration of the compounds in doses equivalent to 100 mg/kg of erythromycin base. Wistar rats (average weight 250 g, 20 animals per compound), fasting for 12 hours before the experiments, were used. Four rats were sacrificed respectively 1, 2, 3 and 5 hours after the oral administration of each of the twelve compounds in 5% gum arabic (20 ml/kg). Four rats were restrained in opposite cages to collect the urine from 0 to 24 hours after the compounds' ad-

Table 1. Plasma levels, $AUC_{0\sim5\,hours}$, urinary excretions of erythromycin base in rats after oral administration of erythromycin esters in doses equivalent to 100 mg/kg as base.

Erythromycin ester	Plasma levels[a] (μg/ml) (mean±SE)				$AUC_{0\sim5\,hours}$ (μg/ml·5 hours)	Urinary excretion[a] (0~24 hours) (mean±SE, %)
	1 hour	2 hours	3 hours	5 hours		
Pivaloyloxymethyl-succinate (**1**)	0.48±0.19	0.18±0.03	0.14±0.02	0.09±0.02	0.96	0.19±0.04
2-Fluorobenzoate (**2**)	0.31±0.01	0.31±0.04	0.27±0.01	0.18±0.01	1.20	0.88±0.03
3-Fluorobenzoate (**3**)	0.11±0.01	0.08±0.01	0.10±0.01	0.04±0.01	0.38	0.15±0.01
4-Fluorobenzoate (**4**)	0.12±0.01	0.08±0.01	0.08±0.02	0.10±0.03	0.47	0.12±0.01
3,4-Dimethoxybenzoate (**5**)	0.45±0.08	0.24±0.05	0.11±0.05	0.17±0.02	1.02	1.16±0.07
3,4-Methylenedioxy-benzoate (**6**)	0.12±0.01	0.14±0.03	0.10±0.02	0.07±0.01	0.48	0.35±0.01
3,4,5-Trimethoxy-benzoate (**7**)	1.51±0.06	1.50±0.13	1.33±0.08	0.67±0.05	5.67	2.13±0.40
2-Thiophencarboxylate (**8**)	0.13±0.01	0.23±0.03	0.12±0.01	ND	0.54	0.18±0.02
1-Adamantanecarboxylate (**9**)	0.23±0.07	0.17±0.02	0.15±0.03	ND	0.62	0.23±0.03
Stearate (salt)	1.91±0.21	1.67±0.10	1.46±0.19	0.52±0.02	6.29	2.02±0.28
Estolate	1.61±0.25	1.34±0.25	1.38±0.13	0.58±0.05	5.60	2.30±0.21
Ethylsuccinate	0.52±0.03	0.35±0.02	0.33±0.01	0.14±0.01	1.51	0.94±0.13

[a] Four animals/time.

ND: Not determinable (<0.04 μg/ml).

Table 2. Lung levels of erythromycin base in rats after oral administration of erythromycin esters in dose equivalent to 100 mg/kg as base.

Erythromycin ester	Lung levels[a] (μg/g of fresh tissue) (mean±SE)	
	90 minutes	180 minutes
Stearate (salt)	15.26±1.86	13.17±1.77
Estolate	13.56±3.23	18.48±1.32
3,4,5-Trimethoxy-benzoate	26.10±2.28	28.11±1.92

[a] Four animals/time.

ministration. Plasma and urinary levels were assayed for erythromycin base by an agar-diffusion method using *Micrococcus luteus* ATCC 9341 as the assay organism[8]. This method measures only erythromycin base; all esters in our experimental conditions were inactive. Area under the curve (AUC) was calculated according to the trapezoidal rule.

Table 1 reports the plasma levels, the $AUC_{0\sim5\,hours}$ and the urinary excretions of the new nine esters in comparison with the three commonly used erythromycin derivatives.

The most interesting ester is the 3,4,5-trimethoxybenzoate (**7**) with a bioavailability similar to those of stearate and estolate.

The 3,4-dimethoxybenzoate (**5**) and the 2-fluorobenzoate (**2**) show a bioavailability similar to that of ethylsuccinate.

In order to investigate the lung levels of erythromycin base, four rats were sacrificed respectively at 90 and 180 minutes after the oral administration of **7**, stearate and estolate esters. The lungs were homogenized in phosphate buffer 0.1 M, pH 8.0 and the antibiotic lung levels were assayed as previously described.

The Table 2 shows that the 3,4,5-trimethoxybenzoate ester allows to obtain lung levels of erythromycin base higher than that of the other two standard esters.

Experimental

Erythromycin pivaloyloxymethylsuccinoyl ester was prepared by the reaction of erythromycin A (17.3 g) in acetone (100 ml) at 20~25°C with pivaloyloxymethylsuccinoyl chloride (7.53 g) in the presence of $NaHCO_3$ (10 g). After stirring at the same temperature for 6 hours, the reaction mixture was poured into phosphate buffer (pH 7.0); the precipitate was filtered and dried under vacuum at 35°C to afford **1** (19.6 g). MP 116~119°C (dec); $[\alpha]_D^{20}$ −73.5° (*c* 2, EtOH). Erythromycin A, determined after enzymatic or chemical hydrolysis of the ester by reversed-phase liquid chromatography (Lichrosorb RP 18 10 Å) using acetonitrile - 0.2 M ammonium acetate - water (65 : 10 : 30) (pH 7.0) as the mobile phase (detection UV 215 nm)[9] was 71.3%.

By a method similar to that described for the synthesis of **1**, were prepared: Erythromycin 2-fluorobenzoate (**2**) mp 141~144°C (dec), $[\alpha]_D^{20}$ −72.8° (*c* 2, EtOH); 3-fluorobenzoate (**3**) mp 131~135°C (dec), $[\alpha]_D^{20}$ −72.6° (*c* 2, EtOH); 4-fluorobenzoate (**4**) mp 123~126°C, $[\alpha]_D^{20}$ −72.6° (*c* 2, EtOH); 3,4-dimethoxybenzoate (**5**) mp 123~124°C, $[\alpha]_D^{20}$ −70.9° (*c* 2, EtOH); 3,4-methylenedioxybenzoate (**6**) mp 125~128°C, $[\alpha]_D^{20}$ −74.5° (*c* 2, EtOH); 3,4,5-trimethoxybenzoate (**7**) mp 172~175°C (dec), $[\alpha]_D^{20}$ −73° (*c* 2, EtOH); 2-thiophencarboxylate (**8**) mp 146~148°C, $[\alpha]_D^{20}$ −74.1° (*c* 2, EtOH); 1-adamantanecarboxylate (**9**) mp 105~109°C, $[\alpha]_D^{20}$ −14.1° (*c* 2, EtOH).

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