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Title: The Absolute Bioavailability of Dyphylline Tablets in Human Subjects

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Abstract approved: James W. Ayres, Ph.D.

Dyphylline has been shown to be rapidly and completely absorbed from oral tablets in an absolute bioavailability study. In a pilot study two healthy male subjects received 1000 mg of dyphylline via intravenous infuson at a rate of 50 mg/min (1.0 ml/min) for 20 minutes. Plasma samples were collected for 16 hours and urine samples were collected for 72 hours. Observed peak plasma concentration were 22.4 mcg/ml for subject one and 25.7 mcg/ml for subject two. No adverse effects were reported by either subject; blood pressure and pulse rate remained normal.

An average of 83% of the administered dose of dyphylline was recovered in the urine. The remaining 17% of the dose may be distributed to "deep tissues" and removed slowly.

Computer based pharmacokinetic modeling of the plasma data, 0-8 hours for subject one and 0-7 hours for subject two, were best described by a two compartment open model with first order elimination from the central compartment. However, sigma-minus plots of time extended urine data reveal nonlinear or multicompartmental pharmacokinetics.

Evidence from the pilot study revealed that intravenous dyphylline can be safely administered to human subjects at the given dose and rate infused. Also, pharmacokinetic data from the pilot study provided background information to proceed with a twelve subject study of the absolute bioavailability of dyphylline.

In a two way crossover design twelve healthy male volunteers received a single 1000 mg dose of dyphylline orally as three conventional tablets (2 x 400 mg, 1 x 200 mg) or as a zero-order intravenous infusion (50 mg/min). A one week washout period separated administration of each dosage form. Plasma samples were collected over a 12 hour period and urine samples were collected for 48 hours post dosing. Urine and plasma samples were extracted and analyzed with high pressure liquid chromatography. Plasma drug concentration vs. time values after intravenous infusion were best described by a two compartment open model with first order elimination (average $r^2 = 0.991 \pm 0.007$). The mean elimination half-life after intravenous administration calculated from the average β-value was 1.99 hours while the

average half-life from the average β-value after oral administration was 1.87 hours. The mean absorption half-life calculated from the mean Ka was 12.2 minutes. Comparison of average AUC values obtained after administration of tablets (2829.7 mcg x min/ml) to average AUC values after intravenous infusion (2944.6 mcg x min/ml) indicates the mean biological availability was 96.5%. This is supported by urinary excretion data as an average of 768 mg of intact dyphylline was recovered in the urine after intravenous infusion and an average of 818 mg was recovered in the urine after oral administration of tablets. The urinary excretion ratio indicates the average oral bioavailability was 109%. It is concluded that dyphylline was rapidly and completely absorbed from the commercial tablet formulation investigated.

The Absolute Bioavailability of Dyphylline Tablets in Human Subjects

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Typed by Thomas K. Huston

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INTRODUCTION

This thesis consists of two parts, the first section is a two subject pilot study of intravenous dyphylline pharmacokinetics and the second section is a twelve subject two way crossover design study of the absolute bioavailability of dyphylline tablets.

A Two Subject Pilot Study of Intravenous Dyphylline Pharmacokinetics

1.1 INTRODUCTION

Theophylline has long been the mainstay of therapy in the treatment of reversible obstructive airway diseases throughout the world. This drug, however, does have undesirable gastrointestinal, central nervous system, and cardiac side effects. Theophylline is also only slightly soluble in water (1 g/120 ml). In attempts to improve theophylline solubility and decrease its side effects, many salt forms and derivatives have been developed with limited success. However, one chemical derivative of theophylline, dyphylline, has been marketed and is reported to be less toxic than theophylline (1,2) in animals and relatively large doses have not produced adverse effects in humans (3,7) except for one report of a headache in association with a peak plasma level of 36.4 mcg/ml in one subject (12).

Dyphylline, 7-(2,3-dihydroxypropyl)theophylline, produces significant bronchodilation with less incidence of side effects than theophylline. Although dyphylline was synthesized in 1946 little information has been available regarding its pharmacokinetic properties until very recently (5-13). Development of sensitive High Pressure Liquid Chromatographic assay methods (11,12) has allowed

extensive evaluation of dyphylline pharmacokinetics following oral administration. Isaksson and Lindholm (10) first reported intravenous administration of dyphylline to people in 1962 but over 17 years elapsed before Lawyer et. al. (8) as well as Zuidema and Merkus (13) reported pharmacokinetic parameters for dyphylline following intravenous administration. Urinary excretion data for the intravenous studies showed 44 percent (8) and 96 percent (13) of the administered dose excreted intact. In studies of orally administered dyphylline an average of 82-83 percent (7,12) of the dose was reported to be excreted intact in the urine and no metabolites were detected.

Early workers (9) reported that plasma dyphylline concentration vs. time profiles following oral administration could adequately be described by assuming a classical one compartment open pharmacokinetic model with first order absorption and elimination. Later workers (7) found following oral administration that the data did not lend support to a one compartment open model because of an initial post-peak rapid decrease in drug concentrations and curvature in the "terminal phase" of the data when plotted on semilogarithmic paper. Further, computer analysis (14,15) of the data indicated that either the two- or three-compartment open models with first order absorption and elimination from the central compartment were compatible

with the data. It was suggested that further research is necessary before an appropriate model can be determined for dyphylline (7).

Intravenous data for dyphylline exhibit at least two compartment behavior in two reports (10,13) and have been evaluated using assumptions associated with both one compartment and two compartment models in another report (8) where the drug was given by infusion over 10 minutes. It was proposed that dyphylline may be an ideal bronchodilator in the management of asthmatics with significant hepatic dysfunction. Further studies were suggested to establish the doses required to produce therapeutic effects for dyphylline.

The purposes of this study were to: (1) determine an appropriate pharmacokinetic model for dyphylline using both plasma concentration and urinary excretion data in two normal healthy male volunteers following intravenous infusion. This model should be useful for determining dosage in the clinical setting as well as providing background information for conducting absolute bioavailability studies in larger populations; (2) compare drug plasma concentration vs. time curves following intravenous administration to predicted values using pharmacokinetic parameters obtained from oral administration of dyphylline; and (3) compare the fraction of the intravenous dose excreted intact in the urine with

similar data obtained after oral dosing to obtain estimates of bioavailability from a specific oral tablet dosage form. Although absolute bioavailability requires comparison of pharmacokinetic parameters obtained from oral and intravenous dosages in the same group of subjects, the comparisons generated herein may provide useful guidelines for determining bioavailability from oral dosage forms using only urinary excretion data. This study may also provide information useful for determining necessary doses for multiple dosing of dyphylline tablets.

1.2 METHODS

The study was approved by the Committee for Protection of Human Subjects, Oregon State University. A physician evaluated two male volunteer subjects for their drug and disease histories. Both subjects were determined to be healthy and were not taking any medications or recreational drugs. Each subject signed an informed consent and agreed not to take any medication or drugs from one week before the study until its completion. The subjects fasted from 10:00 p.m. before dosing at 7:30 a.m. the following day until four hours post dosing, after which food (except xanthines; i.e. chocolate, tea, coffee) and beverages were allowed *ad lib*.

A dose of 1,000 mg of dyphylline[a] diluted in normal saline (0.9%)[b] was administered at a rate of 50 mg/min *via* a constant infusion pump[c] for 20 minutes.

A heparin lock[d] was placed in the back of the hand of each subject by an intravenous therapy nurse prior to sampling of blood.

Blood pressure and electrocardiogram readings were monitored by a physician.

Whole blood (7 cc) was collected from each subject at

time zero just prior to dosing and at 10, 15, 20, 30, 40 minutes and 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 12.0, and 16.0 hours post dosing with a syringe. The heparin lock was cleared of all heparin solution and the lock filled with subject blood by using a disposable syringe prior to sampling. Each sample was then collected in a new disposable syringe and immediately transferred to a heparinized vacuum tube[e]. After each sampling the heparin lock was flushed and filled with heparin (10 u/ml). Once samples had been transferred to vacuum tubes, they were inverted several times, then stored in crushed ice until centrifuged and the plasma collected. Plasma samples were then frozen until assayed.

Urine samples were collected just prior to dosing and at intervals of 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 16, 16 to 24, 24 to 32, 32 to 40, 40 to 48, 48 to 56, 56 to 64, and 64 to 72 hours post dosing. Subjects were provided with separate containers for each collection interval and instructed to void their bladders completely at the end of each interval.

Samples were stored at 4°C until urine volumes were recorded, then aliquots of each sample were frozen until assayed.

Plasma and urine samples were assayed by High Pressure Liquid Chromatography (HPLC)[f] as reported by Gisclon, Rowse,

and Ayres (11).

Plasma samples were prepared for assay by mixing 0.5 ml of plasma with 0.5 ml of acetonitrile containing β-hydroxyethyltheophylline (40 mcg/ml) as internal standard, and vortexed 15 seconds. The plasma sample with its protein precipitate was then centrifuged for 15 minutes at 2800 rpm and 5-40 µl injected into the HPLC.

Urine samples were extracted prior to injection into the HPLC (11). Urine sample (0.5 ml) was mixed with 0.5 ml of β-hydroxypropyltheophylline at a concentration of 35 mcg/ml in acetonitrile, and vortexed for 15 minutes. The sample was passed through a resin column[9] which selectively attaches water soluble organic molecules (e.g., dyphylline). Dyphylline was washed from the column using 15 ml isopropanol-chloroform (1:3) mixture which was collected in a test tube containing 1 ml 0.2 N NaOH, and centrifuged for 15 minutes. The aqueous layer was removed by aspiration and the organic layer evaporated to dryness with a nitrogen stream in a water bath at 55°C. The sample was reconstituted with 1.0 ml of methanol and 5-40 µl injected into the HPLC.

1.3 RESULTS

Figures 1.1 and 1.2 show dyphylline plasma concentration *vs*. time curves for the subjects. Solid circles are the data points which were obtained by duplicate analysis of each sample by different analysts and the solid line represents the predicted values obtained after fitting the data with an AUTOAN 2 computer package (14) which uses CSTRIP and NONLIN.[15] This program determined that the data from time zero to 480 min (8 hr) for subject one and 420 min (7 hr) for subject two were compatible with a two compartment open model with zero order infusion for 20 minutes and first order elimination from the central compartment only. The fit to the data was quite good with $r^2 = 0.996$ for subject one and 0.997 for subject two. The average percent deviation for the predicted lines from the data points was 7.7% and the estimated concentrations averaged 98.9% of the observed concentrations. Table 1.1 shows the estimated pharmacokinetic parameters for the subjects.

Table 1.2 shows subject characteristics and Table 1.3 shows blood pressure and pulse rate data before, during, and after drug infusion. Although the maximum recorded systolic blood

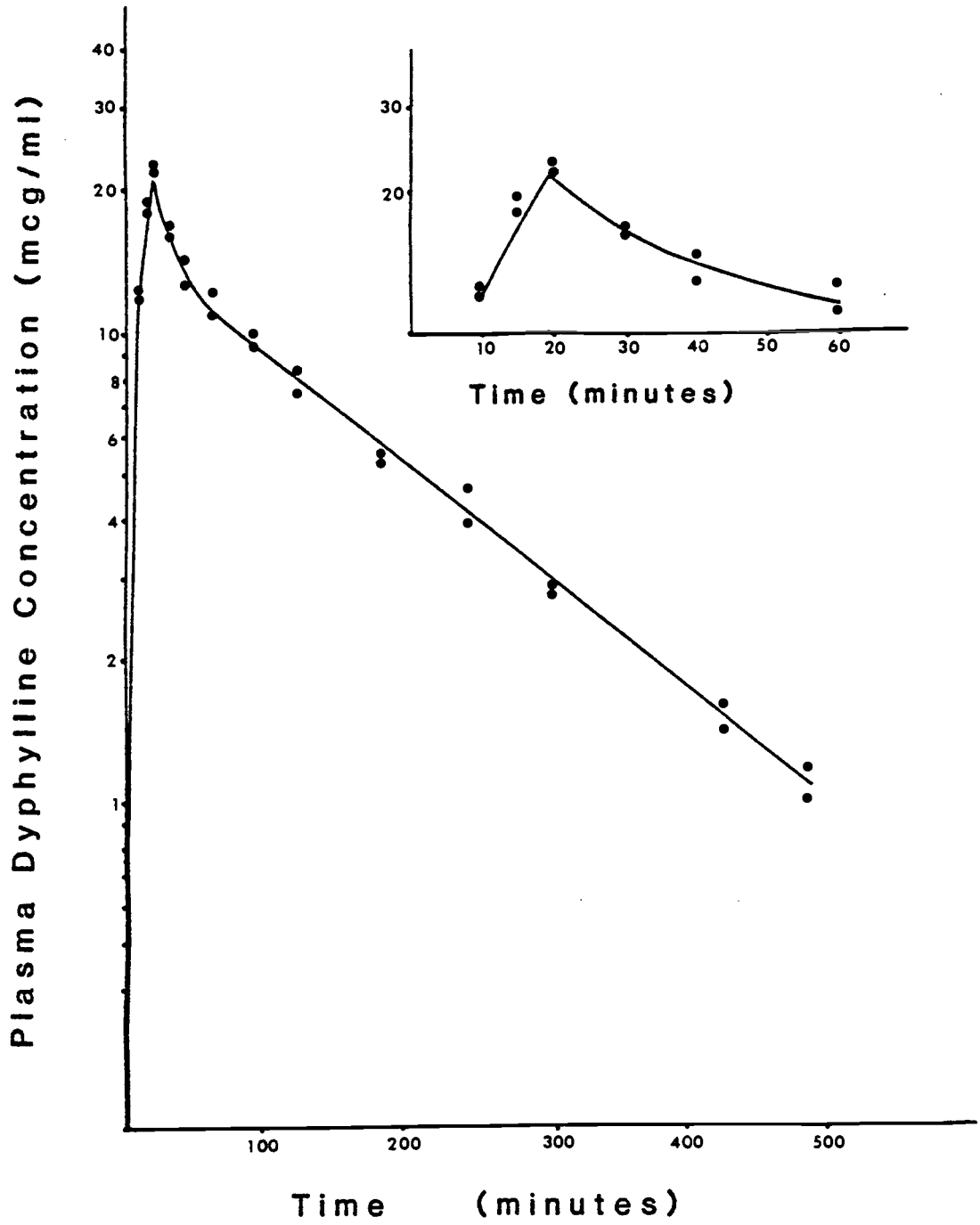

Figure 1.1 Dyphylline Plasma Concentration vs. Time in minutes for subject 1 following infusion of dyphylline 1000 mg represented by the symbol, ● for observed values and a solid fitted line using the computer program, AUTOAN 2 (14).

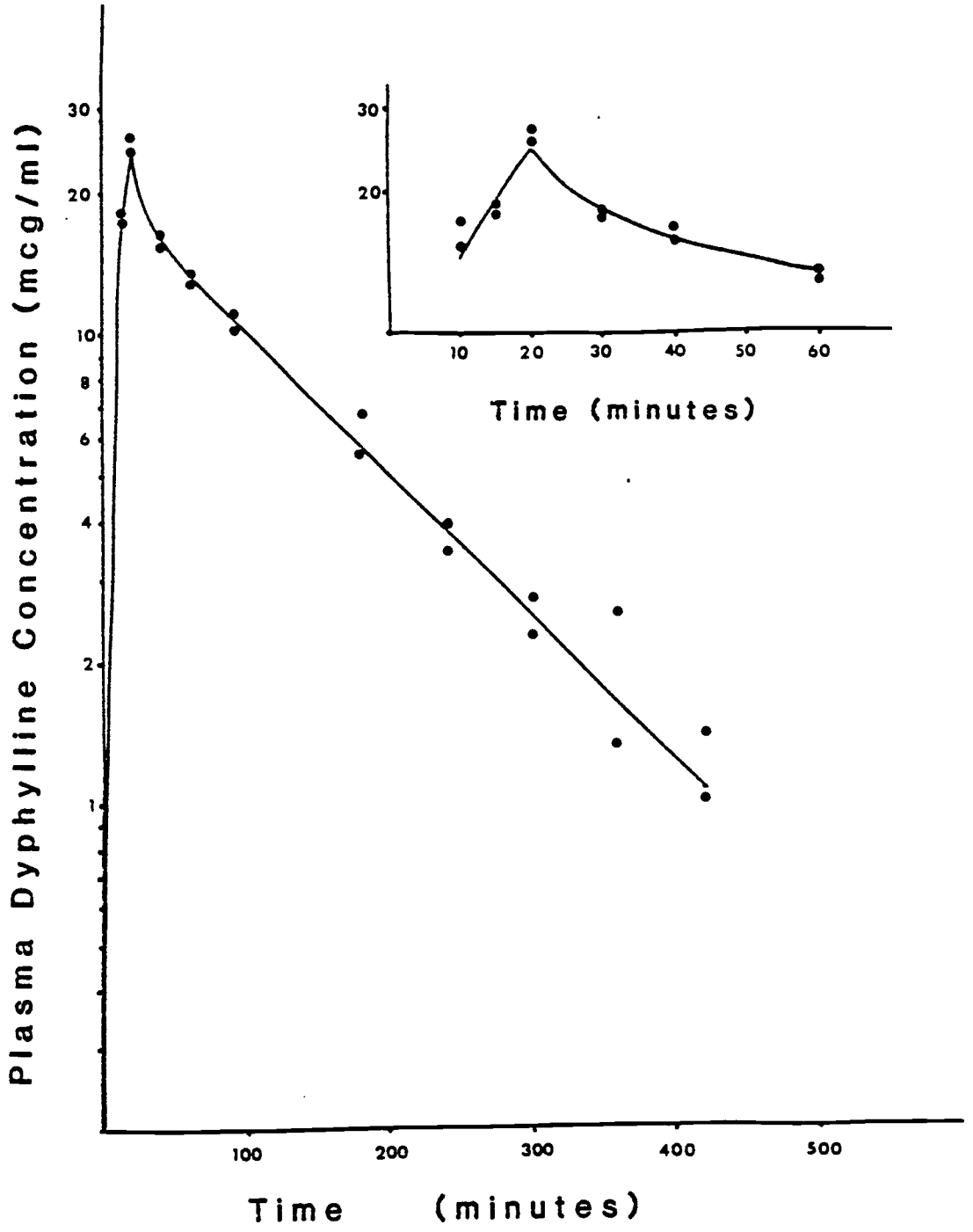

Figure 1.2 Dyphylline Plasma Concentration vs. Time in minutes for subject 2 following infusion of dyphylline 1000 mg represented by the symbol, ● for observed values and a solid fitted line using the computer program, AUTOAN 2 (14).

TABLE 1.1 Dyphylline Pharmacokinetic Parameters[a] Obtained after Intravenous Infusion (20 min.) of 1000 mg in Two Human Subjects.

Parameters	Subject 1	Subject 2
K_{21} (hr^{-1})	2.50	4.39
K_{el} (hr^{-1})	0.62	0.84
K_{12} (hr^{-1})	1.94	4.02
α (hr^{-1})	4.73	8.83
β (hr^{-1})	0.33	0.42
$t_{\frac{1}{2}}$ (hr^{-1})[b]	2.11	1.66
Css (mcg/ml)[c]	142.56	142.13
V_1 (L)	33.92	25.15
V_2 (L)[d]	30.28	25.45
V_d (L)[e]	64.21	50.60

[a]calculated by Autoan 2 (14) assuming first order elimination with 1/y2 weighting

[b]$t_{\frac{1}{2}}$ half-life = $0.693/\beta$

[c]Css, steady state = infusion rate/V_1 K_{el}

[d]V_2, Volume of peripheral compartment = $V_d - V_1$

[e]V_d, total volume of distribution = $(K_{el})\ (V_1)/\beta$

Note: Each value is obtained by AUTOAN 2 fitting of the plasma values obtained by fitting all results of analyst one and analyst two for each sample.

TABLE 1.2 Subject Characteristics

	RACE	SEX	AGE	WEIGHT	HEIGHT
Subject One	Caucasian	Male	31 yrs.	72.7Kg,160lb	5'11",180M
Subject Two	Caucasian	Male	30 yrs.	70.5Kg,155lb	6'2",1.88M

a. Neither subject had any known disease, chronic or acute. Neither subject was taking any medication other than OTC products which (along with alcohol) were not taken from one week prior to the study until the study was completed. Neither subject had any known allergies or history of allergies.

TABLE 1.3 Subjects' Blood Pressure and Pulse Rate

SUBJECT ONE[a]			SUBJECT TWO[b]		
Time	Pulse[c]	BP[d]	Time	Pulse	BP
0650	54	---	0645	77	---
0710	70	---	0710	63	---
0715	60	120/75	0715	65	125/80
0720	57	---	0720	67	---
0725	54	---	0742(10M)[E]	76	120/80
0740(10M)[e]	56	110/75	0752(20M)	66	120/85
0745(15M)	58	115/75	0752(20M)[f]	--	130/80
0750(20M)[f]	57	125/75	0802(30M)	66	120/80
0800(30M)	55	115/70	0812(40M)	67	120/80
0810(40M)	61	120/80	0832(60M)	--	115/80
0830(60M)	53	105/70	0902(90M)	62	115/75
0900(90M)	61	105/70			

[a]Time zero (start of infusion) for Subject one is 0730

[b]Time zero (start of infusion) for Subject two is 0732

[c]Pulse rate (beats/minute) read from electrocardiogram via a Cardiac Nomogram Ruler (manufactured by Lilly 60-MJ-7852-0) using two-cycles

[d]Blood pressure, mm Hg, obtained via blood pressure cuff.

[e]Minutes after the start of the infusion

[f]Dyphylline plasma concentration peaks at 20 minutes which is the time the infusion is stopped

pressure occurred at maximum plasma drug concentration, the effect was small. Blood pressure as well as pulse rate were essentially unchanged during the study. No adverse side effects were observed or reported.

Intact dyphylline recovered in the urine for subject one was 861 mg (86.1%) and 807 mg (80.7%) for subject two. Figure 1.3 shows the amount of drug remaining to be excreted vs. time (sigma-minus plot) on a semi-logarithmic scale (16). The data appear linear from time one hour to 12 hours and the slopes determined by linear regression for this time (1-12 hr) are -0.2592 for subject one ($r = 0.9976$) and -0.3899 for subject two ($r = 0.9979$). These values are similar to the terminal phase slopes associated with the plasma data collected from time zero to 8 hours (Table 1.1). However, the data in Figure 1.3 are curved beyond 12 hours indicating that the "true" terminal elimination phase was not recorded prior to 12 hours. Pharmacokinetic and clinical implications of these data will be considered in the discussion.

All plasma and urine samples were assayed in duplicate by separate analysts on different dates using individually prepared standard curves from published methods (11). The standard curves for plasma for concentrations of 1-200 mcg/ml ($n = 7-9$) were quite good with an average coefficient of variation of 8.84%. Evidence of agreement between the ana-

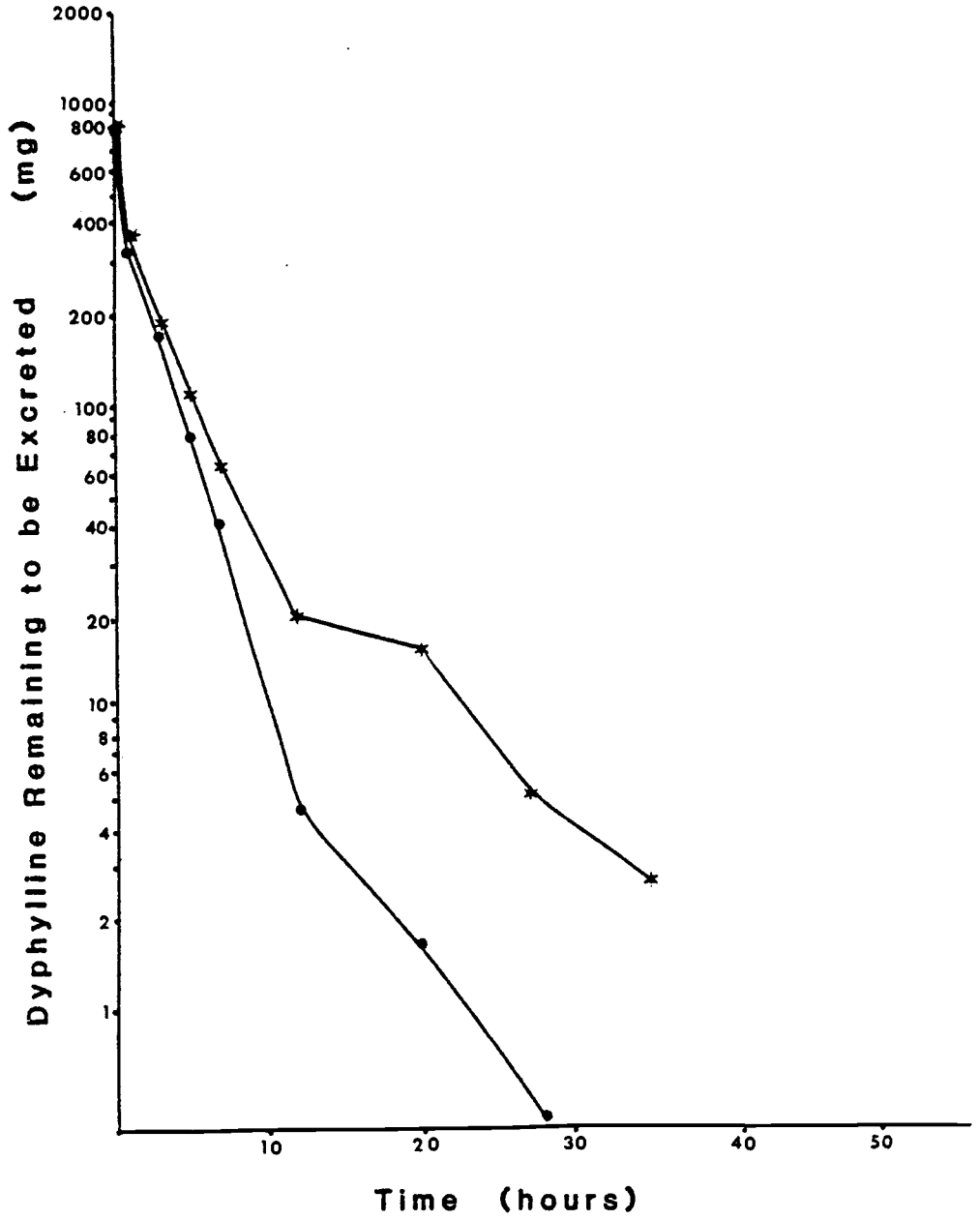

Figure 1.3 Individual Sigma-Minus Plots of the natural Log. of the Amount of Dyphylline Remaining to be Excreted vs. Time in hours following intravenous infusion of dyphylline 1000 mg in subject 1 represented by symbol, ★ and subject 2 represented by symbol, ● .

lytical results can be seen by the closeness of points in Figures 1.1 and 1.2. Further, linear regression of the estimated plasma dyphylline concentrations by analyst one regressed on the estimated concentrations by analyst two have an intercept of 0.4504, a slope of 0.9341, and an r value of 0.9955 (n = 27). The intercept and slope were not statistically significantly different (α = .05) from the theoretical values of zero and 1.0, respectively. Similarly, dyphylline in urine standard curves were quite good for 2.0 to 2000 mcg/ml (n = 13) with r^2 = 0.9994 and an average coefficient of variation of 15.94 percent for analyst two and an average coefficient of variation of 9.23 for analyst one.

1.4 DISCUSSION

A "correct" pharmacokinetic description of dyphylline from time zero to infinity is not available. Both one, two and complex or multicompartmental models have been proposed, as pointed out in the introduction. In each case where a one or two compartment model was used to describe the data, either data was not available for a very long time or there was bias in the fit at late-time values, i.e., a terminal "log-linear phase" was not reached.

The data in Figures 1.1 and 1.2 are well described by a traditional two compartment open pharmacokinetic model with zero-order infusion and first order elimination. However, plasma concentrations were only measured for 8 hours following drug administration. Dyphylline in urine was assayed in samples collected 40 hours after drug administration and inspection of Figure 1.3 reveals that a "terminal" log-linear phase is not reached prior to 12 hours. Thus, "true" dyphylline behavior for more than 12 hours or for multiple dosing must be described by a complex or multicompartmental pharmacokinetic model. This conclusion is consistent with earlier data collected when dyphylline was

administered orally and plasma and urine concentration of dyphylline were evaluated 16-28 hours post dosing (7).

Clinical and pharmacokinetic implications of the above findings are both important. The data in Figures 1.1 and 1.2 are not consistent with predictions made based on pharmacokinetic parameters from one-compartment analysis of data obtained after oral administration. This is true because dyphylline does not behave as a classical one-compartment drug from time zero to infinity even though it has been possible to obtain adequate fits of predicted lines to observed data in some earlier studies. In fact, using one-compartment pharmacokinetic parameters from fitting data reported earlier (7) results in predicted peak plasma dyphylline concentrations of 16.3 mcg/ml for subject one and 16.8 mcg/ml for subject two using volumes of distribution corrected for subject weight (Figures 1.1 and 1.2). The observed peak values were 22.4 mcg/ml and 25.7 mcg/ml respectively which is an average of 7.5 mcg/ml or 45 percent higher than predicted.

The cause of the error in predicting peak plasma dyphylline concentrations after an intravenous dose using oral pharmacokinetic parameters can be identified by reviewing the volumes of distribution obtained for the pharmacokinetic models generated from fitting data from 0-8 hours for both oral and intravenous administration.

Assuming dose independence, orally administered dyphylline's volume of distribution was 0.80 L/kg (7) with a 1200 mg dose which can be compared to intravenously administered dyphylline's volume of distribution of 0.80 L/kg with a 1000 mg dose. Thus, the total volume of distribution is, as it should be, equal for the two models involved. However, the pharmacokinetic model best describing dyphylline behavior after an intravenous dose results in the total volume divided approximately equally between two compartments, a central and a peripheral compartment. The orally administered dyphylline's volume of distribution is described by drug distribution into only one compartment. Since plasma samples are obtained from the central compartment, peak levels during intravenous administration would reflect initial concentration of drug in the central compartment, but after equilibration between central and peripheral compartments (40 minutes post dosing when the total volume of distribution is comparable to that of the oral dosage form) dyphylline plasma concentration approximates predicted peak levels based on oral dyphylline pharmacokinetic parameters. Therefore, the error in predicting intravenous peak dyphylline concentrations from oral pharmacokinetic parameters is a direct consequence which one would anticipate whenever one-compartment pharmacokinetics are used to predict behavior for a drug which follows multi-

compartmental pharmacokinetics. The error of this assumption was of a magnitude which could be serious with drugs like theophylline, but caused no adverse effects with dyphylline.

Intact dyphylline recovered in the urine following intravenous infusion of 1000 mg averaged 83% from time zero to the last measurable sample for both analysis (40 hours) compared to 82% after oral administration (7) of 1200 mg to about the same sampling time. In each case no metabolites were detected and curvature of the terminal urinary excretion sigma-minus plots (Figure 1.3) indicated that small quantities of drug were continuing to be excreted intact. Since the absorption phase for the oral tablet was rapid (7) and completed in about one hour, the approximately equal fraction of the dose excreted intact in urine after 40 hours indicates complete availability from the tablet. The 17-18% not excreted during both studies may be distributed to "deep" tissues, exhibit non-linear protein or tissue binding, be excreted in bile, recycle through saliva or be converted to a metabolite not detectable with the analytical method employed.

Also, the area under the dyphylline plasma concentration-time curve (AUC) from 0-7 hours corrected for body weight and dose from the oral administration of 1200 mg can be compared with the AUC 0-7 hours from the intravenous

administration of 1000 mg. The AUC for orally administered dyphylline was 99.7% of the AUC for intravenously administered dyphylline indicating complete availability. AUC was calculated using the trapezoidal rule and was not corrected for half-life since dyphylline does not appear to follow a simple linear pharmacokinetic model. Renal clearance of 3.99 ml/min x kg after the intravenous dose was comparable to 3.85 ml/min x kg after the oral dose. Although the above data following oral and intravenous administrations were not collected in the same population they were collected in the same laboratory using similar analytical methods and both urinary data and plasma AUC data are indicative that dyphylline was totally available from the oral dosage form used.

The data presented herein will be useful in designing an absolute bioavailability study involving oral and intravenous dyphylline in a larger population, support earlier work which shows that dyphylline can safely be administered by intravenous infusion (8,10) and demonstrate that accurate prediction of dyphylline plasma peak levels after intravenous administration requires pharmacokinetic parameters obtained from intravenous administration rather than oral dosage. Preliminary comparisons support the hypothesis that dyphylline is totally bioavailable from the oral dosage form studied (7).

1.5 ENDNOTES

a Neothylline Injection, 500 mg/2 ml, Lemmon Co., Sellersville, PA 18960

b Abbott Laboratories, North Chicago, IL 60064

c Harvard Infusion Pump

d Heparin Lock

e Vacutainers (143 U Heparin), Becton-Dickson

f Waters Model 6000 A HPLC, Waters C-18 μ- Bondapak reverse phase column, Waters 440 - ultraviolet absorbance detector, Huston dual pen recorder

g XAD-2, Applied Science Laboratories, inc., P.O. Box 440, State College, PA 16801

h Neothylline Tablets, Lemmon Pharmacal Co., Sellersville, PA.

i $(AUC_1 \times \text{body weight}_1)/\text{Dose}_1 = (AUC_2 \times \text{body weight}_2)/\text{Dose}_2$

A Twelve Subject Two Way Crossover Design Study of the Absolute Bioavailability of Dyphylline Tablets

2.1 INTRODUCTION

Dyphylline, 7-(2,3-dihydroxypropyl)theophylline, was synthesized in 1946 to diminish adverse effects associated with theophylline in managing reversible obstructive airway disease while enhancing or maintaining therapeutic efficacy (1,2). Although dyphylline was synthesized over 35 years ago it has enjoyed only limited use in the clinical setting. This is not surprising considering the limited amount of pharmacokinetic information available for the drug and the consequent numbers of hypotheses which filled the vacuum. Until recently it was held dyphylline's effectiveness depended on its metabolism to theophylline (3,10,17). Dyphylline doses were thus calculated on a molar basis with theophylline. Now it is clear from pharmacokinetic and pharmacological studies that dyphylline is an active chemical entity and is primarily excreted unchanged in the urine as first demonstrated in 1979 by Gisclon and Ayres as well as Simons (11,12). Early dyphylline dosing regimens based on the conversion of dyphylline to theophylline assumptions were probably responsible for the stifled growth of dyphylline as a successful bronchodilator alternative to theophylline since such doses first recommended by manufac-

turers may have been subtherapeutic (18).

A single dose double blind crossover study by Hudson with pulmonary compromised subjects demonstrated dyphylline (1000 mg orally) was statistically more effective than placebo but produced less bronchodilation than a 500 mg dose of theophylline. A 500 mg dose of dyphylline produced somewhat greater bronchodilation than placebo but was not statistically significantly different from placebo (4). Average dyphylline peak plasma concentration measured at 1-1.5 hours after administration of the effective 1000 mg dose of dyphylline was 16.3 mcg/ml. A double blind study by Simons et. al. (5) revealed a significantly greater decrease in exercise induced bronchospasm as measured by a small mean greatest percent decrease in FEV after a 15 mg/kg dose of oral aqueous solution compared to 10 mg/kg dose or placebo in seven subjects. Average peak plasma dyphylline concentration measured 40 minutes after the effective 15 mg/kg dose was 12 mcg/ml.

Authors of early pharmacokinetic modeling studies of orally administered dyphylline tablets and solutions concluded that plasma dyphylline concentrations vs. time curves were best described by a one compartment open model (6,9,12,13,19,20). However, a study in 1979 (7) revealed the classical one compartment open model did not accurately describe drug plasma concentration vs. time data after 16

hours for orally dosed dyphylline. Intravenously administered dyphylline appears to follow a classical two compartment model (8,12,19,21).

A relative bioavailability study (9) comparing a tablet, intramuscular injection, and 2 liquid dosage forms determined that all were equally bioavailable. Recently an absolute bioavailability study (19) compared an oral tablet and oral solution with intravenous dyphylline as a reference. The tablets and solution were found to be only 78% and 88% bioavailable respectively. The results of this study seem somewhat surprising when considering previous studies (6,7,9,12,13,20) and dyphylline's physicochemical properties (22). Two reports (7,12) concluded that dyphylline from tablets was at least 82% available based on urinary excretion data. Consideration of the reported absorption half-life of 7.3 minutes (7) leads to the prediction that absorption should be 98.5% complete after only one hour. Also, dyphylline is a polar compound which is readily soluble in aqueous media (1 G./3ml) and it is completely dissolved from tablets in the USP dissolution apparatus in only six minutes (19,22). One would predict that it should be completetly bioavailable from well formulated tablets. Thus, the bioavailability of dyphylline from commercially available tablets was determined by comparing intravenous and oral plasma drug concentrations as well as amount of drug excreted in the urine.

2.2 METHOD

The study was approved by the Committee for Protection of Human Subjects, Oregon State University. Twelve adult male volunteers (Table 2.1) took part in a single dose, two way crossover study. Each subject was interviewed for medical histories by a pharmacist. All subjects were deemed healthy and were not currently taking any medications. Each subject signed an informed consent and agreed not to take any medications or drugs from one week before the study until its completion.

Subjects fasted from 10 pm before dosing the following morning until four hours post dosing, after which food (except xanthines; i.e. chocolate, tea, coffee) and beverages were allowed ad lib.

A 1000 mg dose of dyphylline (see Table 2.1 for dose described as a function of body weight) was administered to each subject in tablet[a] form (2 x 400 mg dyphylline tablets + 1 x 200 mg dyphylline tablet) with 240 ml of water orally or as an intravenous infusion (2 ampules[b] of 500 mg dyphylline per 2 ml diluted with normal saline[c]) administered at a rate of 50 mg/min *via* an infusion pump[d] for 20

Table 2.1 Subject Characteristics and Dose Corrected for Body Weight

Subject Identification Number	Race	Age (yr)	Weight (kg)	Height (M)	Dose[a] (mg/kg)
1	Caucasian	26	68.2	1.85	14.7
2	Caucasian	22	72.7	1.89	13.8
3	Black	30	70.5	1.83	14.2
4	Caucasian	22	84.1	1.78	11.9
5	Caucasian	31	75.0	1.73	13.3
6	Caucasian	23	70.5	1.78	14.2
7	Black	27	78.6	1.78	12.7
8	Black	26	96.8	1.89	10.3
9	Caucasian	26	73.6	1.82	13.6
10	Caucasian	27	106.8	1.85	9.4
11	Caucasian	24	65.9	1.73	15.2
12	Caucasian	23	75.0	1.80	13.3
Mean[b]		25.6	78.1	1.81	13.0
Range		22-31	65.9-106.8	1.73-1.89	9.4-15.2

[a] 1000 mg of dyphylline/subject's weight in kg.

[b] $\Sigma X/N$

minutes followed by 220 ml of water orally. A one week washout period followed the first dosage form. A heparin lock catheter[e] was placed in the forearm of each subject by an intravenous therapy nurse with an infusion catheter[f] also placed in the opposite forearm of subjects receiving the intravenous dosage form prior to drug administration. Changes in cardiovascular function as a possible side effect of dyphylline were monitored with a blood pressure sphygmomanometer[g] and axial pulse rate both before intravenous infusion and when drug plasma concentration peaked.

Whole blood (7 ml) was collected from each subject at time zero (just prior to dosing) and at 10, 15, 20, 30, 40 minutes and 1.0, 1.5, 2.0, 3.0, 5.0, 6.0, 7.0, 8.0, and 12.0 hours post dosing. The heparin lock was cleared of all heparin solution and the lock filled with subject's blood by using a disposable syringe[h] before sampling. Each sample was then collected in a new disposable syringe and immediately transferred to a heparinized vacuum tube.[i] After each sampling the heparin lock was flushed and filled with heparin[j] (10 U/ml). Once samples had been transferred to vacuum tubes, they were inverted several times, then stored in crushed ice until centrifuged and the plasma collected. Plasma samples were then frozen until ready for assay.

Urine samples were collected at time zero and at intervals of 0-2, 2-4, 4-6, 6-8, 8-16, 16-24, 24-32, 32-40, and

40-48 hours post dosing. Subjects were provided with separate containers[k] for each interval and instructed to void their bladders completely at the end of each collection interval. Samples were stored at 9°C until urine volumes were recorded, then 25 ml aliquots of each sample were forzen until assayed. Plasma and urine samples were assayed by high pressure liquid chromatography[l] (HPLC) using a modified published procedure (11).

Plasma samples were prepared for assay by mixing 0.5 ml of plasma sample with 0.5 ml of internal standard (β-hydroxyethyltheophylline,[m] 10 mcg/ml) in acetonitrile,[n] vortexing for 10 seconds, then adding 0.5 ml of 0.2 N sodium hydroxide[o] in water and vortexing for 15 minutes at 2200 rpm. 1.0 ml of the supernatant was added to 4.0 ml of 10% isopropanol[p] in methylene chloride[q] solution and shaken for 15 minutes. This solution was transferred to a centrifuge tube and centrifuged at 2200 rpm for 15 minutes. The aqueous layer (top) was discarded and the organic layer evaporated to dryness in a vacuum oven.[r] The sample was reconstituted with 200 µl in methanol[s] and 5-50 µl injected into the HPLC.

Urine samples were extracted prior to injection into the HPLC (11). Urine (1 ml) and internal standard (β-hydroxypropyltheophylline[t] at a concentration of 29.9 mcg/ml in acetonitrile), 1.0 ml, was vortexed. The urine sample was then passed through a resin column[u] that selec-

tively binds water soluble organic molecules, which was prewashed with 50 ml of methanol followed by 10 ml of deionized water. The prewashed resin column was loaded with sample and then rinsed with 10 ml of deionized water which was discarded, then washed with a 5 ml and 10 ml portion of isopropanol/chloroform^{V} (1:3) which was collected in a centrifuge tube. The resultant solution was shaken for 5 minutes. The top aqueous layer was aspirated and the organic layer evaporated to dryness in a vacuum oven under low heat (30-50°C). The sample was reconstituted with 500 µl of methanol and 8-50 µl injected into HPLC.

Plasma dyphylline concentration was determined by using a pooled linear standard curve of 127 points with dyphylline concentrations from 1 mcg/ml to 30 mcg/ml and 10 points per individual curve. The standard coefficient of determination (r^2) was 0.994 and the average inversely estimated concentration was 100.8% of theory with a 14.1% coefficient of variation.

A separate standard curve was prepared for each day dyphylline urine samples were assayed. Dyphylline urine standards ranged from 5 mcg/ml to 100 mcg/ml with 6 points per curve. Urine samples of anticipated high dyphylline concentrations were diluted prior to assay to fit in the upper range of the standard curve.

Individual drug plasma concentration vs. time data cur-

ves were fit to a two compartment open model using a computer software program[W]. Individual drug plasma concentration *vs.* time data after oral administration were also fit to a one compartment open model. Points were weighted 1/drug concentration.

Dyphylline bioavailability from tablets was determined for each subject by comparing area under the plasma drug concentration *vs.* time curve from time zero to infinity after oral administration of tablets to area under the plasma concentration curve from time zero to infinity after intravenous infusion of dyphylline. Areas under the plasma concentration *vs.* time curves were determined by the linear trapezoidal rule and extrapolated to infinity as follows (23,24)

$$AUC_i = \frac{(C_i + C_{i+1})}{2} \cdot (T_{i+1} - T_i)$$

and

$$AUC_{T\ last} = \frac{C_{last}}{\beta}$$

where C's and T's are drug concentration and time values respectively. C_{last} is the fitted value of the last observed drug concentration point. β is the slope of the terminal phase. Equations for logarithmic trapezoidal rule with extrapolation to infinity were as follows (23,24)

$$AUC_i = \frac{(C_i - C_{i+1})}{Ln(C_i/C_{i+1})} \cdot (T_{i+1} - T_i)$$

and

$$AUC_{T\ last} = \frac{C_{last}}{\beta}$$

Extent of dyphylline absorption, f, for each subject was also measured by dividing area under the plasma drug concentration vs. time curve from time zero to infinity after oral administration of tablets to area under the drug plasma concentration curve from time zero to infinity after intravenous infusion. Analysis of absolute bioavailability was statistically evaluated by two methods which were: a) the 95% confidence interval approach (16,25-27); b) standard hypothesis test comparing area under the curve values obtained after oral administration with area under the curve values after intravenous infusion via paired t-test. The paired t-test statistic was used in standard hypothesis testing when analysis of variance (ANOVA) results showed no period effect indicating no statistical evidence of a carry over effect (refer to Appendix 1). Urine data was analyzed using the paired t-test to compare amount of intact dyphylline recovered in urine after oral dosing to amount of dyphylline recovered after intravenous infusion. Inferences were made on the reliability of urine data by a paired t-test examining volume of urine excreted in each of the two periods of the study. Volume of urine excreted for each period was also compared to the expected mean value using Student's t-test (28).

Several methods were used to study rate of dyphylline

absorption. Comparisons were made between Ka obtained from one compartment and two compartment modeling (29), Wagner-Nelson method, the Loo-Riegelman method (16,24,30) mean absorption time (MAT) calculated by two methods (logarithmic trapezoidal rule and linear trapezoidal rule) (31), and time for drug peak concentrations corrected for lag time. Rate of absorption calculations using MAT and the statistical moment theory were as follows:

$$AUMC = \int tCp\ dt$$

$$AUC = \int Cp\ dt$$

where AUMC was calculated using the linear trapezoidal rule as follows (23):

$$AUMC_i = \frac{(C_1 \cdot T_i - C_{i+1} \cdot T_{i+1})}{2} (T_{i+1} - T_i)$$

and the logarithmic trapezoidal rule as (23):

$$AUMC_i = \frac{(C_i \cdot T_i - C_{i+1} \cdot T_{i+1})}{Ln(C_i/C_{i+1})} \cdot (T_{i+1} - T_i) + \frac{(C_i - C_{i+1})}{2\ Ln(C_i/C_{i+1})} \cdot (T_{i+1} - T_i)^2$$

and both sets of equation are extrapolated to infinity by

$$AUMC_{last\ to} = \frac{C_{last} \cdot T_{last}}{\beta} + \frac{C_{last}}{2}$$

AUC was calculated as described earlier.

$$MRT(inf) = AUMC(inf)_{0-\infty}/AUC(inf)_{0-\infty}$$

$$MRT(iv) = MRT(inf) - T(cut)/2$$

$$MRT(po) = AUMC(po)_{0-\infty}/AUC(po)_{0-\infty}$$

$$MAT = MRT(po) - MRT(iv)$$

$$MAT = 1/Ka$$

$$Ka = 1/MAT$$

Statistical moment theory defines mean absorption time (MAT) as the time it takes 63.2% of the drug molecules to be absorbed into the body's general circulation. Mean residence time (MRT) is considered to be the time it takes for 63.2% of the drug molecules to transit through the body. The MAT and MRT are mathematically described above and MRT for different routes of administration are MRT(inf) for intravenous infusion, MRT(po) for the oral route, and MRT(iv) for the intravenous bolus route. T(cut) is the time when infusion is stopped. AUMC is area under the first moment curve and is the area under the product of concentration of drug in the plasma, Cp, and time, t, curve from time zero to infinity. MRT values were calculated from equations using the logarithmic trapezoidal rule and the linear trapezoidal rule, therefore two values of MAT were obtained.

Total body clearance, Cl, was calculated from both plasma and urine data. Clearance equations from plasma were (24,25),

$$Cl = \beta \cdot Vd$$

where

$$Vd = (Vc)(Kel)/\beta$$

$$Kel = (\alpha)\ (\beta)/K21$$

and equations to calculate clearance from urine data were (24)

$$Cl = (dX_u/dt)/Cp$$

where dX_u/dt is the excretion rate of a given urine collection interval and Cp is the concentration of drug in the plasma at the midpoint of the urine collection interval. The elimination rate constant was also calculated from urine data *via* sigma-minus plots of the percent of dyphylline remaining to be excreted *vs*. time (24).

Before statistical tests were performed on each sample group they were tested for normal distribution patterns by use of the Wilk-Shapiro test (28). All statistical inferences were made at the $\alpha = 0.05$ level.

The power of the tests for some statistical inferences was determined using the appropriate table and the following equation (32).

$$d = \frac{(\mu_1 - \mu_2)}{s/N^{\frac{1}{2}}}$$

2.3 RESULTS

The fraction of dyphylline absorbed from tablets calculated from average logarithmic trapezoidal area under the curve after oral administration divided by average logarithmic trapezoidal area under the curve after intravenous infusion was 96.5%. Calculated fractions of dyphylline absorbed for each subject ranged from 75.1% to 110.1%. Fraction absorbed values calculated by the linear trapezoidal rule were similar with an average of 96.1% of the dose absorbed and a range of 74.9% to 109.4% (Table 2.2).

The paired t-test comparing AUC after intravenous dosing and oral dosing measured by two methods, the linear trapezoidal rule and the logarithmic trapezoidal rule, were not significantly different with p-values of 0.27 and 0.31 respectively. Similar results were obtained using the paired t-test on logarithmic transformations of AUC values measured by two methods, the linear trapezoidal rule and the logarithmic trapezoidal rule, with p-values of 0.24 and 0.28 respectively.

The power of the above statistical test to detect a 20% difference in the amount of dyphylline absorbed from a tablet into the general circulation using the infusion as a reference

Table 2.2 Extent of Dyphylline Tablet Bioavailability

Subject Indentification Number	AUC(Inf)[a] (mcg x min/ml)	AUC(po)[b] (mcg x min/ml)	%f[c]	AUC(Inf), log.[d] (mcg x min/ml)	AUC(po), log.[e] (mcg x min/ml)	%f[f]
1	3271.0	3421.6	104.6	3253.1	3403.2	104.6
2	3105.3	3198.1	103.0	3083.1	3179.5	103.1
3	4665.5	4661.4	99.9	4625.2	4617.3	99.8
4	2586.7	2651.9	102.5	2565.4	2631.1	102.6
5	2855.8	2590.0	90.7	2822.7	2569.3	91.0
6	3257.5	3564.4	109.4	3217.9	3542.2	110.1
7	2414.3	1980.3	82.0	2403.8	1964.7	81.7
8	2277.8	2151.9	94.5	2227.8	2140.1	96.1
9	3248.8	2432.3	74.9	3219.5	2416.4	75.1
10	2067.8	2167.4	104.8	2032.9	2156.3	106.1
11	3606.1	2803.7	77.7	3545.0	2776.3	78.3
12	2366.5	2578.9	109.0	2339.0	2559.7	109.4
Mean $\pm$ S.D.[g]	2976.9 $\pm$ 718.2	2850.2 $\pm$ 757.2	96.1 $\pm$ 12.1	2944.6 $\pm$ 714.3	2829.7 $\pm$ 749.9	96.5 $\pm$ 12.2
%C.V.[h]	24.1	26.6	12.6	24.3	26.5	12.6
Range	2067.8-4665.5	1980.3-4661.4	74.9-109.4	2032.9-4625.2	1964.7-4617.3	75.1-110.1

[a] Area under the plasma concentration time curve after intravenous infusion of dyphylline calculated by the linear trapezoidal rule from time zero to infinity

[b] Area under the plasam concentration time curve after oral administration of dyphylline tablets calculated by the linear trapezoidal rule from time zero to infinity

[c] (value of column b/value of column a) · 100

[d] Area under the plasma concentration time curve after intravenous infusion of dyphylline calculated by the logarithmic trapezoidal rule from time zero to infinity

[e] Area under the plasma concentration time curve after oral administration of dyphylline tablets calculated by the logarithmic trapezoidal rule from time zero to infinity

[f] (value of column e/value of column d) · 100

[g] $\Sigma X/N \pm [\Sigma(X_i-\bar{X})^2/N-1]^{1/2}$

[h] S.D. · $100/\bar{X}$

is quite sensitive with a value greater than 0.99 at α= 0.05. Using the 95% confidence interval approach on the percent ratios of area under the plasma drug concentration time curve after oral administration to area under the plasma drug concentration time curve after intravenous administration, the confidence limits were within the acceptable region of 80% to 120% (refer to Appendix 2 for calculations). The calculated lower confidence limit was 88% and the upper confidence limit was 104%. Bioavailability was also deemed acceptable according to FDA guidelines of the 75/75 rule which states, "In at least 75% of the subjects administered the drug, the test drug product has a bioavailability of greater than 75% relative to that of the administered reference material utilizing each subject as his or her own comparison..." (27). Therefore according to the FDA 75/75 rule and the conclusions from the two statistical tests the intravenous infusion and tablet treatments are considered bioequivalent.

Approximately 767.7 mg and 818.1 mg of dyphylline were recovered in the urine after intravenous and oral administration respectively (Table 2.3). Approximately 45% of the administered dose is excreted in the urine within two hours after dosing and almost all of the recovered drug is excreted in the urine within 24 hours postdosing (Table 2.4 and Figure 2.1). Average amount of dyphylline absorbed when comparing average amount of intact dyphylline recovered in urine after an oral dose with average amount recovered after an intravenous dose

Table 2.3 Cumulative Amount of Dyphylline Recovered in the Urine of Each Subject

Subject Identification Number	$U_{avg.}$, (inf)[a] (mg)	$U_{avg.}$, (po)[b] (mg)	F[c] (%)
1	875.6	722.8	82.6
2	850.2	975.6	114.7
3	724.1	821.9	113.5
4	848.1	705.5	83.2
5	981.6	871.3	88.8
6	633.4	958.0	151.2
7	529.2	685.8	129.6
8	768.8	667.6	86.8
9	742.7	814.8	109.7
10	859.8	862.1	100.3
11	631.2	913.5	144.7
Mean $\pm$ S.D.[d]	767.7 $\pm$ 132.4	818.1 $\pm$ 109.6	109.6 $\pm$ 24.2
%C.V.[e]	17.2	13.4	22.1
Range	529.2-981.6	667.6-975.6	82.6-151.2

[a] Cumulative amount of dyphylline recovered in the urine after intravenous infusion of 1000 mg

[b] Cumulative amount of dyphylline recovered in the urine after a 1000 mg oral dose of tablets

[c] $U_{\infty}(inf) \cdot 100/U_{\infty}(po)$

[d] $\Sigma X/N \pm \{\Sigma(X_i-\bar{X})^2/N-1\}^{1/2}$

[e] $S.D. \cdot 100/\bar{X}$

Table 2.4 Average Amount of Dyphylline Recovered in the Urine

Collection Interval (hour)	$U_{avg.}$, (inf)[a] (mg)	$U_{avg.}$, (inf)[b] (%)	$U_{avg.}$, (po)[c] (mg)	$U_{avg.}$, (po)[d] (%)
0-2	356.6	46.4	357.1	43.6
2-4	207.7	27.1	248.7	30.4
4-6	106.1	13.8	104.9	12.8
6-8	45.6	5.9	59.5	7.3
8-16	43.2	5.6	41.0	5.0
16-24	6.2	0.8	5.3	0.7
24-32	1.4	0.2	0.7	0.1
32-40	0.9	0.1	0.5	0.1
40-48	0.0	0.0	0.4	0.0

[a] Average amount of dyphylline recovered in the urine after intravenous infusion of 1000 mg

[b] $U_{avg.}$,(inf) · 100/$\Sigma U_{avg.}$,(inf)

[c] Average amount of dyphylline recovered in the urine after oral administration of 1000 mg

[d] $U_{avg.}$,(po) · 100/$\Sigma U_{avg.}$,(po)

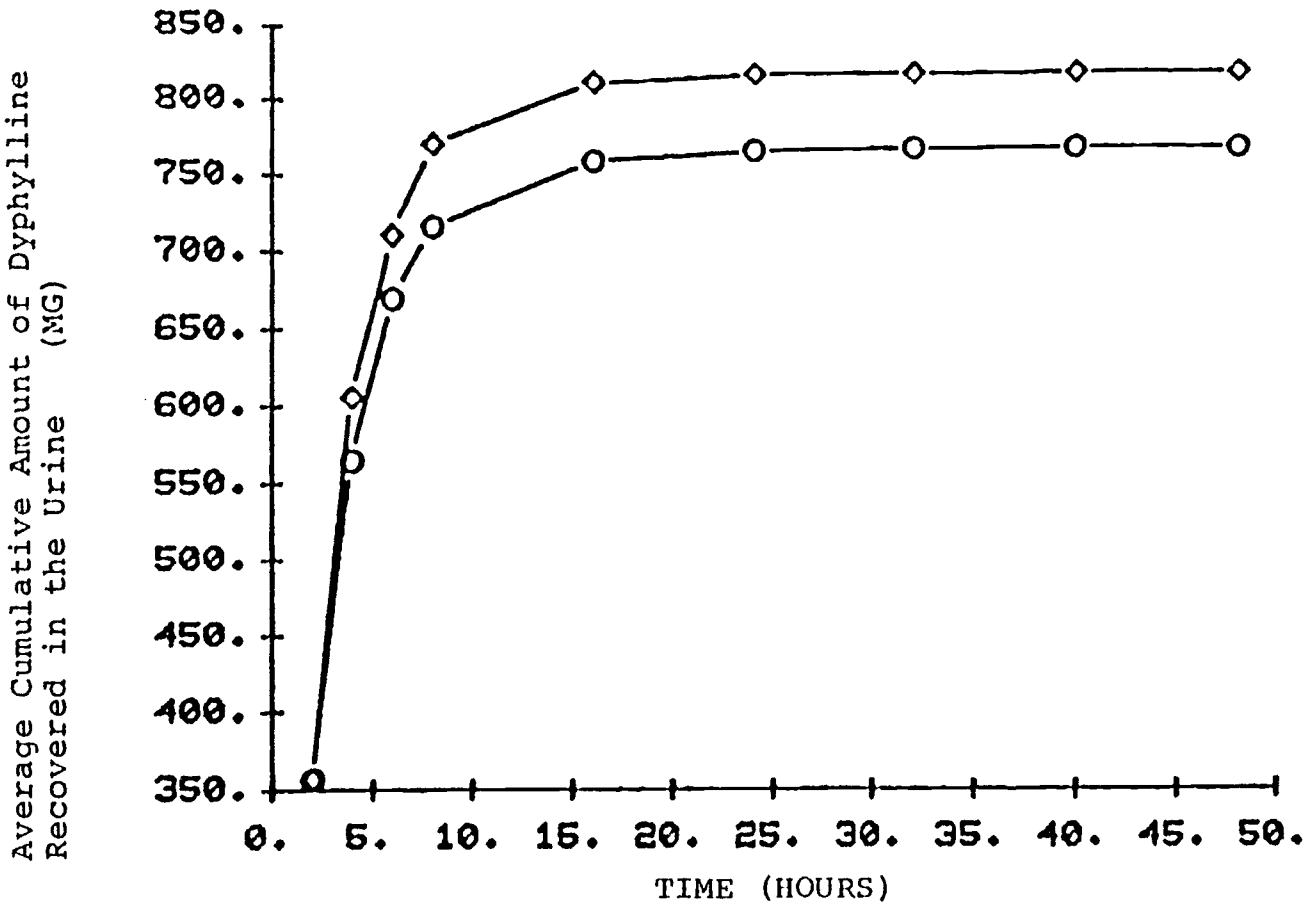

Figure 2.1 Average Cumulative Amount of Dyphylline Recovered in the Urine of Twelve Subjects vs. Time in hours following infusion of dyphylline 1000 mg represented by the symbol ○ and following the administration of dyphylline 1000 mg as conventional tablets represented by the symbol ◇.

in each subject was 106.6% with a range of 82.6% to 151.2% (see Figure 2.1). A paired t-test of amount of intact dyphylline excreted in the urine after intravenous dosing and after oral dosing were not significantly different, p-value is 0.34. However, there was a significant difference (p-value of .008) in urine volumes collected from time zero to 24 hours during week 1 as compared to week 2 as analyzed by the paired t-test at $\alpha = 0.05$.

Plasma data obtained from each subject after intravenous infusion followed a classical two compartment open model. The one compartment open model appeared to best describe the fit of plasma data obtained after oral administration of dyphylline tablets, but a two compartment open model was also used to describe plasma dyphylline data after oral administration (Figure 2.2). Pharmacokinetic parameters are summarized in Tables 2.5 and 2.6. The average elimination half-life after intravenous infusion was 1.99 hours vs. 1.87 hours calculated from the average β-value after oral administration and 1.73 hours calculated from the average Kel value after oral administration. These figures are in close agreement to previous values of 1.75 and 1.71 hours after intravenous administration (8,19) and 1.8, 2.1 and 2.01 hours after administration of tablets (7,12,19) reported in the literature.

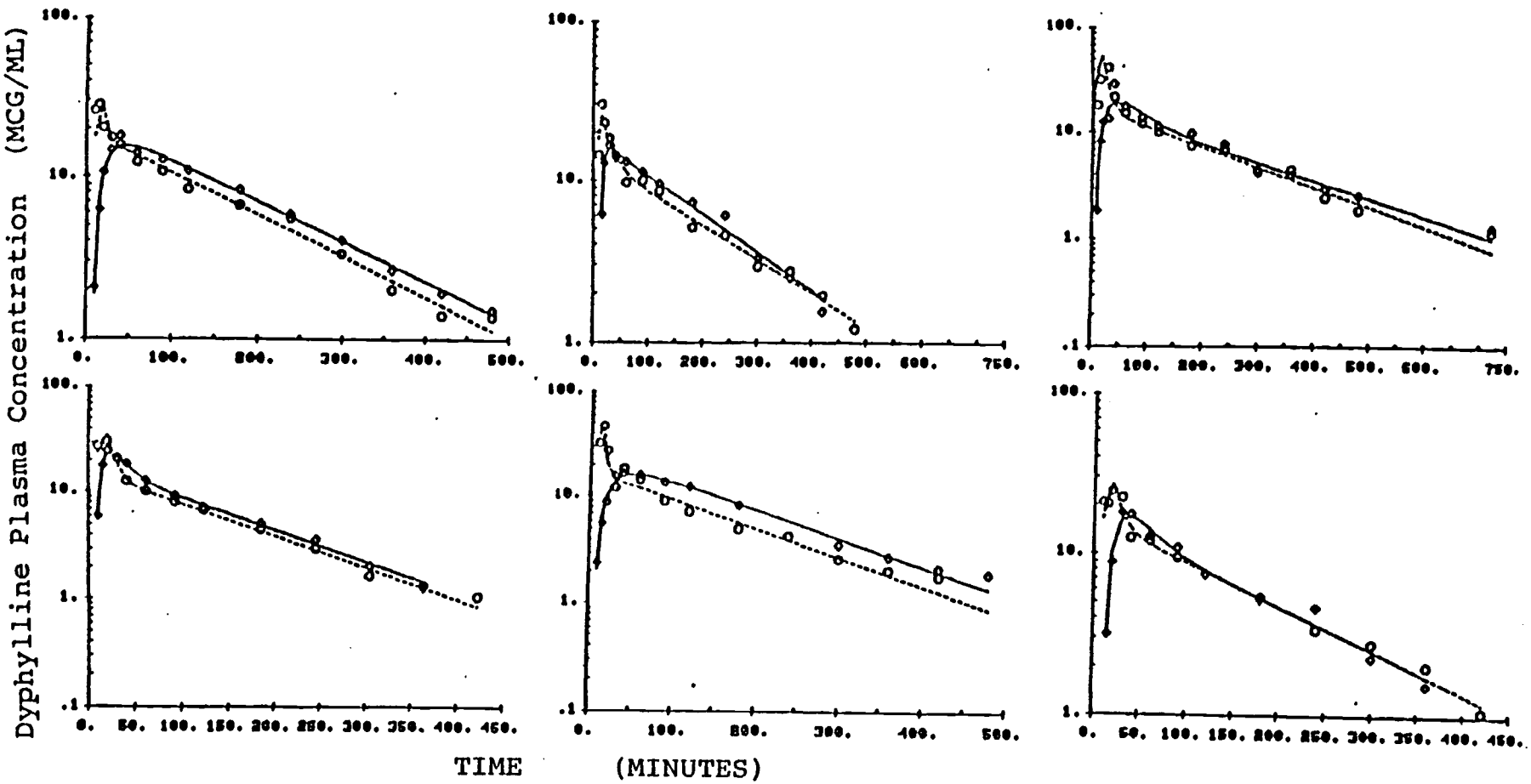

Figure 2.2 Individual Dyphylline Plasma Concentration vs. Time in minutes of subjects: 1, 2, 3, 4, 5, 6 (from left to right) following infusion of dyphylline 1000 mg represented by the symbol O and following the administration of dyphylline 1000 mg as conventional tablets represented by the symbol ◇ .

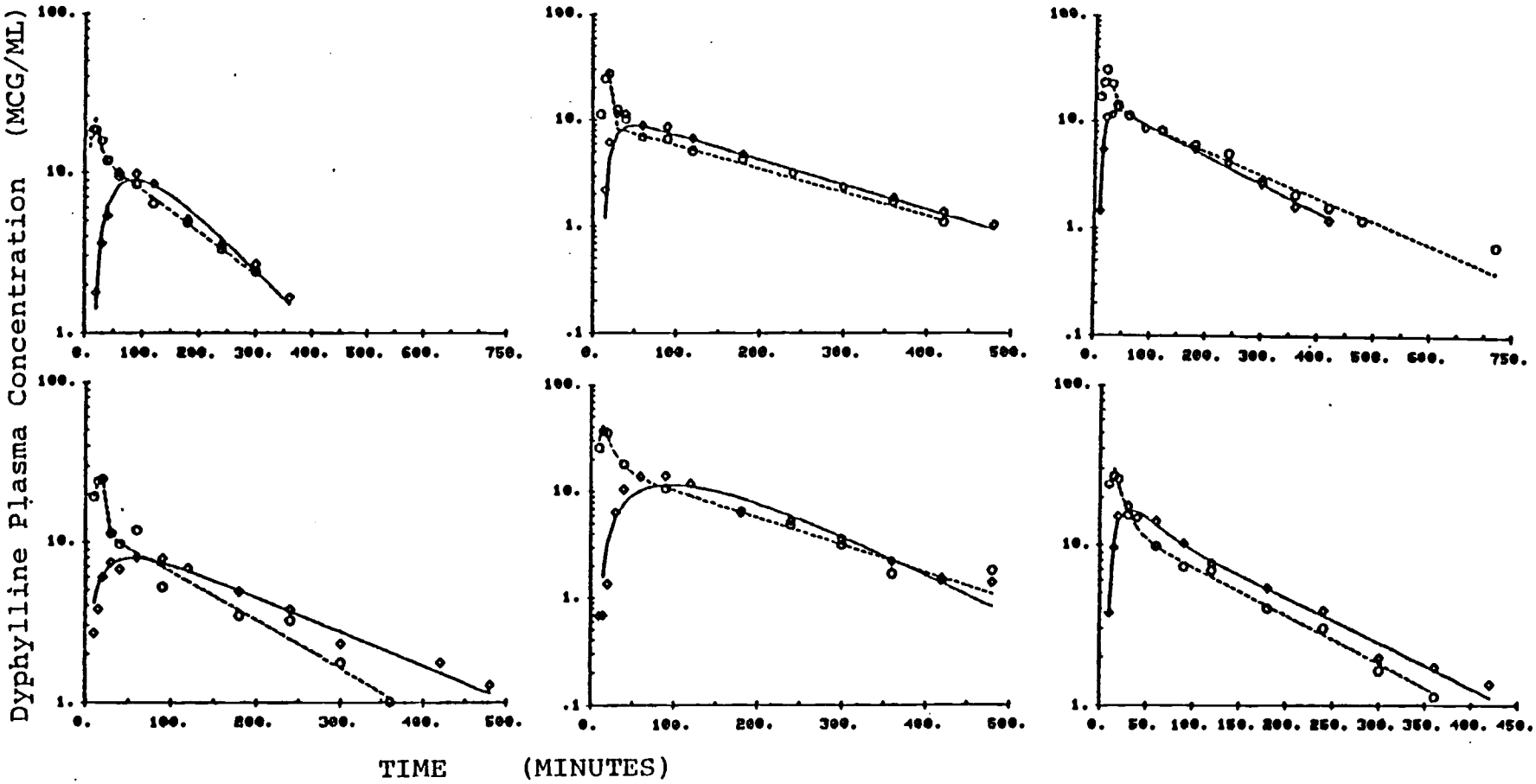

Figure 2.2 (continued) Individual Dyphylline Plasma Concentration vs. Time in minutes of subjects: 7, 8, 9, 10, 11, 12 (from left to right) following infusion of dyphylline 1000 mg represented by the symbol ○ and following the administration of dyphylline 1000 mg as conventional tablets represented by the symbol ◇ .

Table 2.5 Pharmacokinetic Parameters from Two Compartment Modeling of Drug Plasma Data

Subject Indentification Number	α, (inf)[a] (hr^{-1})	α, (po)[a] (hr^{-1})	K21, (inf)[a] (hr^{-1})	K21, (po)[a] (hr^{-1})	Ka[a] (hr^{-1})	β, (inf)[a] (hr^{-1})	β, (po)[a] (hr^{-1})	Kel (inf)[b] (hr^{-1})	Kel (po)[b] (hr^{-1})
1	17.0	10.3	4.5	10.3	4.4	.354	.343	1.332	.343
2	3.4	8.6	1.6	4.6	6.6	.288	.326	.613	.604
3	6.8	2.8	1.2	.9	1.7	.260	.233	1.433	.739
4	7.5	6.1	1.8	1.8	5.0	.405	.411	1.700	1.346
5	6.0	3.6	2.4	1.6	3.6	.369	.372	.946	.843
6	11.8	50.9	2.2	50.9	3.2	.354	.360	1.923	.360
7	9.5	61.0	3.2	61.0	1.4	.367	.486	1.095	.486
8	48.6	4.6	1.7	2.3	3.7	.304	.322	8.834	.647
9	4.2	4.3	1.5	2.2	3.0	.300	.358	.831	.689
10	17.9	7.6	2.6	5.2	3.3	.414	.294	2.886	.429
11	3.2	1.6	1.3	1.6	.9	.342	.561	.760	.561
12	5.2	3.4	1.9	1.8	4.1	.416	.390	1.145	.731
Mean ± S.D.[e]	11.8 ± 12.6	13.7 ± 20.0	2.2 ± 0.9	12.0 ± 20.8	3.4 ± 1.6	.348 ± .051	.371 ± .086	1.958 ± 2.253	.648 ± .270
%C.V.[f]	106.8	146.0	40.9	173.3	47.1	14.7	23.2	115.1	41.7
Range	3.2-48.6	1.6-61.0	1.2-4.5	0.9-61.0	0.9-6.6	.260-.416	.233-.561	.613 8.834	.343-1.346

Endnotes on next page of table

Continuation of Table 2.5

Subject Identification Number	Vl (inf)[a] (L/kg)	Vl (po)[a] (L/kg)	Vd (inf)[c] (L/kg)	Vd (po)[c] (L/kg)	V2 (inf)[d] (L/kg)	V2 (po)[d] (L/kg)	Cl (inf)[a] (ml/min/kg)	Cl (po)[a] (ml/min/kg)
1	.202	.754	.762	.754	.560	0.0	4.50	4.31
2	.441	.441	.937	.819	.496	.378	4.48	4.45
3	.128	.256	.706	.810	.578	.555	3.06	3.15
4	.164	.204	.689	.670	.525	.466	4.65	4.59
5	.304	.373	.779	.840	.475	.475	4.79	5.26
6	.139	.663	.754	.663	.615	0.0	4.43	3.99
7	.291	.475	.868	.798	.577	0.0	5.31	6.49
8	.032	.448	.929	.901	.897	.452	4.72	4.84
9	.311	.495	.859	.951	.547	.457	4.32	5.68
10	.095	.601	.662	.883	.567	.282	4.57	4.38
11	.317	.603	.773	.603	.455	0.0	4.36	5.64
12	.302	.430	.832	.807	.530	.377	5.77	5.24
Mean ± S.D.	.227 ± .118	.479 ± .160	.796 ± .090	.792 ± .103	.569 ± .113	.287 ± .222	4.58 ± 0.64	4.84 ± 0.89
%C.V.	52.0	33.4	11.3	13.0	19.9	77.4	14.0	18.4
Range	.032-.441	.204-.754	.662-.937	.603-.951	.455-.897	0.0-.555	3.06-5.77	3.15-6.49

[a] Pharmacokinetic parameters calculated by a computer software program see endnotes and acknowledgement

[b] $\alpha \cdot \beta / K21$

[c] $Vl \cdot Kel/\beta$

[d] $Vd - Vl$

[e] $\Sigma X/N \pm [\Sigma(X_i - \bar{X})^2/N-1]^{1/2}$

[f] $S.D. \cdot 100/\bar{X}$

Table 2.6 Pharmacokinetic Parameters from One Compartment Modeling of Drug Plasma Data

Subject Identification Number	Kel[a] (hr^{-1})	Ka[a] (hr^{-1})	Vd[a] (L/kg)	Lag-time[a] (min)	t1/2[a] (hr)	Cl[a] (ml/min/kg)
1	.343	4.4	.754	8.6	2.02	4.31
2	.335	13.4	.797	13.0	2.07	4.45
3	.295	3.7	.681	8.4	2.35	3.34
4	.544	20.0	.522	9.1	1.27	4.73
5	.456	7.7	.710	13.7	1.52	5.39
6	.360	3.2	.663	8.1	1.93	3.99
7	.492	1.4	.795	15.6	1.41	6.49
8	.345	7.7	.844	13.5	2.01	4.86
9	.374	5.8	.913	9.0	1.85	5.70
10	.270	2.9	.916	3.7	2.57	4.15
11	.545	.9	.620	11.0	1.27	5.63
12	.445	8.1	.712	8.4	1.56	5.29
Mean $\pm$ S.D.[b]	.400 $\pm$ .093	6.6 $\pm$ 5.5	.744 $\pm$ .117	10.2 $\pm$ 3.3	1.82 $\pm$ 0.42	4.86 $\pm$ 0.88
%C.V.[c]	23.3	83.3	15.7	32.4	23.1	18.1
Range	.270-.545	0.9-20.0	.522-.916	3.7-15.6	1.27-2.57	3.34-6.49

[a] Pharmacokinetic parameters calculated by a computer software program see endnotes and acknowledgement

[b] $\Sigma X/N \pm [\Sigma(X_1-\bar{X})^2/N-1]^{1/2}$

[c] S.D. $\cdot$ $100/\bar{X}$

Clearance was estimated to be 4.6 ml/min/kg after intravenous administration compared to 4.8 ml/min/kg and 4.9 ml/min/kg after oral administration from two compartment and one compartment modeling respectively. Clearance values of 4.6 ml/min/kg after infusion and 4.7 ml/min/kg after oral dyphylline administration were also determined by model independent methods. Additionally, clearance and half-life of the drug were calculated from urinary data, as described in the methods section. The average half-life after intravenous infusion using urinary data was 2.5 hours and after oral administration 2.2 hours. Dyphylline clearance was 4.3 ml/min/kg after oral dosing and 4.2 after intravenous infusion (Table 2.7) based on urinary excretion data.

Volume of distribution after pseudoequilibrium was 0.80 L/kg after two compartment modeling for dyphylline intravenous infusion data and 0.79 L/kg after oral dyphylline administration and 0.73 L/kg after one compartment modeling of the dyphylline plasma data after oral dosing. This is in agreement with values for volume of distribution and clearance calculated from some past studies of dyphylline (7,12,19) and about twice that reported by others (8).

Rates of dyphylline absorption from conventional tablets calculated by several methods are shown in Table 2.8. Average absorption half-life ranged from 6 minutes to 17 minutes depending on the calculation method and assumptions involved.

Table 2.7 Elimination Rate and Clearance Parameters Calculated from Both Plasma and Urine Data

Subject Identification Number	β(inf), U[a] (hr^{-1})	β(inf), P[b] (hr^{-1})	β(po), U[c] (hr^{-1})	β(po), P[b] (hr^{-1})	Cl(inf), U[d] (ml/min/kg)	Cl(inf), P[b] (ml/min/kg)	Cl(po), U[e] (ml/min/kg)	Cl(po), P[b] (ml/min/kg)
1	.371	.354	.535	.343	5.07	4.50	10.38	4.31
2	.259	.288	.348	.326	3.81	4.48	3.78	4.45
3	.265	.260	.332	.233	1.40	3.06	2.43	3.15
4	.217	.405	.412	.411	4.86	4.65	3.09	4.59
5	.195	.369	.195	.372	5.96	4.79	3.03	5.26
6	.261	.354	.295	.360	4.60	4.43	3.01	3.99
7	.298	.367	.269	.486	5.17	5.31	4.90	6.49
8	.217	.304	.185	.322	4.23	4.72	3.88	4.84
9	.277	.300	.278	.358	3.94	4.32	5.47	5.68
10	.350	.414	.311	.294	3.24	4.57	3.57	4.38
11	.325	.342	.353	.561	**	4.36	3.41	5.64
Mean ± S.D.[f]	.276 ± .056	.342 ± .049	.319 ± .098	.370 ± .090	4.23 ± 1.26	4.47 ± 0.54	4.27 ± 2.21	4.80 ± 0.92
%C. V.[g]	20.3	14.3	30.7	24.3	29.8	12.1	51.8	19.2
Range	.195-.371	.260-.414	.185-.535	.233-.561	1.40-5.96	3.06-5.31	2.43-10.38	3.15-6.49

[a] The negative slope value of the plot of % of dyphylline remaining to be excreted vs. time after intravenous infusion

[b] Parameter calculated by a computer software program see endnotes and acknowledgement

[c] The negative slope value of the plot of % of dyphylline remaining to be excreted vs. time after oral administration of tablets

[d] Value of the slope from the plot of excretion rate of a given urine collection interval vs. drug plasma concentration at the midpoint of the given urine collection interval after intravneous infusion

[e] Value of the slope from the plot of excretion rate of a given urine collection interval vs. drug plasma concentration at the midpoint of the given urine collection interval after oral administration of tablets

[f] $\Sigma X/N \pm [\Sigma(X_i-\bar{X})^2/N-1]^{1/2}$

[g] S.D. · 100/$\bar{X}$

Table 2.8 Absorption Rate Constants of Dyphylline from Tablets

Subject Identification Number	Ka, 1C[a] (hr^{-1})	Ka, 2C[b] (hr^{-1})	Loo-Riegelman[c] (hr^{-1})	Wagner-Nelson[c] (hr^{-1})	Ka, (log)MAT[d] (hr^{-1})	Ka, (lin)MAT[e] (hr^{-1})	Tp[f] (min)
1	4.4	4.4	2.3	2.7	1.7	1.7	31.4
2	13.4	6.6	4.0	2.9	7.4	7.1	17.1
3	3.7	1.7	1.5	2.2	1.1	1.2	31.2
4	20.0	5.0	5.8	1.3	2.8	2.8	11.4
5	7.7	3.6	2.7	3.3	2.5	2.4	16.5
6	3.2	3.2	2.9	2.9	1.4	1.4	31.9
7	1.4	1.4	1.4	1.4	2.0	2.1	44.4
8	7.7	3.7	2.5	3.0	1.9	1.7	16.6
9	5.8	3.0	2.5	4.2	4.7	4.9	31.1
10	2.9	2.3	4.0	3.1	.6	.6	55.3
11	.9	.9	.5	1.2	2.0	1.9	79.0
12	8.1	4.1	1.5	4.9	1.6	1.6	21.9
Mean ± S.D.[g]	6.6 ± 5.5	3.3 ± 1.6	2.6 ± 1.4	2.8 ± 1.1	2.5 ± 1.9	2.5 ± 1.8	32.3 ± 19.4
%C.V.[h]	83.3	48.5	53.8	39.3	76.0	72.0	60.1
Range	.9-20.0	.9-5.0	.5-5.8	1.2-4.9	.6-7.4	.6-7.1	11.4-79.0

[a] Absorption rate constant obtained after computer fitting of a one compartment open model

[b] Absorption rate constant obtained after computer fitting of a two compartment open model

[c] Parameter calculated by a computer software program, see endnotes and acknowledgement

[d] Absorption rate constant calculated via the statistical moment theory using the logarithmic trapezoidal rule

[e] Absorption rate constant calculated via the statistical moment theory using the linear trapezoidal rule

[f] Time to peak drug concentrations in the plasma calculated as time to peak observed - lag time

[g] $\Sigma X/N \pm [\Sigma(X_i-\bar{X})^2/N-1]^{1/2}$

[h] S.D. · $100/\bar{X}$

Therefore, time to complete absorption, assuming six half-lives until complete absorption, is from 36 minutes to 1.7 hours. The average time for peak dyphylline plasma concentration after administration of dyphylline tablets was 45 minutes with a range of 20-90 minutes. Lag time averaged 10.2 minutes with a range of 4.7-15.6 minutes (refer to Table 2.9). Maximum dyphylline plasma concentration after the oral dose averaged 16.6 mcg/ml and ranged from 8.0 mcg/ml to 28.8 mcg/ml. In comparison, maximum dyphylline plasma concentration after a 20 minute intravenous infusion averaged 30.3 mcg/ml and ranged from 18.4 mcg/ml to 46.8 mcg/ml (Table 2.9).

Dyphylline cardiovascular effects from a 20 minute intravenous infusion of dyphylline are summarized in Table 2.10. At the time of infusion cessation systolic blood pressure was an average of 9.4 mmHg lower in 8 of 12 subjects (2 subjects had higher systolic levels and in 2 subjects systolic levels remained unchanged) compared to predosing systolic blood pressure levels. There was no apparent effect on diastolic blood pressure in 8 of the 12 subjects (in 3 subjects diastolic blood pressure was lower and in one subject diastolic blood pressure was higher than predosing diastolic blood pressure levels). Average heart rate was higher than at predosing level in 9 of 12 subjects (in 2 subjects the pulse was lower while in one subject it was unchanged). The difference between predose and drug peak effects on systolic blood

Table 2.9 Drug Peak Plasma Concentrations and Time to Peak in Each Subject

Subject Identification Number	C_{max} (Inf)[a] (mcg/ml)	C_{max} (po)[b] (mcg/ml)	tp(po)[c] (min)	lag t[d] (min)	tp(po)[e] corr (min)
1	27.9	18.0	40.	8.6	31.4
2	29.5	18.4	30.	12.9	17.1
3	41.4	28.8	40.	8.8	31.2
4	30.5	24.6	20.	8.6	11.4
5	24.2	17.8	30.	13.5	16.5
6	46.8	16.7	40.	8.1	31.9
7	18.4	10.0	60	15.6	44.4
8	26.3	11.4	30.	13.4	16.6
9	30.3	13.2	40.	8.9	31.1
10	24.9	8.0	60.	4.7	55.3
11	33.3	14.2	90.	11.0	79.0
12	27.0	17.7	30.	8.1	21.9
Mean ± S.D.[f]	30.3 ± 7.8	16.6 ± 5.9	40.0 ± 22.6	10.2 ± 3.1	32.3 ± 19.4
%C. V.[g]	25.7	35.5	56.5	30.4	60.1
Range[h]	18.4-46.8	8.0-28.8	20.-90.	4.7-15.6	11.4-79.0

[a] Drug peak plasma concentrations after a 1000 mg 20 minutes infusion

[b] Drug peak plasma concentrations after a 1000 mg dose of tablets

[c] Observed time to peak after oral dosing

[d] Computer calculated lag time

[e] tp(po) - lag t, the corrected time to peak

[f] $\Sigma X/N \pm \{\Sigma(X_i-\bar{X})^2/N-1\}^{1/2}$

[g] S.D. · $100/\bar{X}$

Table 2.10 Cardiovascular Effect of Intravenously Administered Dyphylline

Subject Identification Number	Blood Pressure[a] t(0)[c] (mmHg)	Blood Pressure[a] T(cut)[d] (mmHg)	Heart Rate[b] t(0) (beats/min)	Heart Rate[b] T(cut) (beats/min)
1	110/70	108/64	52	64
2	100/70	110/70	50	54
3	120/80	110/80	50	56
4	145/80	140/84	70	72
5	125/70	125/70	62	66
6	140/80	120/80	50	54
7	110/60	110/60	50	56
8	135/90	140/90	60	54
9	115/70	100/58	52	46
10	105/60	100/60	50	56
11	115/80	102/62	60	60
12	120/70	115/70	56	60
Mean[e]	120/73	115/71	55	58
Range	100-145/ 60-90	100-140/ 58-90	50- 70	46- 72

[a] As measured by a sphygmomanometer

[b] As measured by palpitation of the axial pulse

[c] Time zero just prior to dosing

[d] Time of cessation of the 20 minute infusion

[e] $\bar{X}/N$

pressure and heart rate were statistically analyzed using the paired t-test and were not significantly different (28).

Since diastolic blood pressure values were not normally distributed as tested by Wilks-Shapiro test at $\alpha = 0.01$ level, a nonparametric statistical test was used to interpret diastolic blood pressure changes. Statistical analysis of predose and drug peak effects on diastolic blood pressure performed by use of the Wilcoxon Signed Rank test were not statistically significant (28).

One side effect, headache, was reported 11 hours post-infusion in subject 5 during phase 1 of the study. No other side effects were reported, and the headache is not thought to be due to either the drug or the treatment protocol.

2.4 DISCUSSION

Dyphylline is the only chemically distinct xanthine alternative to theophylline available on the market in the United States for the treatment of obstructive lung disease. Its pharmacokinetic and physicochemical properties are distinctly different from theophylline. Dyphylline is 40 times more water soluble than theophylline with at least 80% (7,12,19) of the drug eliminated intact by the kidneys through glomerular filtration and active secretion. The first order elimination half-life of dyphylline, about 2.0 hours (range of 1.3 to 3.4 hours), is short compared to theophylline (6,7,8,9,12,13,19,20). At minimum reported therapeutic concentrations of 12 to 16 mcg/ml (4,5) dyphylline has little or no side effects and toxic concentrations in humans have not been defined. Concentrations as high as 50 mcg/ml after a 5 minute intravenous infusion of 400 mg of dyphylline with no side effects reported are possible (19). In contrast, theophylline is poorly soluble (22) but is reported to be a more potent bronchodilator (4,34,35,36) with a greater incidence of toxicity than dyphylline (3,4,8,37). About 90% of a dose of theophylline is metabolized and its half-life is variable from 3 hours to

9.5 hours in normal adults with the half-life decreased as much as 40% in smokers, and as long as 15 to 58 hours in premature infants (33).

Controversy has developed over dyphylline's relative value as an effective bronchodilator (13,39-45) and whether it has acceptable bioavailability (13,38,40-44). Although the controversy of dose levels sufficient to produce effective bronchodilation remains to be clarified, results from this experiment indicate dyphylline is rapidly and completely absorbed from tablets. The analysis of extent of bioavailability was performed using two methods. The traditional approach, linear trapezoidal method for estimating area under the curve, gave similar results to the logarithmic trapezoidal rule. The advantage of the logarithmic trapezoidal rule is to minimize error in estimating area under the curve during the elimination phase. The logarithmic trapezoidal method employs the linear trapezoidal equation from time zero just prior to dosing until peak drug concentrations; then the logarithmic trapezoidal equation is used from drug peak concentrations to the last concentration and then extrapolated to infinity. The linear trapezoidal rule tends to underestimate area under the curve during the absorption phase while overestimating area under the curve during the elimination phase. The net error from using the linear trapezoidal rule for assessing total area under the curve is dependent on the

shape of the curve and frequency of sampling (13). Mathematically expected values for total area under the curve calculated by the logarithmic method are slightly less than area under the curve values calculated by the linear method, however, area under the curve values calculated by both methods were very similar. Similarity of area under the curve values calculated by each method may be due to the short length of the plasma collection interval compared to half-life (collection time interval/half-life, n, is approximately 1.1) during the elimination phase. Chiou (46) has shown that error in estimating area under the curve during the post-absorption phase from the linear trapezoidal rule increases when the ratio of collection time interval to half-life increases. With the drugs (theophylline and doxycycline) he studied, he showed when n = 1., 1.5, 2., 5., percent error = 3.9, 8.8, 15.5, and 84.4 respectively (46).

Assuming no period effect, a paired t-test comparing area under the curve values after a 1000 mg dose of tablets to area under the curve values after a 1000 mg 20 minute infusion of dyphylline failed to show a significant difference at $\alpha = 0.05$ between the two dosage forms. Therefore, from the evidence presented, a short intravenous infusion of 1000 mg of dyphylline would be equivalent to a 1000 mg dose of tablets. However, due to dyphylline's short half-life of approximately 2 hours, treatment of chronic obstructive

pulmonary disease with a conventional oral dose of dyphylline would be impractical in maintaining prescribed therapeutic levels. In addition, due to the wide range of subjects' weight (Table 1.1), 3 of 12 subjects receiving the 1000 mg of dyphylline as tablets (2 x 400 mg + 1 x 200 mg tablet) had peak plasma levels of drug below the recommended therapeutic level, 12 mcg/ml. If these subjects were administered a dose based on body weight of 15 mg/kg as recommended by Simons (5) then all subjects would obtain therapeutic levels. However, such a large dose in a conventional tablet coupled with dyphylline's short half-life would result in impractical demands on the patient to maintain compliance.

Dyphylline is readily absorbed into the general circulation after oral dosing. Several methods (Table 2.8) were employed to estimate the rate of absorpton from the tablet. Of the seven methods used, all except Ka obtained after one compartment modeling of plasma dyphylline data gave similar average sample absorption rate values although each method varied for a given subject. The longest absorption half-life, 17 minutes, was obtained with the statistical moment theory using the logarithmic trapezoidal rule or the linear trapezoidal rule. The shortest half-life, 6 minutes, was obtained from the absorption rate constant after fitting data to a one compartment open model. This last method seems least appropriate for determining the absorption rate

constant. Since infusion data of the drug is best described as a two compartment open model with a very definite α-phase, one would be led to predict similar drug disposition characteristics after oral administration of a conventional tablet. The short absorption half-life obtained from Ka after one compartment modeling of the data may be due to the absorption rate constant masking the α-phase resulting in a larger calculated Ka then "true" Ka. However, in 4 subjects (1,6,7,11) the Ka value from one compartment modeling of plasma data is similar or the same as the Ka value from two compartment modeling. For these subjects two compartment modeling may be inappropriate since Kel ≃ β, K21 ≃ α, K12 ≃ 0, and V2 ≃ 0. Thus the two compartment model has collapsed into a one compartment model (16). Therefore, one compartment modeling of oral dyphylline plasma data for these subjects is the most appropriate method to describe the data. Since the absorption of a drug requires many complex steps (dissolution, stomach emptying, food-drug interaction, drug degradation, etc.) which may not be adequately summarized precisely by one constant, any of the methods described may be appropriate to approximate the relative speed of absorption but precaution must be taken in interpreting Ka values obtained from pharmacokinetic modeling. Additional precautions can be taken in checking values assigned to Ka and α for any drug administered orally when intravenous phar-

macokinetic data is available on the same drug. The intravenous data should identify the approximate α-value which remains the same after oral administration and checking can insure that α and Ka values have not been erroneously interchanged. Examination of α-values calculated by a software program (29) from data of plasma dyphylline concentration vs. time after oral and intravenous infusion and of Ka values after oral administration (Table 2.5), supported the conclusion that these values have been properly assigned to the respective pharmacokinetic variables.

In determining which pharmacokinetic model to use to describe the data the investigator must first decide what his objectives are in fitting the data. If the only objective is to fit a line to the data points the simplest model should be used since a more complex model may do little in improving fit. However, if there are additional objectives such as examining specific pharmacokinetic parameters after modeling a more circumspective approach should be exercised for the above reasons discussed. For the dyphylline data in this study, all plasma drug concentration time data were fit to a two compartment open model because the object of fitting was to obtain accurate pharmacokinetic parameters. However, in 4 aforementioned subjects (1,6,7,11), the two compartment model collapses into a one compartment model, therefore a one compartment model is the appropriate model to describe the data

for these particular subjects.

Data from Table 2.3 indicates an average of about 79% of the administered dose of dyphylline was recovered in the urine 48 hours postdosing each subject with intravenous or oral dyphylline (1000 mg). The unaccounted for 200 mg of dyphylline may be distributed to deep tissues as suggested by Gisclon (7) or possibly metabolized. However, metabolism seems unlikely since no metabolites have ever been identified and because of dyphylline's physicochemical nature of being a very polar compound (1 G dissolves in 3 ml of water)(22). Dyphylline may accumulate in tissues and be slowly eliminated similar to the pharmacokinetic characteristics of the aminoglycosides (47,48,49). A constant infusion of dyphylline to obtain steady state concentrations may determine whether dyphylline is being slowly eliminated from deep tissue stores. If steady state concentration values are higher and steady state takes longer to reach than predicted, this may be due to the presence of a longer half-life not detected from single dose pharmacokinetic modeling.

All the dose administered was not collected in the urine during the collection period. Therefore, to calculate the elimination rate constant from a sigma-minus plot, it was assumed the best estimate of total amount of dyphylline excreted in the urine was not the dose administered but the actual amount of dyphylline recovered in the urine. If the

elimination rate constant is calculated assuming the cumulative amount of dyphylline ultimately excreted in the urine is equal to the dose administered, then the resultant sigma-minus plot curve asymtopically approaches the value of percent of drug not recovered in the urine. The curvature of the line is more pronounced the greater the value of unrecovered dose from the urine. This artifact may misleadingly decrease the value of the elimination rate constant. Therefore, the elimination rate constant was calculated from sigma-minus plots with the assumption that the total amount of drug excreted in the urine during the study period was equal to the cumulative amount of drug actually collected from the urine and not the dose administered. The elimination rate constants, β, calculated from urine data after intravenous infusion (refer to Table 2.7) were statistically different from the elimination rate constant calculated from the plasma data when analyzed by the paired t-test ($p = .009$). The elimination rate constant calculated from the urine data after oral administration was not statistically different from the elimination rate constant calculated from plasma data when analyzed by the paired t-test ($p = .23$). However, half-lives calculated from elimination rate constant of urine data after intravenous infusion and oral administration, 2.5 hours and 2.2 hours, were longer than half-lives calculated from plasma data. The longer half-life obtained from urine

data was detectable due to the longer collection period of 48 hours compared to 12 hours for the plasma samples. Referring to Figure 2.3, curves for subjects 1, 5, 7, and 9 show evidence of curvature suggesting nonlinear or multicompartmental pharmacokinetics. For these subjects the actual half-life for the drug may be longer than values calculated from linear pharmacokinetic modeling indicates. Further examination of these curves by the use of CSTRIP (50), a curve fitting software package, reveals curve(s) for subjects 2, 4, 5, 8, and 9 are best described by two exponential terms rather than one exponential term. This also implies for these subjects that multicompartmental or nonlinear pharmacokinetics are more appropriate than a one-compartment open model. The half-life calculated from the rate constant of the terminal slope for these subjects ranged from 2.4 - 20.4 hours. The average half-life calculated from the average rate constant of the terminal slope in these five subjects was 4.6 hours. While this is interesting pharmacokinetically, it probably has no significance clinically since the plasma concentration is so low before the curvature is observed. Clearance is also slower when calculated with the use of urine data compared to the clearance parameter calculated from model dependent plasma data (see Figure 2.4). However, the differences between the clearance values calculated by the two methods were not statistically different. The clinical significance of these

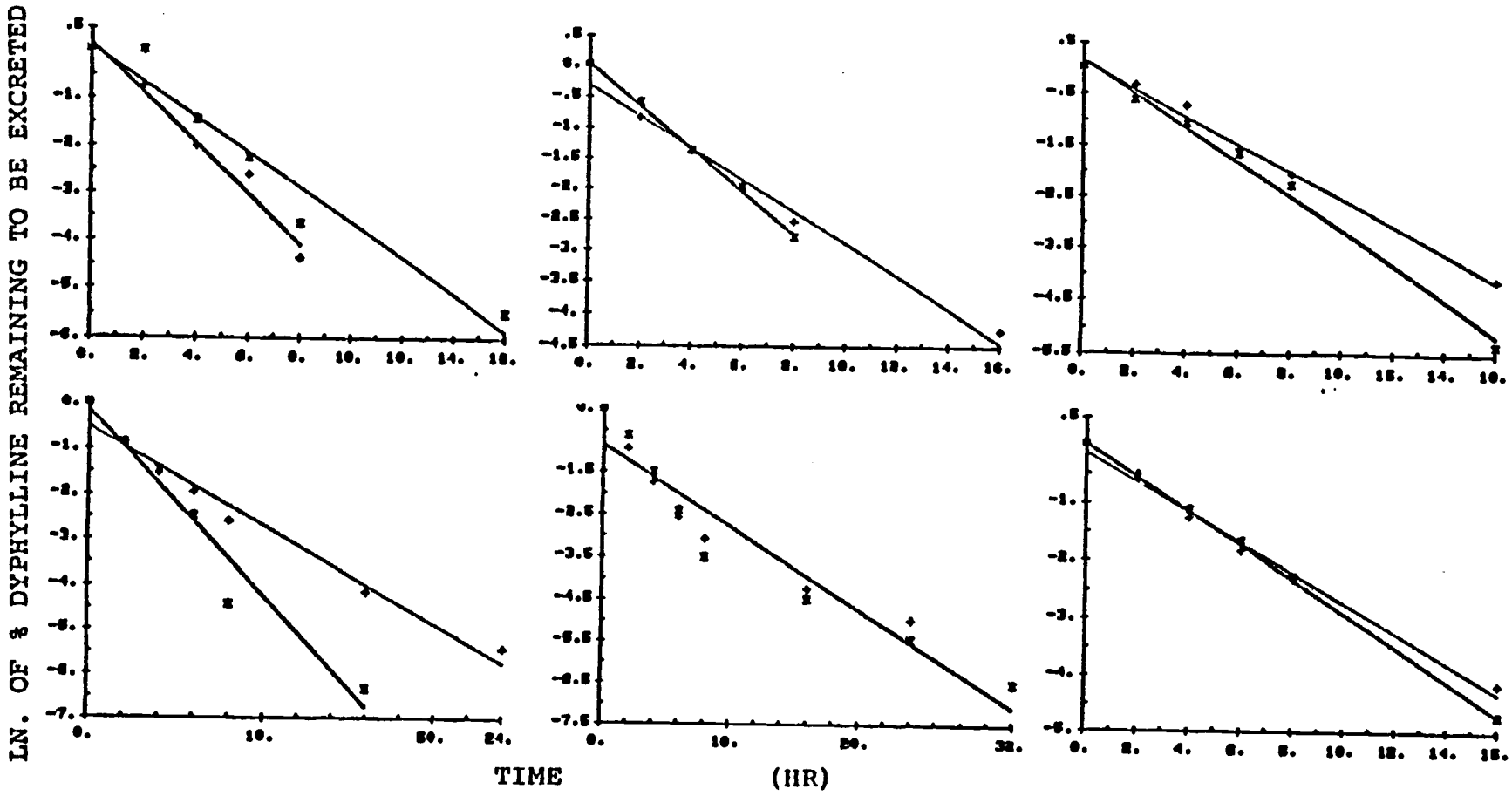

Figure 2.3 Individual Sigma-Minus Plots of the Natural Log. of Percent Dyphylline Remaining to be Excreted vs. Time in hours of subjects: 1, 2, 3, 4, 5, 6 (from left to right) following infusion of dyphylline 1000 mg represented by the symbol + and following the administration of dyphylline 1000 mg as conventional tablets represented by the symbol *.

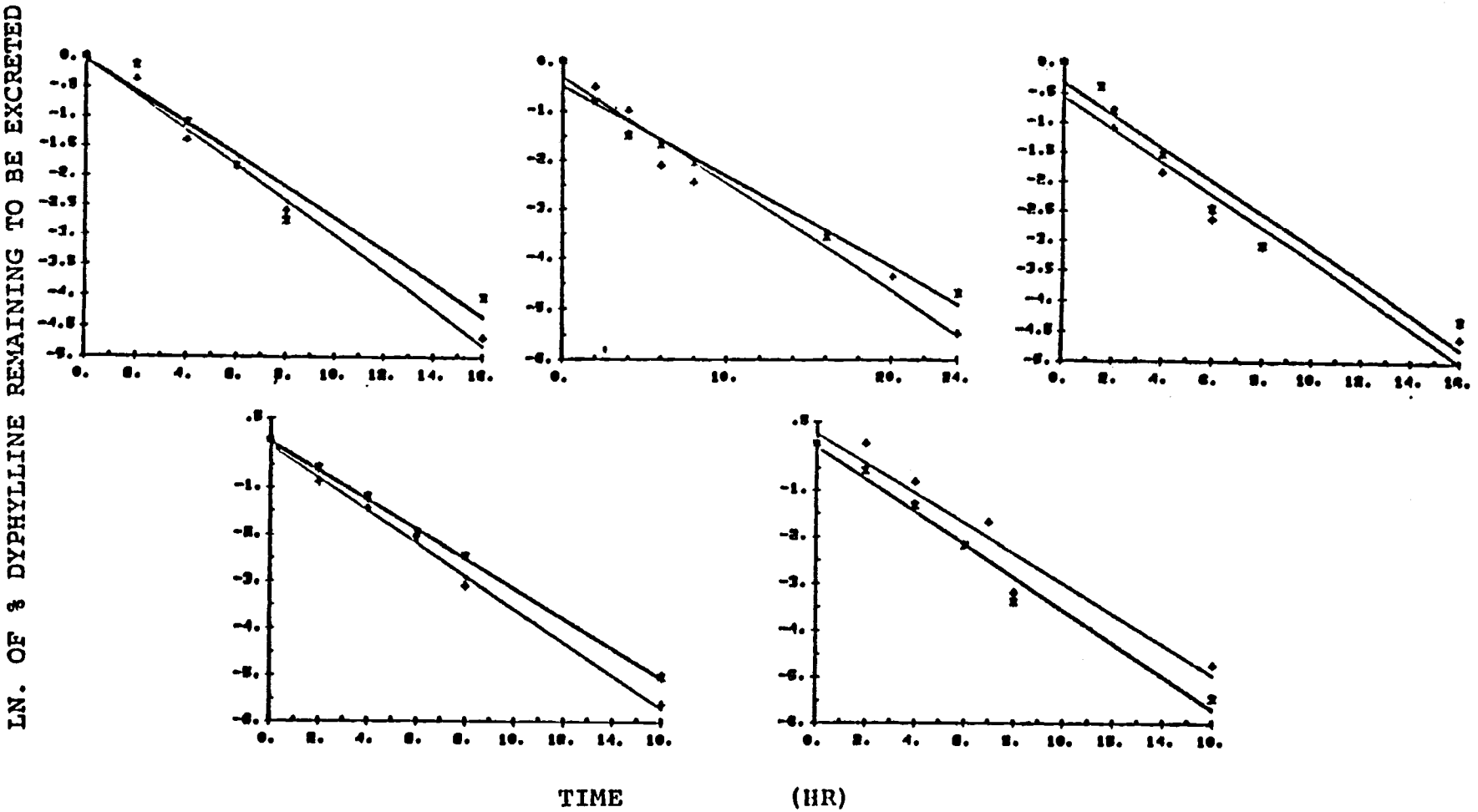

Figure 2.3 (continued) Individual Sigma-Minus Plots of the Natural Log. of Percent Dyphylline Remaining to be Excreted vs. Time in hours of subjects: 7, 8, 9, 10, 11 (from left to right) following infusion of dyphylline 1000 mg represented by the symbol + and following the administration of dyphylline 1000 mg as conventional tablets represented by the symbol *.

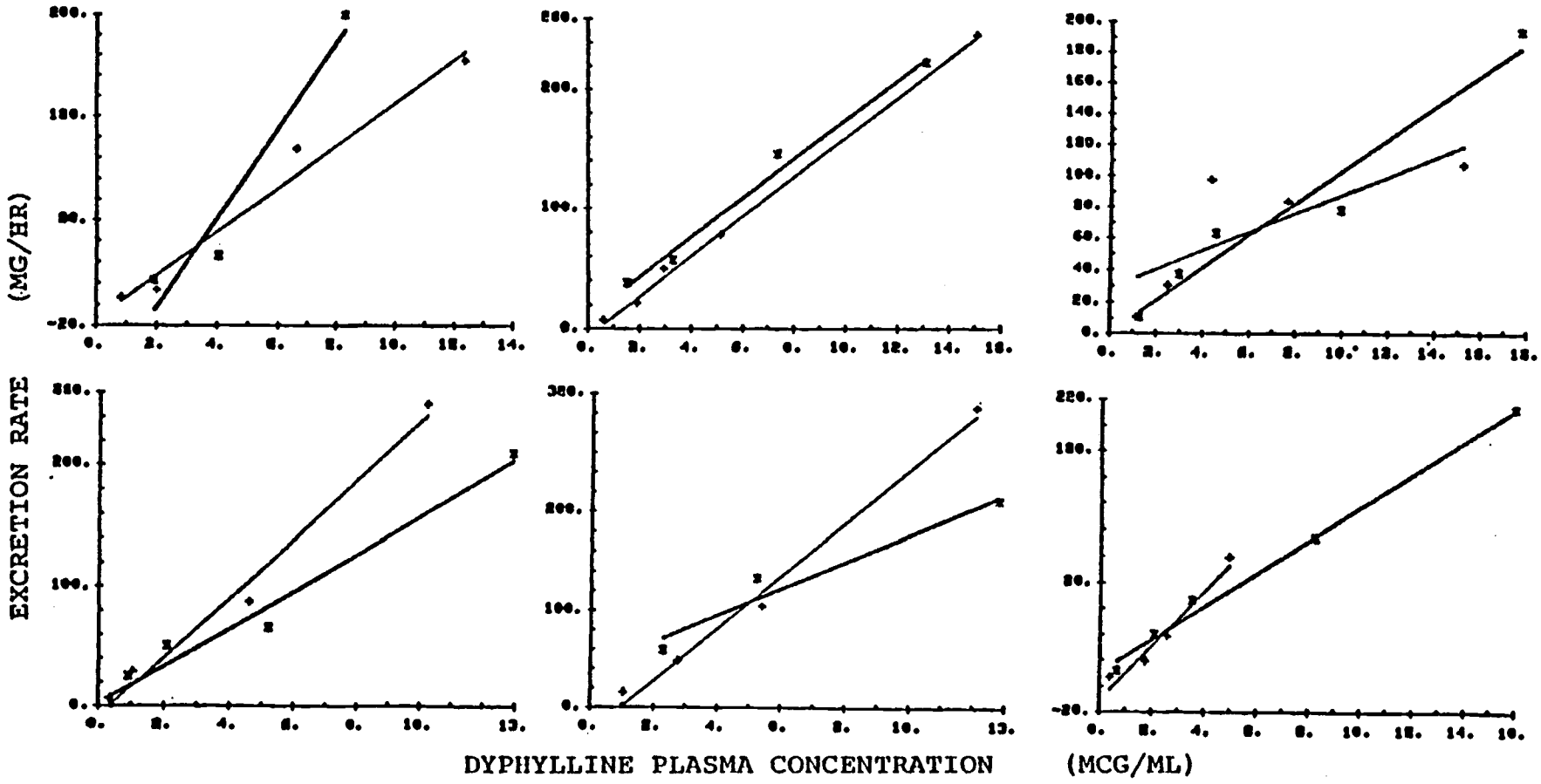

Figure 2.4 Individual Dyphylline Urinary Excretion Rate vs. Dyphylline Plasma Concentration (determined at the midpoint of each urine collection interval) in subjects: 1, 2, 3, 4, 5, 6 (from left to right) following infusion of dyphylline 1000 mg represented by the symbol + and following the administration of dyphylline 1000 mg as conventional tablets represented by the symbol *.

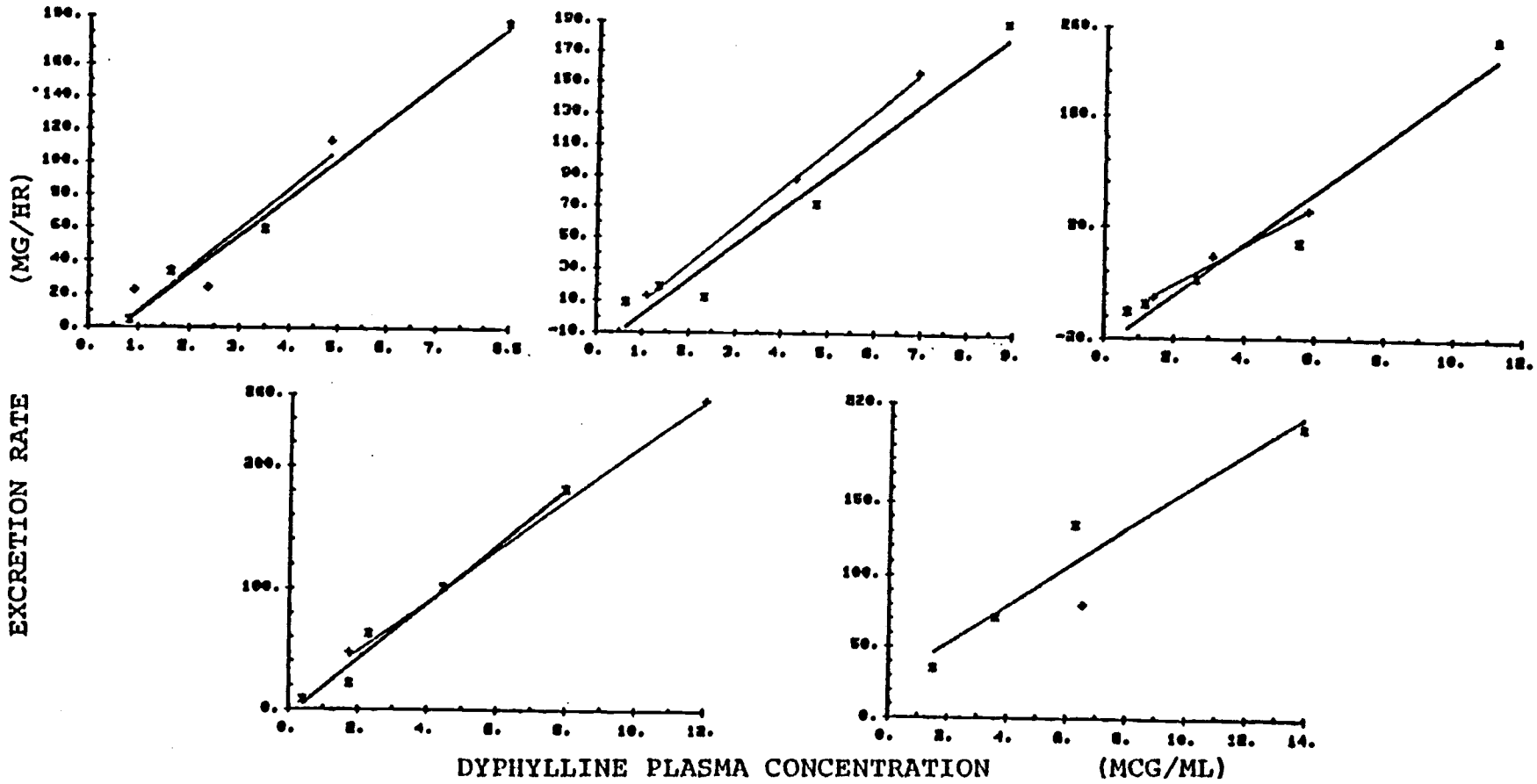

Figure 2.4 (continued) Individual Dyphylline Urinary Excretion Rate vs. Dyphylline Plasma Concentration (determined at the midpoint of each urine collection interval) in subjects: 7, 8, 9, 10, 11 (from left to right) following infusion of dyphylline 1000 mg represented by the symbol + and following the administration of dyphylline 1000 mg as conventional tablets represented by the symbol *.

different values for clearance and elimination half-life of dyphylline will probably be unimportant since dyphylline displays little toxicity and an apparent wide therapeutic index.

Conclusions about bioavailability based on urine data is probably less reliable than that obtained from plasma data. The correlation (r^2 = .02) between percent fraction absorbed, %f, from urine data with %f from the plasma data was poor (see Tables 2.2 and 2.3). Several possible reasons for this discrepancy are proposed. In this group of subjects there was a statistical difference in urine volumes in week 1 vs. week 2 of this study. The difference in urine volumes measured from week to week may be due to incomplete collection of all urine voided or may be due to natural variation in urine output. A major factor effecting dyphylline urine data reliability is the assay procedure. The urine standard curve coefficient of variation averaged 23.4% and ranged from 5.8 to 60.1%. The high degree of variation in the dyphylline standard curve for urine data reflects the range of variance in assigning a concentration value to dyphylline unknown urine samples gathered from each subject using these standard curves. The coefficient of variation for amount of drug collected was about 15%, which is higher than other past dyphylline studies (7,12) where coefficient of

variation was 5-6%, but compares favorable with other drugs' coefficient of variation, 10-40% (51-56).

In addition to pharmacokinetic characteristics of dyphylline, one pharmacological effect of dyphylline was monitored during peak dyphylline plasma concentrations after an intravenous infusion. The cardiovascular effects of dyphylline are reviewed in Table 2.10. The predominant effect of intravenously infused dyphylline of very slightly lowering systolic blood pressure may be due to arterial dilation caused by the direct effect of dyphylline on the vascular smooth muscle or stimulation of the β-adrenoreceptor. The increased heart rate may be a reflex baroreceptor effect due to arterial dilation or due to the direct action of the drug. Although these physiological reactions were not statistically different from predosing cardiovascular condition, there does appear to be a slight cardiovascular pharmacological effect associated with dyphylline administered in these subjects.

2.5 ENDNOTES

a Neothylline Tablets, 400 mg, 200 mg, Lemmon Co., Sellerville, PA 18960

b Neothylline Injection, 500 mg/2 ml, Lemmon Co., Sellerville, PA 18960

c Bacteriostatic Normal Saline, 30 ml ampule, Abbott Laboratories, North Chicago, IL 60064

d Harvard Infusion Pump, model 941, Infusion/Withdrawal pump, Harvard Apparatus, Millis, MA

e Quik-Cath (20 G), Travenol Laboratories, Inc., Deerfield, IL
Sterile Intermittent Infusion Plug, Argyle Co., St. Louis, MO

f Quik-Cath (20 G), Travenol Laboratories, Inc., Deerfield, IL

g Aneroide Sphygmomanometer, Taylor Instruments, c/o Scientific Products, McGaw Park, IL 60085

h 3 ml, 10 ml Plain Disposable Syringes, Becton-Dickson, Rutherford, NJ 07070

i Vacutainer (143 U Heparin), 10 ml, Becton-Dickson, Rutherford, NJ 07070

j Pan-heparin, 1,000 USP units/ml, Abbott Laboratories, North Chicago, IL

k Polyethylene Sample Containers, American Scientific Products, McGaw Park, IL 60085

l M-6000A Pump, Waters Associates, Milford, MA 01757
μ-Bondapak C18 Colmn, Waters Associates, Milford, MA
Model 440 Absorbance Detector, Milford, MA
Dual Pen Recorder, Soltec Co., Encino, CA
WISP 710B, Waters Associates, Milford, MA

m β-hydroxyethyltheophylline, Pierce, Rockford, NJ 61105

n Acetonitrile, HPLC Grade, J.T. Baker Chemical Co., Phillipsburg, NJ 08865

o Sodium hydroxide, Mallinckrodt Chemical Works, St. Louis, Mo 63160

p Isopropanol, Reagent Grade, J.T. Baker Chemical Co., Phillipsburg, NJ

q Methylene chloride, Reagent Grade, J.T. Baker Chemical Co., Phillipsburg, NJ

r Vacuum Oven, Lab-Line Instruments, Inc., Melrose Park, IL

s Methanol, Reagent Grade, J.T. Baker Chemical Co., Phillipsburg, NJ

t β-hydroxypropyltheophylline, Aldrich Chemical Co., Inc., Milwaukee, WI 53233

u Amberlite, XAD-2, Mallinckrodt Inc., Paris, KT 40361

v Chloroform, Reagent Grade, J.T. Baker Chemical Co., Phillipsburg, NJ

w Drugfun, See Acknowledgement of Prophet and Reference 29.

CONCLUSION

Dyphylline is completely and quickly absorbed from the conventional tablets tested. However, the relative large dose and short half-life may suggest dyphylline's spectrum of oral use is best for those subjects unable to tolerate aminophylline or theophylline. A more useful application of dyphylline would be as the intravenous dosage form where its short half-life, rapid onset of action, and low toxicity would be ideal in the critical care area or emergency situation. More studies are necessary to elucidate dyphylline's effective dose and its pharmacokinetic behavior during constant steady state infusion.

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APPENDIX

Appendix 1. Analysis of Variance of Extent of Absorption in a Two-Way Crossover Design

The two-way crossover design of the absolute bioavailability of dyphylline tablets in humans is as follows in Table 1.

Table 1. The Two-Way Crossover Design

		Time Period[a]	
Group	Subjects[b]	I	II
1	1-6	A[c]	B[d]
2	7-12	B	A

[a] A one week washout period separated the two time periods.

[b] Numbers correspond to subject identification number.

[c] Treatment A is 1000 mg of dyphylline (Neothylline®) administered at 50 mg/min (concentration of approximately 50 mg/ml of normal saline) followed by 220 ml water orally.

[d] Treatment B is 1000 mg of dyphylline (Neothylline®) administered orally as two 400 mg tablets and one 200 mg tablet followed by 240 ml of water orally

The ANOVA (analysis of variance) table of AUC calcuated by the linear trapezoidal rule for a two-way crossover design is as follows:

Table 2. Analysis of Extent of Absorption of Dyphylline Tablets

Source of Variation	d.f.	SS	MS	F	Sig. level
Total	23	12076618.1	---	---	---
Subjects	11	11191539.8	1017412.71	17.40	p<.005
Groups	1	3947461.6	3947461.6	67.51	p<.005
Subject/Group	10	7244078.2	724407.8	12.39	p<.005
Time Period	1	203946.5	203946.5	3.49	N.S.
Treatments	1	96418.8	96418.8	1.65	N.S.
Residual	10	584713.0	58471.3	---	---

Reviewing the ANOVA table it can be concluded from this study there is no statistical difference in extent of absorption after Treatment A, the intravenous infusion of 1000 mg of dyphylline and Treatment B, the oral administration of a 1000 mg dose of dyphylline tablets. Neither did this study show any evidence of a statistically detectable period effect or carry over of drug from one week to the next.

Similar results and conclusions can be drawn from analysis of variance of AUC calculated by the logarithmic trapezoidal rule.

Appendix 2. The 95% Confidence Interval Approach, Calculation of Confidence Interval Limits

In assessing bioavailability of a drug using the 95% Confidence Interval Approach, there is general agreement in the literature for drugs not exhibiting dangerously narrow therapeutic windows that the acceptable confidence limits of a 95% confidence interval are 80%, the lower limit, and 120% the upper limit (16,24,25,26).

The general formula used to calculate the confidence limits of a 95% confidence interval is

$$\bar{X} \pm t_{.05} (S/\sqrt{N})$$

Where $\bar{X}$ is the sample mean; $t_{.05}$ is the Student's t-test at $\alpha = 0.05$; S is the standard deviation of the sample; and N is the number of subjects.

The following example illustrates calculations of the 95% confidence interval from experimental data in this text.

Example: Using the data of AUC ratios from column 3 of Table 2.2 (page 41).

$$\bar{X} = 96.1; \quad S = 12.1; \quad N = 12; \quad t_{.05,\,11} = 2.201$$

$$96.1 \pm 2.201 (12.1/\sqrt{12}) = 88.4, 103.8$$

The lower and upper limit of the 95% confidence interval of ratios of AUC values (calculated by the linear trapezoidal

rule) after oral administration compared to dyphylline after intravenous infusion are 88.4% and 103.8% respectively. Since this interval is within the predefined limits of 80-120%, it can be concluded the extent of bioavailability of 1000 mg dose of dyphylline tablets is not significantly different from a 1000 mg intravenous dyphylline infusion.