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Single- and Multiple-Dose Kinetics of Estazolam, a Triazolo Benzodiazepine

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Abstract. The pharmacokinetic properties of estazolam, a triazolo benzodiazepine hypnotic agent, were assessed in a series of healthy volunteers following single and multiple doses. After single oral doses of 2–16 mg, peak plasma concentrations were reached within 6 h. Values of elimination half-life ranged from 8.3–31.2 h (mean 17.0 h) and did not vary significantly with dose. During 3 weeks of therapy, steady-state plasma concentrations increased approximately in proportion to increasing doses, and accumulation was essentially complete within 3 days of each dose change. The mean observed accumulation ratio was 1.84, which was slightly larger than the predicted ratio of 1.53. Exposure to multiple-dose estazolam therapy had no significant influence on the kinetics of a single dose of antipyrine, suggesting that estazolam neither stimulates nor inhibits enzyme activity in humans. Thus the accumulation and elimination kinetics of estazolam can be classified as intermediate to those of the short-acting (such as oxazepam) and the long-acting (such as diazepam) benzodiazepine derivatives.

Key words: Benzodiazepines – Estazolam – Drug accumulation – Pharmacokinetics

Estazolam (TA-8, D-40TA) (Takeda Chemical, Osaka) is a triazolo benzodiazepine hypnotic agent (Momose et al., 1976; Isozaki et al., 1976) (Fig. 1). The present study was undertaken to determine characteristics of absorption and elimination of estazolam following a single oral dose, as well as the pattern of estazolam accumulation during 3 weeks of therapy. The effect of estazolam treatment on subjects' drug metabolizing capacity was also tested by measurement of antipyrine clearance before and after 2 weeks of estazolam treatment.

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Materials and Methods

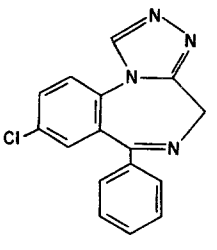
Overall Design. The study consisted of 1) a single-dose estazolam kinetic study lasting 72 h; 2) a multiple- dose kinetic study, consisting of 21 consecutive days of estazolam ingestion, followed by a 4-day wash-out period; and 3) two single-dose studies of antipyrine kinetics, each lasting 24 h, performed before and within 24 h after 14 consecutive days of estazolam ingestion.

The study was performed at the Quincy Research Center, Kansas City, Missouri, U.S.A. All plasma samples were frozen, packed in dry ice, and shipped to the Clinical Pharmacology Unit at Massachusetts General Hospital for laboratory analysis.

Single-Dose Estazolam Kinetics. Twenty-one healthy male volunteers ranging in age from 22–53 years (mean 37.4 years) participated in this part of the study (Table 1). After an overnight fast, subjects ingested either 2, 4, 6, 8, 12, or 16 mg estazolam with water. Venous blood samples were drawn prior to dosing and at 0.5, 1.0, 1.5, 2.0, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 60, and 72 h after dosing.

Multiple-Dose Estazolam Kinetics. Estazolam was administered to six healty male volunteers (Table 2) as a single daily dose at the same time of day for 21 consecutive days. Their ages ranged from 18–51 years (mean 37.0 years). Three of these subjects (numbers 02, 05, and 29) had previously participated in the single-dose study. Doses administered were: Days 1–7, 2 mg; days 8–14, 4 mg; and days 15–21, 6 mg. A venous blood sample was drawn just prior to the dose on each treatment day. On days 1, 8, 15, and 21, additional samples were drawn 0.5, 1.0, 1.5, 2.0, 2.5, 3, 4, 6, 8, 12, and 18 h after the dose. After the final dose (day 21) blood samples were also drawn at 24, 36, 48, 60, 72, 84, and 96 h.

Study of Antipyrine Kinetics. Estazolam (2 mg) was ingested nightly for 14 consecutive nights by six healthy male subjects (Table 3). None



ESTAZOLAM

Fig. 1. Structural formula of estazolam

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Table 1. Subject characteristics and single dose pharmacokinetics of estazolam

Subject number	Age	Weight (kg)	Estazolam dose (mg)	Maximum plasma concentration (ng/ml)	Time of maximum concentration (h after dose)	Absorption half-life (min)	Elimination half-life (h)
2	22	107	2	101	0.5	0.25	21.1
3	29	64	2	93	2.0	33.8	11.6
4	26	80	2	75	6.0	36.2	31.2
5	42	89	2	75	2.0	–[a]	17.5
8	45	86	4	157	0.5	0.6	16.6
11	22	90	4	188	4.0	–[a]	24.9
13	27	49	4	213	2.5	13.2	9.0
15	27	67	4	170	0.5	03	10.5
17	44	109	6	190	6.0	13.0	24.5
24	44	99	6	152	2.5	23.4	23.6
27	44	73	6	243	6.0	7.5	20.1
29	51	56	6	394	1.0	9.3	11.9
22	29	74	8	271	3.0	7.5	12.5
37	35	88	8	332	2.5	39.0	19.5
40	53	68	8	348	0.5	0.2	20.7
41	33	58	12	745	6.0	70.7	8.3
49	53	65	12	664	0.5	0.1	17.2
50	24	58	16	839	2.5	44.1	13.0
53	32	82	16	656	6.0	–[a]	16.8
55	52	85	16	465	0.5	≈ 0	15.0
58	52	71	16	579	1.0	8.8	13.9
					Mean ± SEM	17.1 ± 4.7	17.1 ± 1.3

[a] Absorption pattern not explained by a first order process

of these subjects had participated in the previous single- or multiple-dose studies. They ranged in age from 29 – 45 years (mean 38.5 years). Prior to the first dose, and within 24 h of the final dose of estazolam, a sterile aqueous solution of antipyrine (200 mg/ml) was given IV by a constant-rate infusion pump over a period of 10 min. The dose of antipyrine was 20 mg/kg body weight. Venous blood samples were obtained prior to the infusion, at the end of the infusion, and at 0.25, 0.5, 1.0, 2, 3, 4, 6, 8, 12, 18, and 24 h after infusion.

Analysis of Body Fluids. Concentrations of estazolam were determined by electron capture gas-liquid chromatography (Greenblatt, 1978a, b; Greenblatt et al., 1978) after the addition of either flurazepam or desmethyldiazepam as an internal standard. Plasma samples were buffered to pH 9.0 using borate buffer (de Silva and Puglisi, 1970), then extracted twice with 10 ml of benzene/hexane (50:50). The combined extracts were evaporated to dryness and redissolved in 50 µl of toluene (containing 15% isoamyl alcohol), of which 1 – 3 µl was injected into the chromatograph. Unter conditions described previously (Greenblatt, 1978a, b; Greenblatt et al., 1978), approximate retention times were, for desmethyldiazepam, 3.0 min, for flurazepam, 4.1 min, and for estazolam, 9.0 min (Fig. 2).

Standard curves were prepared daily using known concentrations of estazolam. The peak height ratio (estazolam to internal standard) was plotted versus the added concentrations of estazolam (Fig. 3). The slope of this curve, determined by linear regression analysis, was used to calculate estazolam concentrations in unknown samples.

The method has a sensitivity limit of 5.0 ng/ml or better. The coefficient of variation for identical samples containing 25 ng/ml is 11% or less, and the recovery is approximately 100%. A hydroxylated biotransformation product of estazolam (Kanai, 1974), identified as the major endogenous human metabolite, did not yield an identifiable chromatographic peak. Hence, the method provides no information on plasma concentrations of metabolic products.

Plasma antipyrine contentrations were determined by spectrophotometric assay (Brodie et al., 1949; Greenblatt and Locniskar, 1979).

Analysis of Data. Plasma estazolam concentrations following single doses were analyzed by weighted iterative nonlinear least-squares regression techniques (Greenblatt et al., 1977, 1979a, b). Data points were fitted by computer (Marquardt, 1963; Usanis, 1972) to the following two funtions: $C = B(e^{-\beta t} - e^{-k_a t})$ (equation 1) and $C = -(A + B)e^{-k_a t} + Ae^{-\alpha t} + Be^{-\beta t}$ (equation 2). C is the plasma estazolam concentration at time t after dosage. In equation 1, B is a hybrid intercept term, k_a is the apparent first-order absorption rate constant, and β is the apparent first-order elimination rate constant. In equation 2, A and B are hybrid intercept terms, k_a has the same meaning as in equation 1, and the exponents α and β are hybrid quantities representing rate constants for distribution and elimination, respectively. The choice between equations 1 and 2 as functions of best fit was based upon the scatter of actual data points about the fitted function, and by comparison of weighted residual errors (Boxenbaum et al., 1974). Apparent first order half-lives for drug absorption ($t_{1/2a}$) and elimination ($t_{1/2\beta}$) were calculated from their respective rate constants.

For the three subjects who participated in both the single- and multiple-dose kinetic studies, appropriate linear combinations of the fitted function from the single-dose trial were used to predict plasma concentrations during the multiple-dose study (Greenblatt, 1979; Gibaldi and Perrier, 1975; Wagner, 1975), assuming dose-independent kinetics and linear superimposability of individual doses. Oberved accumulation ratios were calculated as the area under the 24-h plasma concentrations curve (AUC) at steady state (day 21)

Table 2. Multiple-dose pharmacokinetics of estazolam

	Subjects					
Number	02	05	29	15C	16C	18C
Age (years)	22	43	51	38	18	50
Weight (kg)	107	91	56	70	77	78
Days 3–8 (2 mg)						
Dose (mg/kg)	0.019	0.022	0.036	0.029	0.026	0.026
Mean predose plasma concentration (ng/ml)	40.3	28.0	27.8	20.9	25.0	25.5
(%CV)[a]	(20.9)	(14.2)	(4.2)	(12.1)	(21.7)	(33.7)
Days 10–15 (4 mg)						
Dose (mg/kg)	0.037	0.044	0.071	0.057	0.052	0.051
Mean predose plasma concentration (ng/ml)	95.3	77.6	83.7	33.8	66.7	78.0
(%CV)[a]	(10.4)	(9.7)	(14.4)	(14.6)	(7.7)	(15.0)
Days 17–21 (6 mg)						
Dose (mg/kg)	0.056	0.066	0.107	0.086	0.078	0.077
Mean predose plasma concentration (ng/ml)	142.8	157.3	148	68.4	98.6	145.5
(% CV)[a]	(11.4)	(17.6)	(14.2)	(11.8)	(12.3)	(11.7)
Wash-out half-life (h)	17.6	16.8	12.7	15.7	15.4	12.4
Single-dose $t_{1/2\beta}$ (h)	21.1	17.5	11.9	–	–	–
Predicted accumulation ratio	1.83	1.62	1.53	1.51	1.51	1.35
Observed accumulation ratio	2.02	1.89	1.91	1.37	1.56	2.30

[a] Coefficient of variation (SD divided by mean, expresed in percent)

Table 3. Pharmacokinetics of IV antipyrine before and after estazolam treatment

Subjects			Antipyrine kinetics					
Number	Age (years)	Weight (kg)	Elimination half-life (h)		Volume of distribution (l/kg)		Total clearance (ml/min/kg)	
			Before	After	Before	After	Before	After
AP-1	45	64	5.34	7.97	0.62	0.72	1.34	1.05
AP-2	43	64	6.21	5.23	0.98	0.71	1.90	1.57
AP-5	36	67	6.93	6.45	0.56	0.59	0.93	1.06
AP-6	42	62	14.59	7.99	0.57	0.57	0.45	0.82
AP-9	29	80	6.89	7.65	0.52	0.54	0.88	0.81
AP-12	36	72	10.88	8.84	0.63	0.68	0.67	0.89
Mean ± SEM			8.47 ± 1.44	7.35 ± 0.52	0.64 ± 0.06	0.63 ± 0.03	1.02 ± 0.21	1.03 ± 0.11
Paired *t*-test			0.87 (NS)		0.21 (NS)		−0.04 (NS)	

divided by the 24 h AUC after the first dose, with appropriate adjustment made for the differences in doses (2 mg on day 1 and 6 mg on day 21). These ratios were compared to those predicted based upon $t_{1/2\beta}$ observed during the single-dose study, or the wash-out half-life observed after termination of chronic dosage (Greenblatt, 1979; Gibaldi and Perrier, 1975; Wagner, 1975; Greenblatt and Koch-Weser, 1975).

Plasma antipyrine concentrations were analyzed using the one-compartment open model approximation. The terminal log-linear portion of each plasma concentration curve was fitted by least-

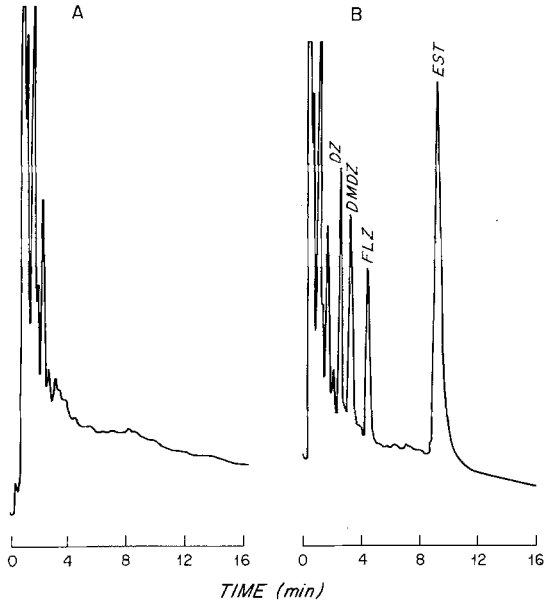

Fig. 2. A Chromatogram of a drug-free control plasma extract. **B** The same plasma sample to which was added diazepam (DZ) (25 ng/ml), desmethyldiazepam (DMDZ) (25 ng/ml), flurazepam (FLZ) (25 ng/ml), and estazolam (EST) (100 ng/ml)

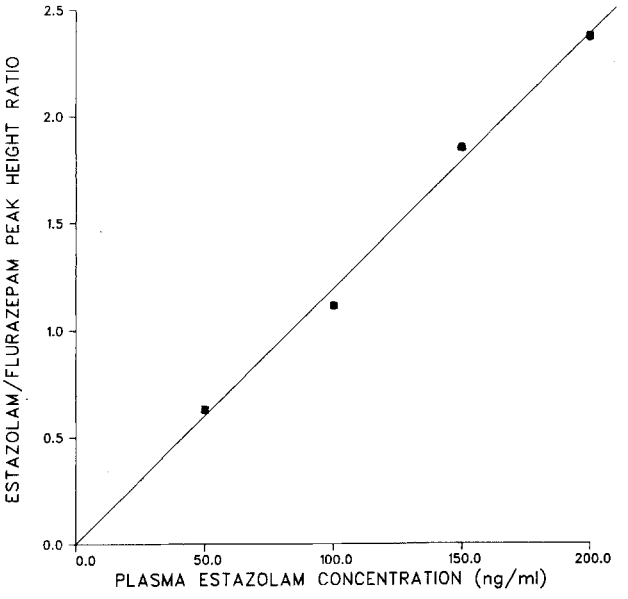

Fig. 3. Calibration curve showing relation of estazolam plasma concentration to estazolam: flurazepam peak height ratio

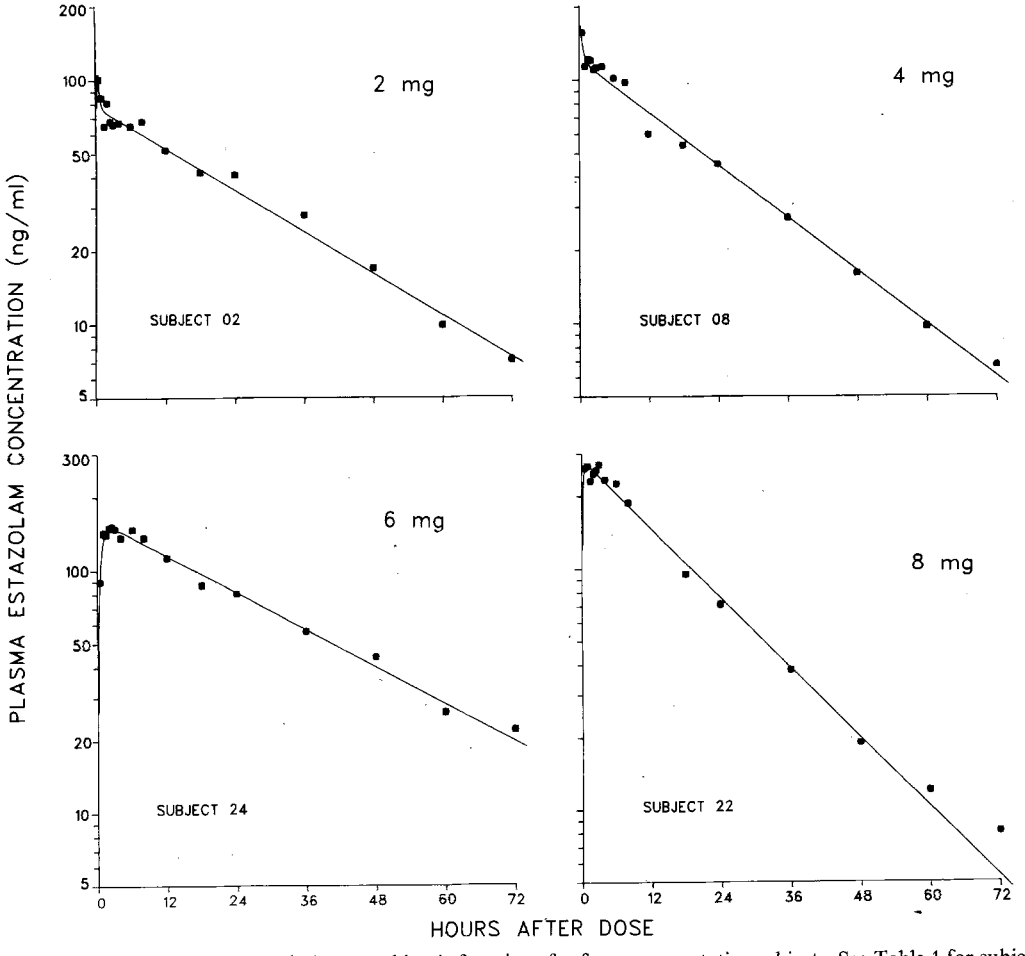

Fig. 4. Plasma estazolam concentrations and pharmacokinetic functions for four representative subjects. See Table 1 for subject identification and kinetic analysis. Note that subject 02 also participated in the multiple-dose study

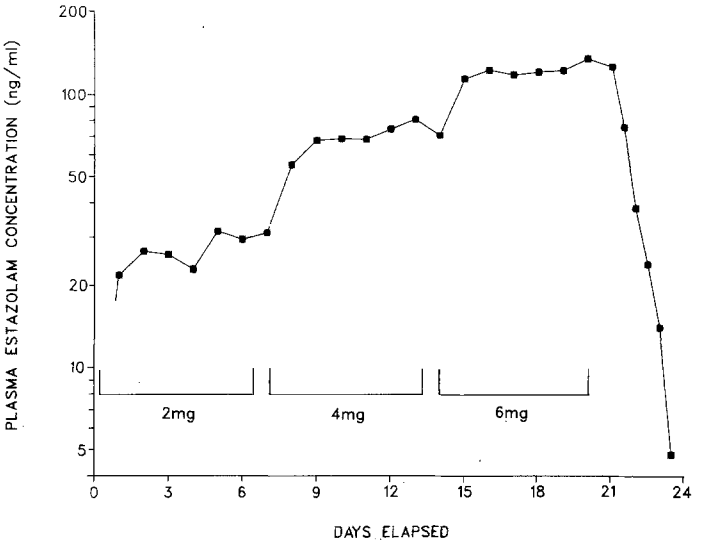

Fig. 5
Predose plasma estazolam concentrations during and after the multiple-dose study. Each point is the mean for all six subjects at the corresponding time. Standard errors, omitted for clarity, are available upon request from the authors

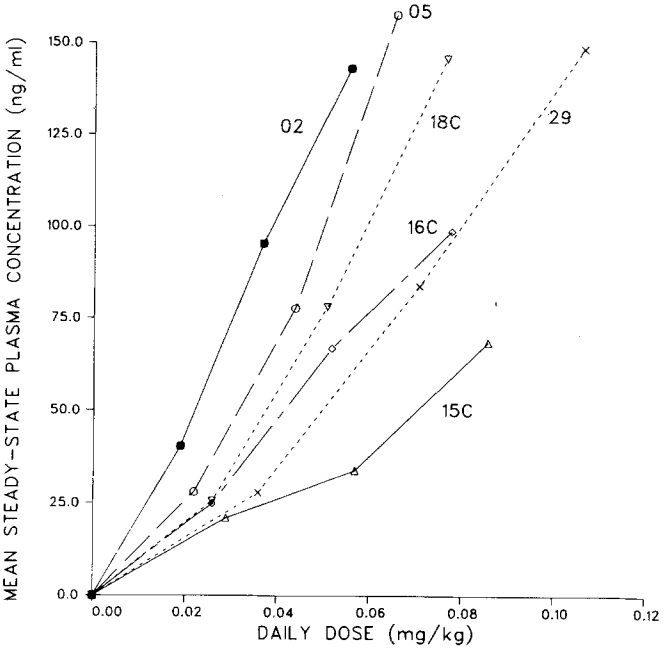

Fig. 6
Relation of daily dose (mg/kg) to mean steady-state plasma estazolam concentrations during the multiple-dose study. See Table 2 for complete analysis

squares regression analysis to a function of the form $C = Be^{-\beta t}$ (equation 3), where C is the plasma antipyrine concentration at time t after the end of the infusion. B is the extrapolated intercept term, which was corrected for the infusion period (Loo and Riegelman, 1970); β is the apparent first order elimination rate constant. The dose, the corrected intercept, and β were used to calculate the elimination half-life ($t_{1/2\,\beta}$), volume of distribution (V_d), and total clearance.

Differences in kinetic variables for antipyrine between the two trials were assessed using Student's t-test.

Results

Single-Dose Kinetics. Peak plasma concentrations of estazolam were reached within 6 h of dosage in all 21 subjects (Table 1, Fig. 4). The mean $t_{1/2a}$ was 17.1 min, but varied considerably among subjects. Values of $t_{1/2\,\beta}$ ranged from 8.3 – 31.2 h (mean 17 h). One-way analysis of variance indicated that the elimination half-life was not dependent upon dose ($F = 0.84$, $df = 5,15$).

Multiple-Dose Kinetics. Steady-state plasma estazolam concentrations increased approximately in proportion to the increasing dose (Table 2, Figs. 5 and 6). The predicted accumulation ratio averaged 1.53. In four of the six subjects, predicted and observed ratios were essentially identical (Table 2); in the other two, observed accumulation exceeded that predicted. The overall mean observed ratio (1.84) was larger than the pre-

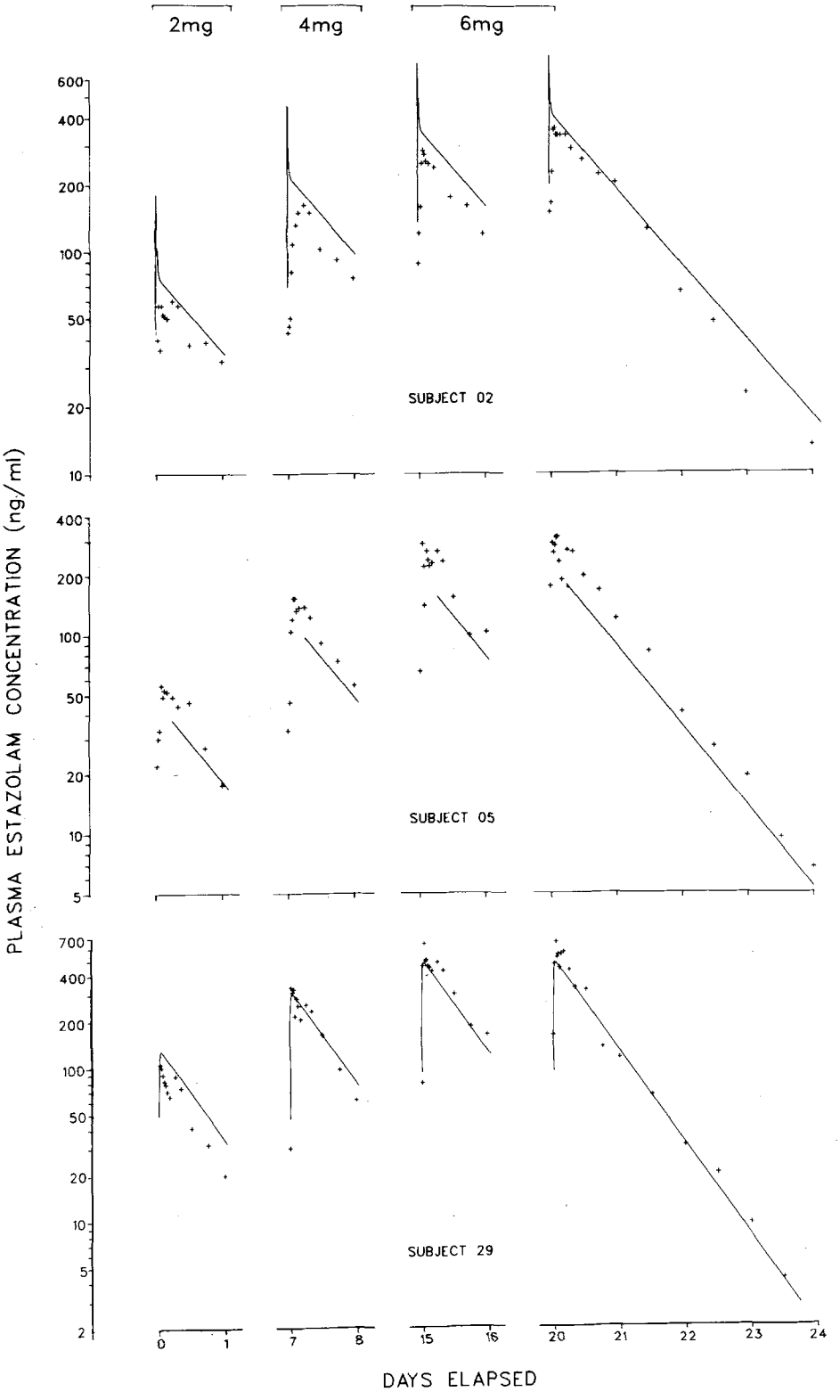

Fig. 7
Plasma estazolam concentrations, and predicted functions based upon the single-dose study, in the three subjects who participated in both single- and multiple-dose studies. For subject 05 only the terminal log-linear portion of the single-dose plasma concentration profile was used for prediction. See Table 1 and 2 for complete analysis

dicted ratio, but the difference did not reach significance (paired $t = 2.02$).

Figure 7 shows actual data points and functions predicted from the single-dose trial in the three subjects who participated in both of these studies.

Antipyrine Kinetics. The mean $t_{1/2\,\beta}$ for antipyrine following 2 weeks of estazolam ingestion was slightly shorter than before treatment, but the difference was not significant (Table 3, Fig. 8). V_d and total clearance were essentially identical between the two trials, sug-

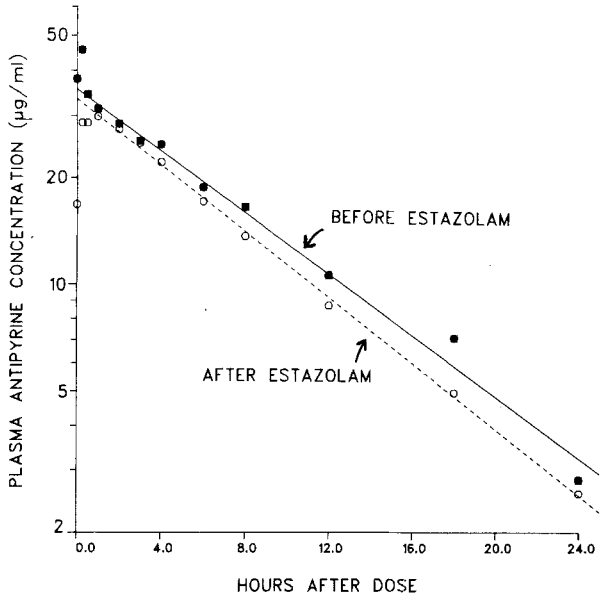

Fig. 8. Plasma antipyrine concentrations and pharmacokinetic functions in subject AP-5 before and after 2 weeks of estazolam treatment. See Table 3 for complete analysis

gesting that 2 weeks of treatment with therapeutic doses of estazolam has no important effect upon the distribution, elimination, or clearance of antipyrine.

Discussion

The single-dose kinetic profile of estazolam suggests that absorption of the compound following oral dosage is reasonably rapid. Peak concentrations were attained within 6 h in all subjects, with values of $t_{1/2\,a}$ averaging 17 min. The mean $t_{1/2\,\beta}$ for estazolam averaged 17 h, which is similar to that of lorazepam (Greenblatt et al., 1979a, b). Thus, the elimination kinetics of estazolam can be classified as intermediate, with values of $t_{1/2\beta}$ larger than those of the rapidly eliminated benzodiazepines (such as oxazepam), but shorter than those of the long-acting compounds (such as diazepam, desmethyldiazepam, or desalkylflurazepam) (Greenblatt and Shader, 1978).

The multiple-dose kinetic profile established the rate and extent of estazolam accumulation. Consistent with the observed values of $t_{1/2\,\beta}$ after single doses, accumulation was essentially complete within 3 days after starting therapy or changing the dose. Observed and predicted accumulation ratios did not differ significantly for the group as a whole, and, in four of six subjects, observed and predicted values were nearly identical. The mean steady-state plasma concentration was highly correlated with dose, further suggesting that pharmacokinetics of estazolam are dose-independent. Finally, $t_{1/2\,\beta}$ values following termination of 21 days of therapy were similar or identical to those observed after a single dose. It should be emphasized, however, that our study provides no information on the pharmacokinetics of estazolam metabolites that might have pharmacologic activity (Kanai, 1974).

Clearance of exogenously administered antipyrine is commonly used an index of hepatic drug-hydroxylating capacity. As in a previous study of lorazepam (Greenblatt et al., 1979a), 2 weeks of estazolam had no significant influence on the kinetics of a single dose of antipyrine. This suggests that chronic treatment with estazolam neither stimulates nor inhibits enzyme activity in humans. The similar values of estazolam $t_{1/2\beta}$ following single- and multiple-dose therapy also suggests that estazolam neither stimulates nor inhibits its own elimination. In most clinical studies, benzodiazepine therapy has essentially no effect on hepatic enzyme activity (Greenblatt and Shader, 1974), though antipyrine clearance in humans was impaired by 7 days of prazepam therapy (Vesell et al., 1972).

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