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Absorption and Disposition of Ethambutol in Rabbits

M. M. CHEN *, C. S. LEE ‡x, and J. H. PERRIN *

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Abstract □ The absorption and disposition of ethambutol was examined in six rabbits in a three-way crossover study. Each rabbit received 45-mg/kg doses of ethambutol in three treatments: one intravenous injection, and two oral solutions, ethambutol alone and ethambutol in the presence of aluminum hydroxide (40 mg/kg). Half-lives of ethambutol ranged from 2.26 to 5.20 h when administered alone and 2.18 to 4.00 h when coadministered with the antacid; the difference was not significant ($p > 0.3$). Mean clearance after the oral administrations (189.2 mL/min/kg) was significantly greater than the mean intravenous clearance (43.7 mL/min/kg) ($p < 0.01$), suggesting a first-pass metabolism of ethambutol when administered nonparenterally to rabbits. The volume of distribution ranged from 5.5 to 17.8 L/kg, suggesting an extensive distribution of ethambutol outside the central compartment and, possibly, a localized deposit within the body tissues. Mean bioavailability of ethambutol was ~28% and was not affected by the presence of aluminum hydroxide. The rate of ethambutol absorption, however, was slightly delayed by the antacid.

Keyphrases □ Ethambutol—pharmacokinetics in rabbits, coadministration with aluminum hydroxide □ Aluminum hydroxide—effect on ethambutol absorption in rabbits □ Bioavailability—absorption of ethambutol in rabbits, coadministration with aluminum hydroxide

Ethambutol (I), an antitubercular agent, is prescribed alone or in combination with other drugs for the treatment of tuberculosis. The absorption and excretion of I has been studied in rats and mice (1), dogs (2, 3), and humans (4–6). The median lethal dose (LD_{50}) of racemic I in noninfected adult mice was 12,800 mg/kg when administered orally, 1600 mg/kg when administered subcutaneously, 800–1600 mg/kg when administered intraperitoneally, and 200–400 mg/kg when administered intravenously (1). Since I is well absorbed in mice and the drug metabolites are pharmacologically inactive (3), the remarkable discrepancies in LD_{50} following different administration routes suggest a first-pass metabolism of the drug when taken nonparenterally. A part of this study was thus designed to investigate the effect of first-pass metabolism on the availability of I in rabbits.

Pharmacokinetic studies comparing the intravenous and oral administrations of I to humans have demonstrated rapid and adequate absorption, with bioavailabilities of 70–80% (6, 7). The oral solution and tablets of I were equally well absorbed in humans (6), suggesting a gastric emptying rate- rather than dissolution rate-limited absorption of the drug. Aluminum ion is a known inhibitor of gastric emptying, and its effect on drug absorption has been documented (8). Mattila *et al.* (9) have investigated the effect of aluminum hydroxide on the absorption of I in humans; the results were erratic. Since the results of Matilla *et al.* (9) were inconclusive, this study also seeks to clarify the effect of aluminum hydroxide on the absorption of I using the rabbit model.

EXPERIMENTAL SECTION

Materials—All materials used in this study were the same as those used in a previous investigation (10).

Animal Experiments—Six male New Zealand White rabbits (weight, 2.8–4.1 kg) were studied in a three-way crossover manner. Each rabbit received 45-mg/kg doses of I in three separate treatments: as an intravenous injection, as an oral solution, and as an oral solution in the presence of aluminum hydroxide (40 mg/kg). Gastric emptying of solid food residues was induced by fasting for 38–42 h before drug administration. Water was allowed *ad libitum* during fasting; food and water were withheld over the experimental period. A 2-week washout interval was implemented between the crossover studies.

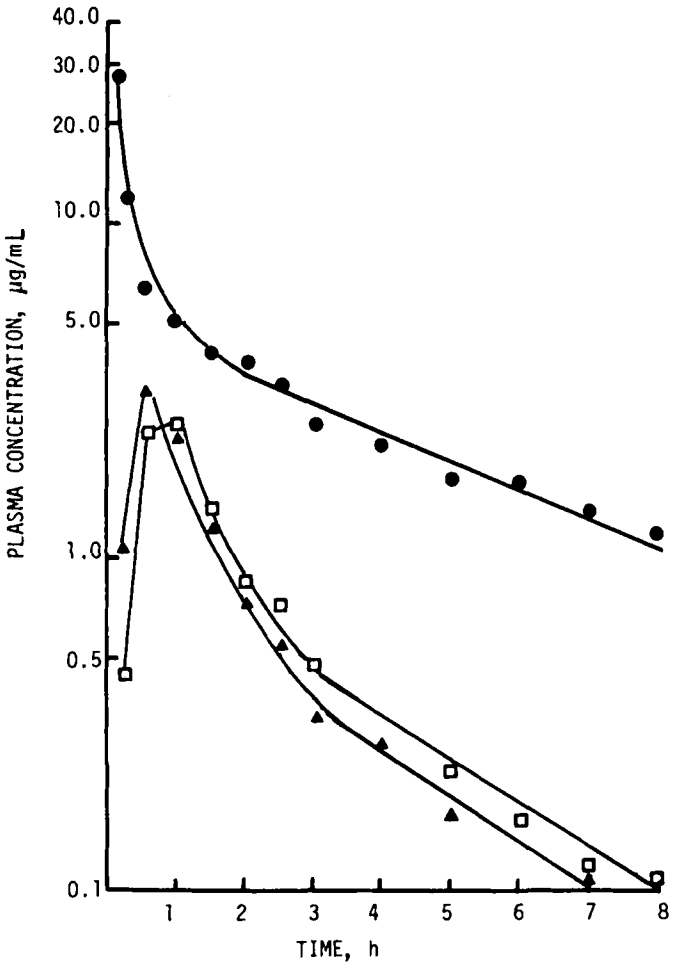

Figure 1—*Semilogarithmic plasma concentration* versus *time plots for rabbit 6 administered ethambutol (45 mg/kg) intravenously (●) and orally (▲, without aluminum hydroxide; □, with the antacid).*

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Table I—Mean Plasma I Levels after Single Doses of I (45 mg/kg) as Three Treatments

Treatment[a]	Plasma I Concentration, μg/mL												
	0.15 h	0.25 h	0.5 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	5.0 h	6.0 h	7.0 h	8.0 h
A													
Mean	20.23	10.84	5.34	4.09	3.11	2.21	2.13	1.64	1.66	1.35	0.90	0.77	0.70
SD	6.20	3.09	1.62	2.11	1.81	1.13	0.73	0.81	1.20	0.61	0.49	0.49	0.47
B													
Mean	—	0.74	2.44	1.52	0.76	0.55	0.37	0.29	0.26	0.18	0.18	0.13	0.12
SD	—	0.20	0.96	0.44	0.27	0.17	0.12	0.07	0.07	0.07	0.07	0.07	0.07
C													
Mean	—	0.74	1.73	1.56	0.81	0.56	0.46	0.37	0.24	0.19	0.15	0.12	0.09
SD	—	0.44	0.61	0.56	0.37	0.20	0.17	0.10	0.07	0.12	0.05	0.05	0.05

[a] Treatments: (A) intravenous injection; (B) oral solution with I alone; (C) oral solution with I and aluminum hydroxide; $n = 6$.

In the case of the intravenous injection, I was administered *via* the ear vein by a slow push over 5 min. As oral solutions, I was prepared in 70 mL of warm, deionized water and administered *via* the mouth by intubation. The intubation line was flushed with 30 mL of warm water to ensure that the complete dose was deposited into the stomach. Blood samples were collected from the ear vein at 0 (blank), 0.15 (injection only), 0.25, 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 h. Hourly urine aliquots were collected from two rabbits by urethral catheter for the first 8 h; thereafter, cumulative 16-h urine samples were collected by natural voiding while rabbits were housed in the metabolism cage.

Blood Cell-to-Plasma Partition Ratio—Aliquots (1 mL) of heparinized whole blood were incubated with I (concentrations of 0.5, 1, and 2 μg/mL) at 37°C for 30 min. Immediately after incubation, plasma was separated by centrifugation and stored at −20°C until analysis. The blood cell-to-plasma partition ratio was calculated as $[C_b/C_p - (1 - H)]/H$, where H is the hematocrit.

Analytical Procedure—A GC procedure, as previously described, was used for sample analysis (10). The GC procedure normally provides a lower detection limit of 0.25 μg/mL. Since plasma levels of I after administration of the oral solutions were usually low, the procedure was manipulated to accommodate the concentration of 0.1 μg/mL.

Pharmacokinetic Analysis—The β-phase half-life ($t_{1/2\beta}$) was obtained by fitting the plasma data to a two-compartment model by the NONLIN program (11). Total body clearance (CL_t) and the noncompartmental steady-state volume of distribution (Vd_{ss}) were determined as:

$$CL_t = \text{dose}/\text{AUC}_\infty \qquad \text{(Eq. 1)}$$

$$Vd_{ss} = \text{dose}(\text{AUMC}_\infty)/(\text{AUC}_\infty)^2 \qquad \text{(Eq. 2)}$$

where AUC is the area under the plasma concentration *versus* time curve, and AUMC is the area under the first moment of the plasma curve (12). Both areas were calculated by the trapezoidal rule in conjunction with an area extrapolation method. The first plasma datum was assumed to be zero in the case of the oral administrations; the first plasma datum after intravenous administration was a NONLIN-generated initial concentration.

Bioavailability was determined by the AUC method, taking into account the half-life change due to alternate routes of drug administration:

$$F = \left(\frac{\text{AUC}_{po}}{\text{AUC}_{iv}}\right) \times \left(\frac{t_{1/2iv}}{t_{1/2po}}\right) \qquad \text{(Eq. 3)}$$

where iv indicates the intravenous administration route, and po indicates the oral administration route. In the two rabbits for which urine was collected at 24 h, bioavailability was also determined by the urinary recovery method:

$$F = \frac{(Ae,24 + CL_r \cdot \text{AUC}_{24\to\infty})_{po}}{(Ae,24 + CL_r \cdot \text{AUC}_{24\to\infty})_{iv}} \qquad \text{(Eq. 4)}$$

Table II—Half-lives of I after Single Doses of I (45 mg/kg) as Three Treatments [a]

Rabbit	Half-life, h		
	Treatment A	Treatment B	Treatment C
1	4.67	5.20	3.77
2	4.08	3.58	3.37
3	4.49	3.66	4.00
4	3.42	2.65	2.18
5	1.96	2.26	2.58
6	4.59	2.83	2.62
Mean ± *SD*	3.87 ± 1.03[b]	3.36 ± 1.05[b]	3.09 ± 0.73[b]

[a] Treatments: (A) intravenous injection; (B) oral solution with I alone; (C) oral solution with I and aluminum hydroxide. [b] Half-lives of I after treatments A, B, and C are not significantly different ($p > 0.3$); $n = 6$.

where $Ae,24$ is the amount of drug excreted in the 24-h urine sample, and CL_r is the average renal clearance as determined from the drug excretion rates during the first 8 h. The extrapolated area ($\text{AUC}_{24\to\infty}$) was calculated as $C_{p,24}/\beta$, where the 24-h plasma concentration was extrapolated from $C_{p,8}$ using the elimination rate constant, β.

Statistical Analysis—Mean pharmacokinetic parameters between any two treatments were tested for significance of difference by paired *t* test statistics.

RESULTS AND DISCUSSION

Table I describes the mean plasma data for rabbits that received I after treatment protocols A, B, and C. At the end of the 8-h sampling period, the residual level of I after intravenous injection was six times that after administration of the oral solutions. Figure 1 depicts a representative plasma profile in rabbit 6. During the first hour of intravenous injection, plasma concentrations of I dropped rapidly, indicating a fast distribution of the drug into the peripheral tissue compartment. Overall, the log-linear phase began at 1–3 h after drug injection and exhibited a half-life range of 1.96–4.67 h. After the oral administrations of I, a rapid distribution of the drug was also observed in all rabbits. Peak plasma concentrations were reached within 1 h, and a slightly delayed peak time was observed for I in combination with aluminum hydroxide. The log-linear phase did not begin until 1.5 h after oral solution administration, and the time to reach distribution equilibrium was as late as 3 h in some rabbits. It should be noted that the delayed stomach emptying as induced by fasting might lead to prolonged absorption and overestimation of half-life measurements (13). In the presence of aluminum hydroxide, I exhibited half-lives of 2.18–4.00 h, as compared with 2.26–5.20 h for I administered alone. The mean half-life of I was not significantly altered by the coadministration of aluminum hydroxide (3.36 ± 1.05 *versus* 3.09 ± 0.73 h; $p > 0.3$). In Table II, the mean half-lives of I in the three treatments are compared; all rabbits exhibited similar half-lives, irrespective of the route of administration and the presence of aluminum hydroxide.

Values for the steady-state volume of distribution of I, derived on the basis of venous data after intravenous administration, are shown in Table III. A wide range was observed for the volume of distribution of I (5.5–17.8 L/kg; mean, 8.5 ± 1.9 L/kg). Chiou (14) has reported that Vd_{ss} estimated with venous data could be 20–120% higher than that derived on the basis of arterial concentration. By taking into consideration the arteriovenous difference, the volume obtained is still several times the body weight of the rabbit (assuming a density of 1 g/mL). This indicates that in addition to the extensive distribution, I was probably deposited locally in the rabbit body tissues. Ethambutol has been known to be extensively distributed into the viscera and tissues of animals and humans. Pujet and Pujet (15) have reported a preferential distribution of I in the deep layers of the lung. Furthermore, *in vivo* and *in vitro*

Table III—Total Body Clearance and Volume of Distribution of I after Single Doses of I (45 mg/kg) as Three Treatments [a]

Rabbit	CL_t, mL/min/kg			Vd_{ss}, L/kg
	Treatment A	Treatment B	Treatment C	Treatment A
1	70.4	120.6	139.4	17.8
2	35.3	196.3	199.5	7.8
3	28.6	175.6	182.5	6.4
4	29.8	261.3	272.7	5.5
5	76.1	178.9	249.2	8.1
6	22.3	148.2	145.8	5.6
Mean ± *SD*	43.7 ± 23.2[b]	180.2 ± 46.0[b]	198.2 ± 54.0[b]	8.5 ± 4.6

[a] Treatments: (A) intravenous injection; (B) oral solution with I alone; (C) oral solution with I and aluminum hydroxide. [b] Oral clearances are not significantly different ($p > 0.1$); however, oral clearance and intravenous clearance are statistically different ($p < 0.01$); $n = 6$.

studies have demonstrated a favorable partition of I into erythrocytes in humans (4, 6). The blood-cell-to plasma partition ratio averaged 1.25 in all rabbits for the concenration range thus investigated.

Values for total body clearance of I after the three treatments are summarized in Table III. Two oral studies, I alone and I in combination with aluminum hydroxide, yielded virtually the same clearance (180.2 ± 47.9 *versus* 198.2 ± 54.0 mL/min/kg), indicating that aluminum hydroxide had no effect on the elimination of I. However, the total clearance after oral administration was significantly greater than the intravenous clearance (189.2 *versus* 43.7 mL/min/kg; $p < 0.01$), suggesting a first-pass metabolism for I. Previous studies with mice, rats, and dogs did not document first-pass metabolism of I since intravenous and oral studies were not carried out in a crossover manner (2, 3). The difference in LD_{50} among various routes of administration in mice could be due partly to the rate of absorption into the circulatory system. However, the magnitude of difference in LD_{50} observed (1) between oral and intravenous administrations (12,800 *versus* 400 mg/kg) supported our proposition of first-pass metabolism in rabbits. Poor absorption of I in rabbits could be an alternative argument; however, we found <15% of orally administered I in rabbit feces.

Renal excretion of I was studied in two rabbits after intravenous and oral administrations. Renal clearances, computed as $Ae,8/AUC_{0\to8}$, averaged 2.5 mL/min/kg. There was no significant change in renal clearance between studies utilizing the oral and intravenous administration routes. The fraction of I dose excreted unchanged after intravenous studies averaged 5.2%, which is considerably lower than the corresponding figures of 30 and 70% in dogs and humans, respectively (10).

The bioavailability data for I, alone and in combination with aluminum hydroxide, were calculated with Eq. 3 and are shown in Table IV. It should be noted that the validity of Eq. 3 is based on the assumption that $Vd_\beta(CL_t/\beta)$ remains the same, in spite of a change in β (16). Although the AUC method was used in all rabbits for bioavailability determination, the urinary recovery method was simultaneously applied to two rabbits for cross-examination. For rabbit 1, the urinary recovery method yielded a somewhat higher bioavailability than the AUC method (62.9 *versus* 57.5%), whereas for rabbit 5, both methods yielded virtually the same results (30.1 *versus* 30.0%). On the average, 28% of the oral I dose was bioavailable to the circulatory system. Without correction for half-life change with Eq. 3, the mean bioavailability was somewhat lower (25%). The low bioavailability also supported the hypothesis of first-pass metabolism of I in rabbits, as previously suggested on the basis of clearance measurements (Table III). As the hepatic blood flow (Q_b) in rabbits has been reported to be 60 mL/min/kg (17), the extent of hepatic first-pass effect for I was approximated as CL_t/Q_b where CL_t is the mean plasma clearance after intravenous administration. A mean of 73% (43.7 of 60) of the dose of I was estimated to undergo first-pass metabolism. This is consistent with the bioavailability of 25–28% in rabbits reported here.

The aluminum hydroxide dose of 40 mg/kg, as used in this study, was three times the therapeutic dose of the antacid used in humans on a weight basis. In the study of Mattila *et al.* (9), a 60-mg/kg dose was used. *In vitro*, aluminum ion at 5×10^{-4} M inhibits the contractile response of human and rat gastric strips to acetylcholine by >50% (18). The *in vitro* effect of aluminum ion on the contractile response of rabbit gastric strips has not been investigated. The aluminum concentration achieved *in vivo* in the stomach of rabbits receiving aluminum hydroxide (2×10^{-2} M) was much higher than that used *in vitro*. It was felt that such a concentration would be sufficient to significantly delay gastric emptying in rabbits.

With regard to the absorption of I, the rabbit may not be an ideal model for comparison with human data; however, rabbits demonstrated a consistent, although insignificant, response to aluminum hydroxide. On the contrary, humans responded to aluminum hydroxide erratically, making it difficult to make a conclusion on the effect of the antacid on the bioavailability of I. In summary, the concomitant administration of aluminum hydroxide appeared to have slightly delayed the absorption of I in some rabbits, as reflected by a prolonged peak time; the extent of absorption, however, was insignificantly altered by the presence of the antacid.

Table IV—Bioavailability of I after Two Oral Treatments [a]

Rabbit	Bioavailability, % of Intravenous Dose	
	Treatment B	Treatment C
1	52.40 (67.57)[b]	62.52 (58.30)[b]
2	20.48	21.38
3	19.97	17.58
4	14.71	17.12
5	36.87 (30.28)[b]	23.17 (29.98)[b]
6	24.44	26.82
Mean ± *SD*[c]	28.15 ± 14.01	28.10 ± 17.24

[a] Treatments: (B) solution with I alone; (C) solution with I and aluminum hydroxide. [b] Numbers in parentheses denote bioavailability determined by the urinary recovery method. [c] $n = 6$.

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