DOSE INDEPENDENT PHARMACOKINETICS OF CAFFEINE AFTER INTRAVENOUS ADMINISTRATION UNDER A CHRONIC FOOD-LIMITED REGIMEN

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SUMMARY

Several studies have shown that caffeine follows non-linear pharmacokinetics in both rats and humans. Recent data have demonstrated that caffeine may following linear pharmacokinetics when administered orally and intraperitoneally to food-limited rats. In this study the pharmacokinetics of caffeine was analyzed following intravenous (IV) administration to rats under a food-limited regimen. Four rats were administered four doses of caffeine and a standard dose of the caffeine metabolites, paraxanthine, theobromine, and theophylline. Caffeine pharmacokinetic parameters were dose independent following intravenous doses ranging from 1 to 20 mg/kg. Furthermore, the caffeine area under the curve (AUC) increased linearly as a function of dose. The mean fraction of caffeine converted to paraxanthine, theobromine, and theophylline was 16%, 16%, and 7%, respectively. The linear pharmacokinetics demonstrated in the present study may be attributed to the induction of hepatic metabolism under a chronic food-limited regimen.

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INTRODUCTION

Caffeine is the most commonly self-administered psychoactive agent /1/. Caffeine is found in many dietary substances and prescription as well as nonprescription products. On average, adults in North America consume 200-250 mg of caffeine per day, generally to induce wakefulness and alleviate the fatigue and boredom of performing tedious work for long periods of time /2/.

Although caffeine's mechanism of action is complex and not fully elucidated, its pharmacodynamic profile has been well described. Central nervous system, cardiac, and diuretic effects are associated with the use of caffeine. At small doses caffeine's central nervous system effects include cerebral cortex stimulation, whereas higher doses stimulate the medullary respiratory, vasomotor, and vagal centers /3/. This stimulatory effect results in improvements in alertness, mood, and fatigue. Over time caffeine has reinforcing effects resulting in habitual use, tolerance and withdrawal symptoms upon discontinuation /4/. Following caffeine administration cardiovascular effects include a small increase in blood pressure, decrease in heart rate, and cerebral artery vasoconstriction. During stress, however, caffeine can actually increase heart rate and cardiac output /5/. In addition to its CNS and cardiovascular effects caffeine is also a weak diuretic /6/.

Caffeine is metabolized by various enzymatic pathways in the liver. Its biotransformation is primarily dependent on cytochrome P450 1A2 /7/. Metabolism of caffeine yields pharmacologically active demethylated products and inactive metabolites. The metabolic routes for caffeine are markedly dependent on species. The primary metabolite of caffeine in humans and rabbits is paraxanthine (1,7-dimethylxanthine) /8/. It is estimated that, in humans, approximately 35-80% of caffeine is metabolized to paraxanthine and 6-11% and 3-4% is metabolized to theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethylxanthine), respectively /7,9/. In monkeys theophylline, and in mice theobromine, are the primary metabolites /8/. In contrast, theophylline and theobromine production are of the same order, while paraxanthine

formation is lower in the rat /8/. Caffeine also undergoes ring oxidation to form trimethyluric acid. Due to further metabolic biotransformation the primary caffeine metabolites found in the urine are 1-methylxanthine and 1-methyluric acid.

Following caffeine administration dose-dependent pharmacokinetics have been reported in rats and humans, suggesting a saturation of hepatic metabolism /9-13/. However, recent work has demonstrated that the pharmacokinetics following oral and intraperitoneal administration of caffeine in rats under a food-limited regimen are dose-independent (i.e., linear) /14/. It is unknown whether this is due to an alteration in caffeine absorption or hepatic metabolism. Therefore this study was performed to evaluate the pharmacokinetic profile of caffeine following intravenous administration in rats under a food regimen similar to the one used previously /14/.

MATERIALS AND METHODS

Animals

Four male, albino, virus-free Sprague-Dawley rats from Harlan Sprague Dawley, Inc. (Indianapolis, IN) were used. They were housed individually in a temperature-regulated room with a daily cycle of illumination from 7:00 a.m. to 7:00 p.m. They were reduced to 80% of their initial, adult free-feeding body weights (mean = 383 g; range: 380-386 g) by receiving limited daily food rations (5 g for the first day, 10 g for the next 5 days) and were then maintained at their 80% body weights by receiving a daily food supplement (range: 14-16 g). They were held at these weights for 2 weeks before the start of the experiment, and remained at these weights for the duration of the experiment. Water was continuously available in the living cages. Experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publ. No. 85-23, revised 1985).

Catheterization

Right jugular vein cannulation was performed under sterile conditions and has been described previously /15/. The proximal end of the silastic catheter was inserted into the jugular vein; the distal end of the catheter was threaded subcutaneously, and exited through a small

incision in the back of the animal. The catheter was flushed with 0.9% saline containing 20 units of heparin per ml and was sealed with fishing line when not in use. The animals were allowed to recover for at least 2 days following the jugular vein catheterization prior to drug administration.

Drug administration and blood sampling

Caffeine, theobromine, paraxanthine, and theophylline were purchased from Sigma Chemical Co. (St. Louis, MO). Caffeine was dissolved in 0.9% NaCl. Theobromine, paraxanthine, and theophylline were dissolved in 0.9% NaCl containing 0.14 N NaOH, 0.14 N NaOH, and 0.05 N NaOH, respectively. All the methylxanthines were administered intravenously, in an injection volume of 1 ml/kg body weight. The drug solution was delivered in 15 s and was followed by 0.3 ml 0.9% saline in 15 sec. The animals first received a series of various caffeine doses (1, 5, 10 and 20 mg/kg). This was followed by a second series consisting of administration of the primary caffeine metabolites (5 mg/kg of theobromine, paraxanthine and theophylline). Each dose was separated by 3-5 days in a random order within a series.

Blood samples (100 µl) from the jugular vein catheter were obtained following caffeine administration at 0.08, 0.25, 0.5, 1, 2, 4, 8, 12, 16, 24, and 28 hours post-injection. Blood samples were only obtained through the 12 hour time point following administration of each of the caffeine metabolites. In order to maintain the feeding regimen and also to avoid the effect of food on methylxanthine pharmacokinetics, we gave the drug doses 6 hours prior to the feeding time.

Determination of methylxanthines by HPLC

Serum microsample HPLC methods for the determination of caffeine and its active metabolites have been described previously /16/. The separation of caffeine was performed on a Beckman Ultrasphere C_{18} column (5 µm particle size, 150 x 2 mm I.D.). Programmable absorbance UV detectors 785A (Applied Biosystems Instruments, Foster City, CA, USA) were operated at 270 nm. The capacity factors for theobromine, paraxanthine, theophylline, β-hydroxyethyltheophylline (used as an internal standard), and caffeine were 1.31, 2.52, 2.97, 3.73, and 6.45, respectively.

PK analyses

PK analysis was performed using the SAAM II software system (SAAM Institute, 1997). Serum concentration-time profiles were analyzed using compartmental modeling (Figure 1). Serum drug concentrations were expressed in moles for the calculation of the pharmacokinetic parameters. Assessment of the goodness of fit of each proposed model to experimental data was based on the correlation matrix, residual and weighted residual plots, visual plots, and error in parameter estimation (SD) which is derived from the covariance matrix.

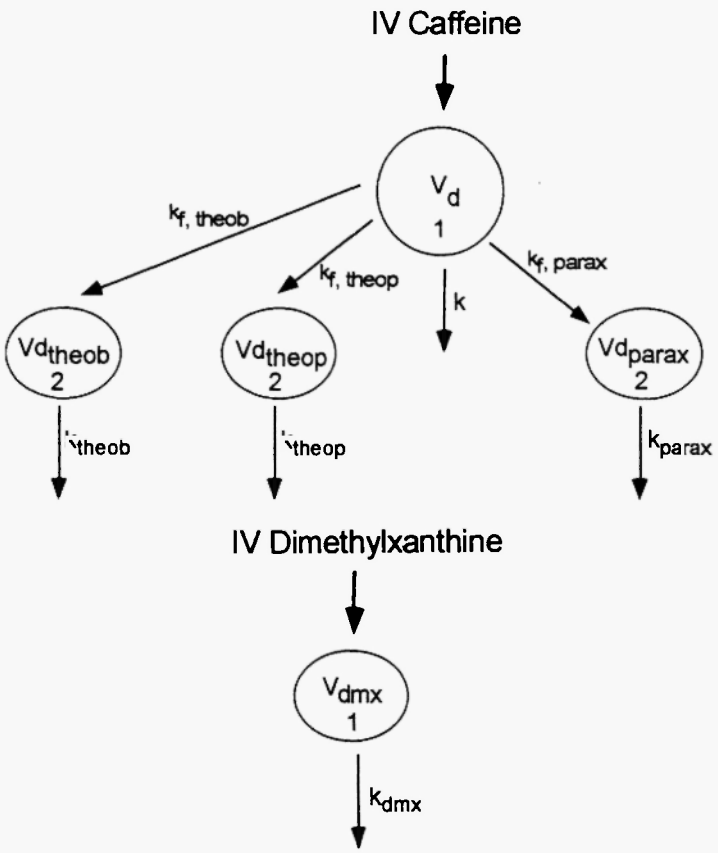

Fig. 1: Compartmental model describing disposition of caffeine and its major metabolites.

Serum caffeine, theobromine, and theophylline concentration-time profiles were analyzed using a one-compartment, open model following IV administration (Fig. 1). For each rat, the caffeine pharmacokinetic parameters (i.e., the volume of distribution, Vd, and the elimination rate constant, k) were estimated following each dose. The pharmacokinetic parameters were then estimated simultaneously for all the doses. Standard equations were used to calculate the concentration at time zero (X_0) from the values of Vd and k for each caffeine dose. The half-life ($t_{1/2}$) for the elimination phase was calculated by the following equation: $t_{1/2} = 0.693/k$. The area under the serum caffeine concentration-time curve from time 0 to infinity ($AUC_{(0-\infty)}$) and the area under the first moment of the serum caffeine concentration-time curve from time 0 to infinity ($AUMC_{(0-\infty)}$) were calculated by the following formulas: $AUC_{(0-\infty)} = X_0/k$ and $AUMC_{(0-\infty)} = X_0/k^2$. Total clearance (Cl) was then defined as dose/$AUC_{(0-\infty)}$. The mean residence time (MRT) for IV caffeine was obtained from $AUMC_{(0-\infty)}/AUC_{(0-\infty)}$.

The conversion of caffeine to the three dimethylxanthine metabolites was studied by assuming that the loss of caffeine was from the central compartment with formation constants for each dimethylxanthine (i.e., $k_{f,\ theob}$, $k_{f,\ theop}$, and $k_{f,\ parax}$). It was also assumed that the distribution and elimination properties of each dimethylxanthine metabolite did not differ from those of dimethylxanthine when it was given as a parent compound by the IV route. The fraction (Fm_{dmx}) of caffeine converted to each of the three dimethylxanthines following an IV caffeine dose was calculated according to the following equation /9/:

$$AUC_{(0-\infty)dmx} / AUC_{(0-\infty)caf} = f_{dmx} * Cl_{caf}/Cl_{dmx}$$

where $AUC_{(0-\infty)dmx}$ and $AUC_{(0-\infty)caf}$ are the AUCs for a dimethylxanthine and caffeine, respectively; and Cl_{dmx} and Cl_{caf} are the clearance values for a dimethylxanthine and caffeine, respectively.

One-way repeated measures ANOVA followed by Newman-Keuls tests using SigmaStat (Jandel, San Rafael, CA) were performed as appropriate. Metabolite pharmacokinetics following caffeine administration and separate administration were compared using Student's t-test.

RESULTS

The pharmacokinetic parameters of caffeine following four different intravenous doses are found in Table 1. Mean concentration versus time curves for caffeine and each of the major metabolites following the four doses are depicted in Figure 2. The volume of distribution and clearance were not significantly different between the various doses ($p>0.05$). The plot of caffeine AUC versus dose demonstrated that the caffeine pharmacokinetic characteristics in this study were not dose dependent (Figure 3, $r^2 = 0.9978$).

The pharmacokinetic parameters of the primary metabolites of caffeine (theobromine, paraxanthine, and theophylline) following 5 mg/kg doses of each are found in Table 2. These values were used to calculate the fraction of caffeine converted to each of the respective metabolites.

Table 3 lists the formation parameters for each of the primary metabolites after the four different intravenous doses of caffeine. Although the values did not reach statistical significance, the metabolite elimination rate constants were greater and the half-lives longer when analyzed following caffeine administration versus administration as the parent drug ($p= 0.10$). This difference is probably related to the requirement of metabolite formation following caffeine administration which can affect calculated elimination rate constants. The mean fraction of caffeine converted to theobromine, paraxanthine, and theophylline was 16%, 16%, and 7%, respectively. The fraction of caffeine converted to theophylline and theobromine did not significantly differ as the dose was increased, however, the fraction converted to paraxanthine decreased as the dose increased from 5 to 10 mg/kg ($p= 0.05$).

The psychomotor stimulant effects of caffeine observed during blood sampling were a function of the dose. There were no apparent side effects noticed at any dose.

DISCUSSION

This study was designed to investigate the dose dependence of caffeine pharmacokinetic parameters following intravenous administration. It also analyzed the formation of each of the primary caffeine metabolites. Following intravenous caffeine administration the sub-

TABLE 1

Mean pharmacokinetic parameters (± SD)
after four different i.v. caffeine doses (n = 4)

Dose (mg/kg)	**1**	**5**	**10**	**20**
Dose (μmol/kg)	5.15	25.75	51.49	102.99
Vd (l/kg)	1.95 ± 0.83	1.95 ± 0.40	1.71 ± 0.23	1.48 ± 0.12
Cl (l/hr/kg)	0.380 ± 0.056	0.290 ± 0.038	0.328 ± 0.068	0.306 ± 0.064
k (hr^{-1})	0.217 ± 0.084	0.213 ± 0.043	0.251 ± 0.034	0.251 ± 0.034
$t_{1/2}$ (hr)	3.78	3.38	2.79	2.79
$AUC_{(0-\infty)}$ (nmol*hr/ml)	13.81 ± 2.36	90.15 ± 11.39	161.93 ± 31.20	346.82 ± 63.68
MRT (hr)	14.15 ± 16.11	6.87 ± 2.11	5.31 ± 0.80	4.94 ± 0.71

TABLE 2

Mean pharmacokinetic parameters (± SD) after an IV dose for each
dimethylxanthine (n = 4)

	Theobromine	**Paraxanthine**	**Theophylline**
Dose (mg/kg)	5	5	5
Dose (μmol/kg)	27.75	27.75	27.75
Vd (l/kg)	1.21 ± 0.18	0.803 ± 0.054	0.636 ± 0.10
Cl (l/hr/kg)	0.158 ±0.016	0.278 ± 0.020	0.132 ± 0.018
k (hr^{-1})	0.141 ± 0.007	0.375 ± 0.045	0.226 ± 0.032
$t_{1/2}$ (hr)	4.92	1.87	3.12
$AUC_{(0-\infty)}$ (nmol*hr/ml)	163.77 ± 14.9	92.91 ± 6.45	198.27 ± 29.71
MRT (hr)	7.15 ± 0.39	2.70 ± 0.34	4.50 ± 0.65

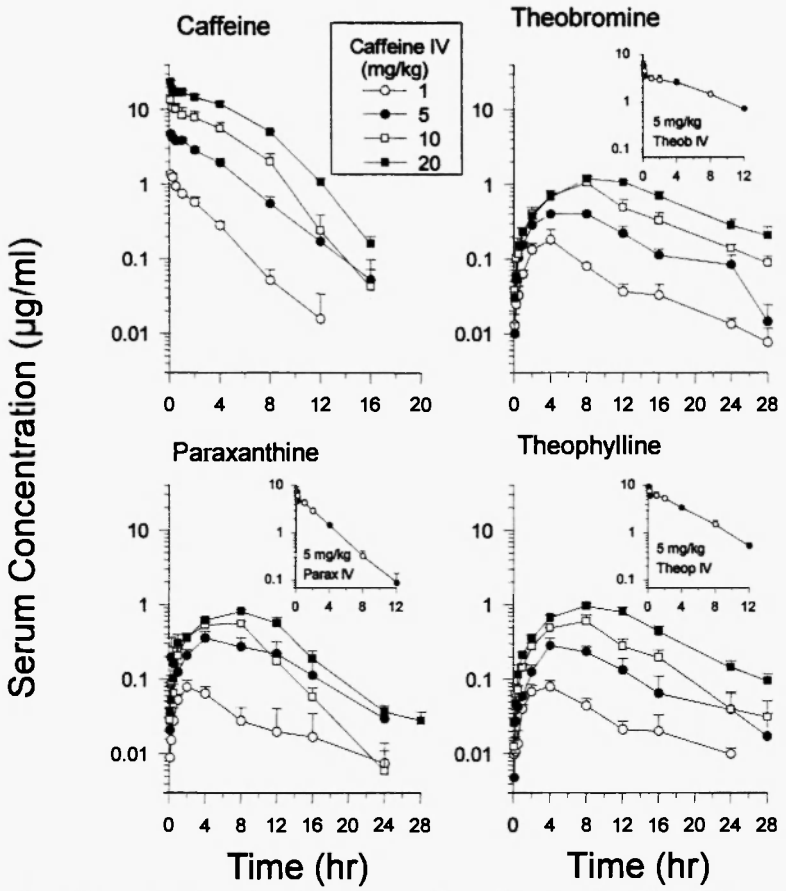

Fig. 2: Mean concentration-time curves for caffeine and each of its major metabolites (paraxanthine, theobromine, and theophylline) following four doses of caffeine. Insets demonstrate concentration-time curves for the metabolites following their administration alone.

sequent calculated pharmacokinetic parameters were dose independent. Previous studies have demonstrated that caffeine pharmacokinetics are dose dependent in both rats and humans /9-13/. Although there is insubstantial evidence to definitively conclude how large a caffeine dose is needed to saturate metabolic processes, preliminary data would suggest that non-linearity occurs following doses greater than 1-10 mg/kg /10,11,13/. Therefore the dosages in this study should have demonstrated non-linearity.

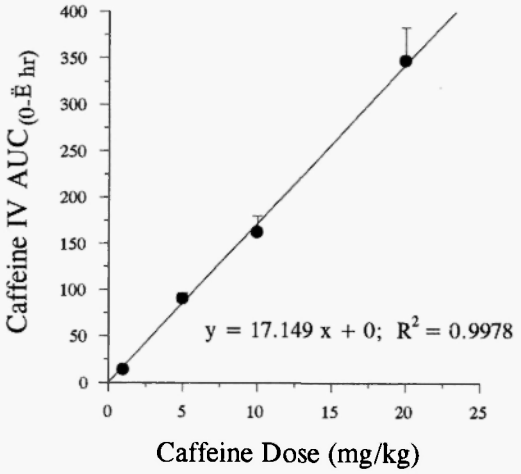

Fig. 3: Plot of caffeine AUC versus dose following intravenous administration of four doses of caffeine.

A possible explanation for the difference in this study from previous studies is the underlying characteristics of the rats. In this study rats were administered limited daily food rations and their weight decreased to 80% of their free feeding weight. Food deprivation regimens are frequently used to study the effects of various psychoactive drugs on food-reinforced behavior. Food deprivation, however, may alter metabolic processes in the liver. The effects of short-term fasting and long-term food restriction on the hepatic cytochrome P450 system have been extensively studied in rats /17-19/. For example, food restriction for 28 days in male rats enhanced hexobarbital metabolism by increasing the hepatic microsomal metabolizing enzymes and produced a significant decrease in hexobarbital sleeping time /20/. The induction of the cytochrome P4501A2 family by the food-limited regimen might account for the inability to demonstrate dose dependent pharmacokinetics following caffeine administration. In order to confirm this deduction more sophisticated studies would need to be done, such as measuring microsomal or cytochrome P4501A2, or measuring hexobarbital sleeping time. These tests were not completed in this small study.

Another possibility is that alternate pathways of caffeine metabolism were induced secondary to food deprivation. The fraction of

TABLE 3

Mean pharmacokinetic parameters (± SD) of the formation of dimethylxanthines after four different i.v. doses of caffeine (n = 4)

Dose (mg/kg)	**1**	**5**	**10**	**20**
Dose (μmol/kg)	5.15	25.75	51.49	102.99
k_{theob} (hr^{-1})	0.142 ± 0.046	0.103 ± 0.032	0.127 ± 0.036	0.089 ± 0.018
$t_{1/2}$ theob (hr)	5.34	7.19	5.81	8.01
k_{parax} (hr^{-1})	0.221 ± 0.12	0.157 ± 0.060	0.234 ± 0.10	0.190 ± 0.030
$t_{1/2para}$ (hr)	3.82	5.13	3.34	3.73
K_{theop} (hr^{-1})	0.131 ± 0.045	0.163 ± 0.079	0.112 ± 0.026	0.112 ± 0.026
$t_{1/2\ theop}$ (hr)	5.84	4.85	6.48	6.48
$k_{f,\ theob}$ (hr^{-1})	0.093 ± 0.062	0.042 ± 0.017	0.042 ± 0.017	0.027 ± 0.007
$k_{f\ parax}$ (hr^{-1})	0.054 ± 0.029	0.026 ± 0.008	0.024 ± 0.01	0.018 ± 0.006
$k_{f\ theop}$ (hr^{-1})	0.022 ± 0.017	0.015 ± 0.005	0.012 ± 0.002	0.012 ± 0.002
$AUC_{(0-\infty)}$ **theob** (nmol*hr/ml)	6.58 ± 3.10	28.81 ± 10.19	48.75 ± 12.34	79.37 ± 14.0
$AUC_{(0-\infty)}$ **parax** (nmol*hr/ml)	4.03 ± 1.14	19.81 ± 8.89	19.99 ± 13.45	38.69 ± 10.27
$AUC_{(0-\infty)}$ **theop** (nmol*hr/ml)	3.33 ± 1.91	13.17 ± 4.16	25.56 ± 4.68	54.93 ± 8.77
f_{theob}	20.90 ± 12.11	18.11 ± 8.38	15.03 ± 4.12	12.09 ± 1.58
f_{parax}	21.80 ± 6.66	21.28 ± 9.09	10.63 ± 6.73	10.38 ± 2.65
f_{theop}	8.45 ± 4.89	6.67 ± 2.12	6.56 ± 1.69	7.07 ± 1.85

caffeine converted to paraxanthine and theobromine decreased as doses increased (only paraxanthine decreased significantly), suggesting a saturation of one of the major metabolic pathways for caffeine elimination. However total clearance of caffeine did not decrease as doses increased. Although oxidative N-demethylation of caffeine to theobromine, paraxanthine, and theophylline is a significant metabolic process, direct ring oxidation to caffeine also occurs, resulting in the formation of a methyluric acid. Since this metabolite (or other minor metabolites) were not measured it is impossible to determine whether their formation increased with dosage increases.

CONCLUSIONS

In conclusion, caffeine demonstrated linear pharmacokinetics when administered in various doses to rats under a chronic, food-limited feeding regimen. Because rats are frequently administered a food-limited regimen in order to perform pharmacodynamic studies using psychoactive agents, this pharmacokinetic effect has important implications. Obviously, investigators performing caffeine pharmacodynamic studies in food-limited rats must plan for dose independent caffeine clearance. This pharmacokinetic effect, however, could impact many other drugs. The cytochrome P450 enzyme system is involved in the metabolism of over 100 pharmacologic agents used clinically. Therefore, pharmacokinetic analysis of drugs administered to rats under a chronic food-limited feeding regimen should be performed in order to adequately study the drug's pharmacodynamic effect.

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