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Pharmacokinetics of Ethanol in Pigtailed Macaques: Intersubject Variability and Effect of Subchronic Administration

THOMAS F KALHORN, DOUGLAS M BOWDEN AND JOHN T SLATTERY[1]

Regional Primate Research Center and Departments of Anesthesiology, Pharmaceutics, Psychiatry and Behavioral Sciences and Pharmacology, University of Washington, Seattle, WA 98195

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KALHORN, T F , D M BOWDEN AND J T SLATTERY *Pharmacokinetics of ethanol in pigtailed macaques Intersubject variability and effect of subchronic administration* PHARMACOL BIOCHEM BEHAV 24(3) 485–489, 1986 —The pharmacokinetics of IV ethanol (0 6 g/kg) were examined in 11 male colony-bred pigtailed macaques *(Macaca nemestrina)* aged 3 to 13 years The animals were either chaired during blood sampling (4 hr) or connected to a tether system that allowed injections and blood sampling while the animal moved freely about its cage In all instances, ethanol pharmacokinetics could be described by a single Michaelis-Menten function, inclusion of a parallel first-order rate constant to account for non-alcohol dehydrogenase elimination of ethanol did not improve the fit Volume of distribution was 0 802±0 054 L/kg (mean±SD), K_m (the apparent *in vivo* Michaelis constant) was 0 063±0 022 μg/ml, and V_{max} was 0 199±0 039 g/kg/hr The pharmacokinetic parameter values of chaired and tethered monkeys did not differ Three of the tethered monkeys received 3 g/kg of ethanol daily for two weeks by IV infusion (subchronic administration) Ethanol pharmacokinetics, determined on five occasions before and five occasions after subchronic ethanol administration, showed that the treatment did not alter the volume of distribution or K_m in any of the three monkeys The value of V_{max} increased approximately 23% in one of the three monkeys that received subchronic ethanol, this increase may have been due to a single, inadvertent administration of a 4 5-g/kg dose over a 20-min period V_{max} did not change in the other two monkeys

Ethanol Monkeys Pharmacokinetics Enzyme induction

NONHUMAN primates provide culture-free models in which to test hypotheses regarding the influence of ethanol on social behavior Dose-related changes in both solitary and social behavior have been observed in several studies of alcohol consumption in primate subjects [1–4, 8, 9, 14, 16, 19, 22] Ataxia, impairment of operant performance, and increased social grooming, social play, sexual activity, and passive-submissive behavior have commonly been described Increased levels of aggressive behavior have also been reported in some cases

Because the blood level of ethanol cannot be determined without disrupting the socio-emotional context in which behaviors of interest are to be observed, investigators have been unable to test directly for correlations between blood level and behavioral state in monkeys that were allowed to consume ethanol voluntarily while living in a social group We have used the pigtailed macaque model originally developed by Elton and colleagues [5,6] to study the effects of social factors on voluntary alcohol consumption [11] Our modification [12,26] of Elton's apparatus, which provides minute-by-minute, round-the-clock drinking records from individual animals living in social groups, may offer potential for testing for correlations between level of blood alcohol and social state If an animal's minute-to-minute ethanol consumption could be translated into a reliable minute-to-minute estimate of blood ethanol concentration for several hours during which behavior is recorded, tests for such correlations would be possible A mathematical model based on the pharmacokinetics of ethanol in the pigtailed macaque might provide such a running estimate

Ethanol elimination in man has been described successfully by a mathematical model consisting of a single Michaelis-Menten process [27,28] The feasibility of using a simulation model for on-line calculation of blood levels in the monkey would depend on the stability within subjects and comparability among subjects in the pharmacokinetic parameters that govern the elimination of ethanol from the circulation

The present study addressed two issues First, we assessed the pharmacokinetics of ethanol in pigtailed

[1]Requests for reprints should be addressed to John T Slattery, Ph D , Department of Pharmaceutics BG-20, University of Washington, Seattle, WA 98195

macaques under conditions that have been typical in our studies of voluntary consumption We focused on intrasubject and intersubject variability in the major pharmacokinetic parameters apparent volume of distribution, K_m, the concentration at which the rate of ethanol elimination is one-half the maximum possible, and V_{max}, the maximal rate of elimination Second, inasmuch as chronic ethanol ingestion in large doses is known to cause enzyme induction and accelerated elimination in virtually all species tested [17], we addressed the question of whether subchronic exposure to ethanol in amounts equal to the higher voluntary intake levels encountered in our studies (3 g/kg/day) alters the pharmacokinetic parameters

METHOD

Subjects were 11 male colony-bred pigtailed macaques (*Macaca nemestrina*), 3 to 13 years of age, weighing 4 to 12 kg A wide age and weight range was selected to match the variability ordinarily encountered in studies of social behavior Six animals were studied under temporary chair restraint, the animal was anesthetized with 10 mg/kg ketamine IM and placed in the chair, and an indwelling catheter was placed in one saphenous vein Ethanol was administered one hour after the animal recovered from anesthesia The other five animals were studied in their home cages (1×1×1 3 m), where a vest-and-tether device allowed them to move about the cage during measurements The tether, connected to a swivel outside the cage, carried a cannula that was chronically implanted through either a jugular vein or femoral vein with the tip in the vena cava [13,20]

The animals received Purina Monkey Chow and water ad lib We showed in a previous study that food does not alter the pharmacokinetics of ethanol administered IV [13] Ethanol was administered at 0900 hr in all studies For the determination of intersubject variability in the pharmacokinetics of ethanol, a single 0 6-g/kg (20% v/v) dose of ethanol was administered IV as a bolus through the sampling catheter, and was followed by a 10-ml rinse of heparinized (1 μl/ml) saline Catheter patency was maintained with a constant infusion of 15 ml of heparinized saline per hour Preliminary studies showed that this procedure did not introduce artifacts into the determination of ethanol concentrations

The effect of subchronic administration of ethanol on its pharmacokinetics was determined in three of the tethered monkeys Baseline studies were carried out on five successive days Each day, 0 6 g/kg of 20% v/v ethanol was administered IV and serial blood samples were obtained as described above The animals then received IV infusions of ethanol by syringe pump (0 5 g/kg/hr in 8 ml saline for two 3-hr periods separated by 3 hr for a total of 3 g/kg) each day for 14 days This regimen corresponded to the highest rate of spontaneous ingestion by monkeys in our previous behavioral experiments [11] Then determinations of pharmacokinetic parameter values were repeated over another four days as in the baseline studies

Blood samples (0 75 ml) were collected serially for 4 hr at intervals of 15 to 30 min They were anticoagulated with 1 0 mg EDTA and stored on ice until the end of sampling Blood was then centrifuged at 12,000 g for 3 min, and 100 μl of plasma was transferred to a small vial containing 0 4 ml of 400 μg/ml n-propanol

The samples were refrigerated until analyzed for plasma concentration by gas chromatography (within 72 hr) One

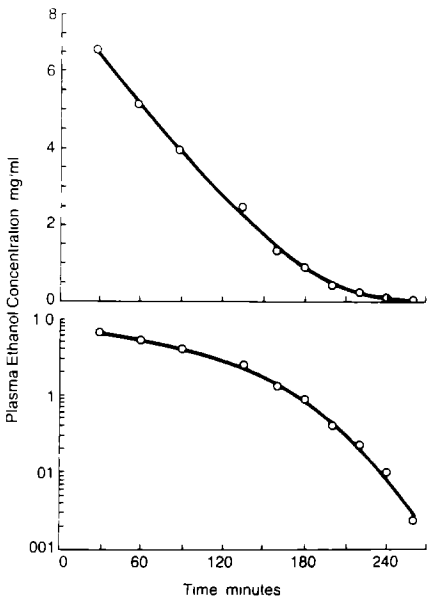

FIG 1 Time course of ethanol concentration in plasma following an IV dose (0 6 g/kg) to monkey 208, plotted on linear (top) and semilogarithmic (bottom) coordinates

microliter was injected into a Varian Aerograph Series 1400 GC equipped with a flame ionization detector and a 1 8×2 mm silanized glass column packed with 5% carbowax on 60/80 mesh Carbopak (Sulpeco) Temperatures were 160°, 93°, and 200°C for the injector, column and detector, respectively Column eluent was quantified by a Hewlett-Packard 3390A recording integrator operating in the peak height mode Response was linear from 4 to 3000 μg/ml plasma, coefficient variation was less than 5%

Pharmacokinetic parameter values were determined with the MKMODEL program [10] of the PROPHET computer system (Bolt, Beranek and Newman, Inc , Cambridge, MA) Values of C_0, V_{max} and K_m were determined from the IV data by fitting the equation

$$dC/dt = C_0 - (V_{max} * C/(K_m + C))$$

where C is ethanol concentration at time t, V_{max} is the maximum velocity of elimination, and K_m is the apparent *in vivo* Michaelis constant C_0 is the concentration at time zero extrapolated from the linear portion of the plot of concentration versus time, i e , the initial rapid drop of ethanol concentration, presumed to be a distributional phase, was disregarded in obtaining estimates of V_{max} and K_m Volume of distribution was determined by dividing C_0 by dose

RESULTS AND DISCUSSION

The time course of change in ethanol concentration following administration of the standard dose (0 6 g/kg) to a representative monkey is presented in Fig 1 The concentration declines at a constant rate until it approaches 0 2 mg/ml, at which point the kinetics exhibit more of a first-order character Substantial data have been accumulated to show that ethanol is oxidized to acetaldehyde by several enzyme systems, alcohol dehydrogenase (ADH), catalase,

TABLE 1

PHARMACOKINETICS OF ETHANOL IN MALE PIGTAILED MACAQUES

Monkey Number	Weight kg	Volume of Distribution L	Volume of Distribution L/kg	K_m mg/ml	V_{max} g/hr	V_{max} g/kg hr
003	4 2	3 52	0 839	0 043	0 71	0 169
479	4 2	3 31	0 793	0 052	0 76	0 181
208*	4 5	3 54	0 786	0 053	1 10	0 232
369*	4 5	4 10	0 912	0 026	0 90	0 201
029	5 1	4 44	0 870	0 068	1 31	0 258
329*	5 4	4 22	0 782	0 064	1 31	0 243
023	6 5	4 76	0 732	0 097	1 01	0 156
375	7 1	5 50	0 775	0 102	1 27	0 179
267	7 6	5 99	0 788	0 068	1 44	0 184
038*	10 3	8 36	0 812	0 062	2 34	0 245
066*	12 7	9 31	0 733	0 062	1 83	0 144
Mean		5 19	0 802	0 063	1 27	0 199
SD		1 99	0 054	0 022	0 48	0 039
Coefficient of Variation		38 4%	6 77%	34 3%	37 7%	19 7%

*Tethered monkeys, all others were chaired Parameter values were not significantly different between chaired and tethered monkeys, t-test, $0.15<p<0.9$

and a microsomal ethanol oxidizing system (MEOS) all contribute to the elimination of ethanol [15, 18, 21, 23–25, 28] ADH accounts for the majority of ethanol elimination, but has a K_m substantially lower than that of the other systems [28] We found, however, that the fit of the data to a function that described the decline of ethanol concentration with a single Michaelis-Menten term was not improved by adding a first-order term to represent the contribution of enzyme systems other than ADH In fact, the fitted curves for each monkey were essentially identical to those assuming a single Michaelis-Menten process only

The results of the intersubject variability studies are summarized in Table 1 The value of V_{max} agrees with the value determined by others in this species [7] Pharmacokinetic parameter values did not differ between chaired and tethered monkeys The coefficients of variation for volume of distribution and V_{max} were quite large As might be expected, however, the values of these parameters correlated positively with body weight (r^2 for volume of distribution=0 98, for V_{max}=0 71), and when parameter values were expressed relative to body weight, their coefficients of variation were remarkably low (6 73% for volume of distribution, 19 6% for V_{max}) The large coefficient of variation for K_m (34 3%) was not reduced by reference to body weight

Even though the coefficients of variation for volume of distribution and V_{max} are small, it might seem that the relatively large coefficient of variation for K_m would confound attempts to predict ethanol concentration given an administration history However, if the ethanol concentration exceeds 0.5 mg/ml when ethanol is administered in a given study, the interanimal variability in the value of K_m would not substantially affect the plasma concentrations observed This fact is illustrated in Fig 2, which shows the results of simulations carried out with mean values of V_{max} and volume of distribution, and the extreme values of K_m listed in Table 1

The effects of subchronic administration of ethanol daily

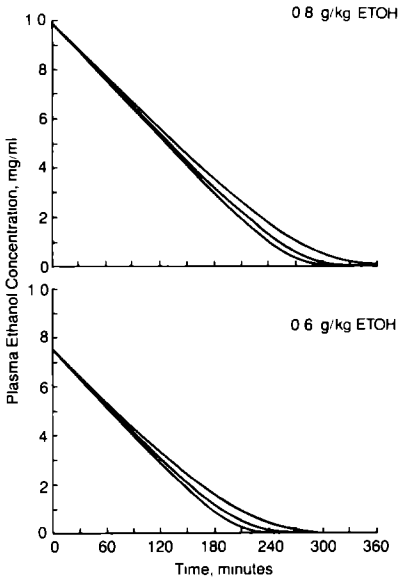

FIG 2 Simulated time course of ethanol concentration in plasma following 0 8 g/kg (top panel) and 0 6 g/kg (bottom panel) K_m=0 1 mg/ml (upper line), 0 06 mg/ml (middle line) and 0 04 mg/ml (lower line) V_{max}=0 25 g/kg/hr, volume of distribution=0 80 L/kg

for two weeks at doses similar to those ingested spontaneously in behavioral studies are represented in Table 2 Values of K_m and volume of distribution did not change from the preexposure to the postexposure determination in any monkey The value of V_{max} was unchanged in two monkeys, but increased by 23% in monkey 329 Owing to a malfunction of the infusion pump and timing device, this monkey received a

TABLE 2

PHARMACOKINETIC PARAMETER VALUES BEFORE AND AFTER INTENSIVE EXPOSURE TO ETHANOL*

Monkey Number	Weight, kg		Volume of Distribution L/kg		K_m, mg/ml		V_{max}, g/kg hr	
	Before	After	Before	After	Before	After	Before	After
038	10 3	11 5	0 812	0 790†	0 062	0 059†	0 244	0 221†
			±0 056	±0 028	±0 004	±0 011	±0 020	±0 007
			(6 85)	(3 59)	(6 20)	(19 3)	(8 35)	(3 02)
329	5 4	5 8	0 800	0 816†	0 064	0 072†	0 243	0 298‡
			±0 080	±0 013	±0 015	±0 016	±0 012	±0 034
			(10 0)	(1 65)	(23 0)	(22 7)	(4 84)	(11 3)
208	4 5	4 5	0 786	0 757†	0 053	0 041†	0 245	0 232†
			±0 033	±0 038	±0 014	±0 012	±0 038	±0 017
			(4 15)	(4 97)	(26 9)	(28 8)	(15 4)	(7 44)

*Data are mean, standard deviation, and, in parentheses, coefficient of variation in %, n=5 except for animal 038 (n=4)
†Not significantly different from before, $p>0.05$
‡Significantly different from before, $p<0.05$

dose of approximately 4 5 g/kg over a 20-min period on day 10 of the subchronic administration regimen The monkey was comatose after this dose, it received glucose for hypoglycemia and was fully recovered two days later, at which time ethanol administration was resumed for four days

The results suggest that 3 g/kg/day ethanol administered over a two-week period does not increase the clearance of the drug However, a single large dose within this regimen apparently can increase clearance While enzyme induction has not previously been reported following a single dose of ethanol in primates, MEOS activity is stimulated in mice by a single injection of ethanol (4 g/kg) [23]

Enhanced ethanol metabolism due to repeated high-dose ethanol administration has been clearly demonstrated by other studies in humans and nonhuman primates The clearance of ethanol in nonjaundiced alcoholics has been reported to be 70% [18] to 100% [15] greater than that of nondrinking control subjects and jaundiced alcoholics The enhanced clearance in alcoholic patients has been shown to involve greater activity of an NADPH-dependent ethanol oxidizing system (presumably MEOS), ADH activity does not differ between alcoholics and healthy controls [18] Other studies in nonhuman primates have produced results compatible with those in humans [21, 24, 25] The dose used in those studies was quite large (as much as 50% of total caloric intake for two to seven years) [21,25] and resulted in an approximately 34% increase in ethanol clearance, which was attributed to increased MEOS activity [21]

The results of our study of subchronic exposure do not support the conclusions drawn by Elton *et al* [7] from their study of chronically exposed monkeys They found a higher value of V_{max} in a pigtailed macaque that drank an average of 3 1 g/kg/day for 230 days than in a monkey that drank 2 15 g/kg/day over the same period and in two monkeys that were not exposed to ethanol The differences ranged from 6% when the high-dose animal was compared with one of the naive monkeys to 25% when the high-dose monkey was compared with the other exposed animal The authors attributed those differences to induction Either the period of treatment used in our study was insufficient to cause induction in the two monkeys that received ethanol according to our protocol, or the higher value of V_{max} observed in the earlier study represented intersubject variability rather than induction The latter interpretation appears more likely, given the range of values of V_{max} presented in Table 1

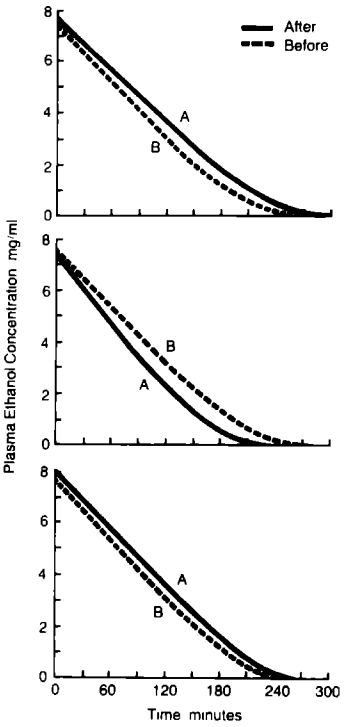

FIG 3 Simulated time course of ethanol concentration in plasma following 0 6 g/kg to monkeys 038 (top), 329 (middle) and 208 (bottom), before (B) and after (A) subchronic administration of ethanol

Enhanced clearance of ethanol is relevant to behavioral studies only insofar as the effect on ethanol concentration is

significant in a behavioral context To aid the behavioral scientist in assessing the implications of the results of this study, the simulated time course of plasma ethanol concentration using the mean parameter values before and after ethanol exposure for each of the three monkeys in our study (Table 2) is shown in Fig 3 In the two monkeys that showed no changes in parameter values, the curves were slightly above the curve simulated on the basis of values obtained before administration of ethanol Even with a 23% increase in V_{max} after exposure to ethanol experienced by animal 329, the simulated time course of concentration was quite similar to that obtained before exposure to ethanol It remains to be determined whether repeated exposure to ethanol concentrations higher than those produced in our subchronic regimen would cause a significant degree of induction

The results of this study show that the intersubject variability of the pharmacokinetics of ethanol in colony-bred pigtailed macaques is small, except for K_m, and that the influence of K_m on estimates generated by the model in the dose-range typically encountered in behavioral experiments is minimal Subchronic exposure to ethanol in doses spontaneously ingested by monkeys in behavioral studies are insufficient to cause alterations in the pharmacokinetics of the drug, unless interspersed with occasional very large doses Thus, to the extent that the concentration/time profile depends on the pharmacokinetics of elimination, one can calculate it to an acceptable degree of accuracy from a detailed record of consumption, body weight, and a distributed input model based on Michaelis-Menten kinetics This constraint is met only when the drug is administered IV The results of a study of additional factors that can affect the absorption of orally administered ethanol are reported separately [13].

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