Abstract approved: Redacted for privacy

James W. Ayres, Ph.D.

Improved assay methods for determination of dyphylline levels in plasma, saliva, and urine utilizing high pressure liquid chromatography have been developed. Detection of levels as low as 25 ng/ml from 0.5 ml plasma and 50 ng/ml from 0.5 ml saliva were possible. Both procedures utilized the same extraction process. A separate extraction process was used for dyphylline analysis in urine due to the presence of interfering substances occurring with previous methods. The method was applied to samples of one subject's plasma, saliva and urine after administration of dyphylline.

Plasma, saliva, and urine dyphylline concentrations were measured after administration of a single oral dose of 15 mg/kg to six healthy male subjects. Improved analytical methods have allowed sampling over longer time periods than have previously been reported. The resultant plasma,
saliva and urine data show that dyphylline pharmacokinetics cannot be described by traditional one or two compartment open models. Model independent pharmacokinetic parameters, including renal clearance, drug excreted, time to plasma peak, and peak plasma values have been determined. In contrast with a previous report, the analysis of dyphylline in urine has established that dyphylline is primarily eliminated unchanged in the urine.

The observed rapid and reproducible rise in plasma dyphylline levels is consistent with prediction of rapid onset of action following oral dosing with the tablet investigated.

A high degree of correlation was found between saliva and plasma dyphylline concentrations of individuals using parabolic equations, the $r^2$ values ranging from 0.935 to 0.996. This relationship suggests nonlinear protein binding of dyphylline occurs within the range of plasma levels attained by the dose given in the study.

These observations should prove to be useful for designing future pharmacokinetic studies and clinically when monitoring of plasma dyphylline levels via saliva sampling is anticipated.
Assay and Pharmacokinetics of Dyphylline Following Oral Administration in Humans

by

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section One</th>
<th>Saliva, Urine and Plasma Analysis of Dyphylline via HPLC.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abstract</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Materials and Methods.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Sample Preparation.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Analysis Procedure.</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Human Data.</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Results and Discussion</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Section Two</th>
<th>Pharmacokinetics of Dyphylline Administered Orally to Humans</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abstract</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Methodology</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Results and Discussion</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>29</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section 1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Typical HPLC chromatogram.</td>
</tr>
<tr>
<td>2</td>
<td>Typical semilogarithmic graph of plasma and saliva dyphylline concentration vs time for one individual</td>
</tr>
<tr>
<td>Section 2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Semilogarithmic graph of mean plasma dyphylline concentration vs time, and mean saliva dyphylline concentration vs time.</td>
</tr>
<tr>
<td>2</td>
<td>Graph of mean saliva dyphylline concentration vs mean plasma dyphylline concentration from 1 to 12 hours post-dosing</td>
</tr>
<tr>
<td>3</td>
<td>Graph of saliva dyphylline concentration vs plasma dyphylline concentration for 4 individuals from the saliva peak to 12 hours post-dosing.</td>
</tr>
<tr>
<td>4</td>
<td>Graph of mean dyphylline urinary excretion rate in mg/hr vs time at mid-point of collection interval.</td>
</tr>
</tbody>
</table>

LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inversely estimated concentrations from individual standard curve data using different pooled standard curves for dyphylline in human plasma</td>
</tr>
</tbody>
</table>
ASSAY AND PHARMACOKINETICS OF DYPHYLLINE FOLLOWING ORAL ADMINISTRATION IN HUMANS

SECTION ONE

SALIVA, URINE AND PLASMA ANALYSIS OF DYPHYLLINE VIA HPLC

Lee Gisclon, Kim Rowse and James Ayres
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Corvallis, Oregon

Abstract

Improved assay methods for determination of dyphylline levels in plasma, saliva, and urine utilizing high pressure liquid chromatography have been developed. Detection of levels as low as 25 ng/ml from 0.5 ml plasma and 50 ng/ml from 0.5 ml saliva were possible. Both procedures utilized the same extraction process. A separate extraction process was used for dyphylline analysis in urine due to the presence of interfering substances occurring with previous methods. The method was applied to samples of one subject's plasma, saliva and urine after administration of dyphylline.

Introduction

Dyphylline (dihydroxypropyltheophylline) is a distinct chemical entity which is an analog of theophylline and not thought to be metabolized to theophylline, but is reported to produce brochodilation by the same mechanism as theophylline, i.e., inhibition of phosphodiesterase, preventing the
breakdown of cyclic 3'5'-AMP (Simons, et al. 1975a). Dyphylline has been found to have a low incidence of adverse effects even when given in large doses (Hudson, et al. 1973; Simons, et al. 1975b). Significant bronchodilation has been demonstrated in asthma and in exercise induced bronchospasm (Simons, et al. 1975a; Hudson, et al. 1973); however, larger than conventional doses were required. Further research is indicated to thoroughly define the efficacy and toxicity of dyphylline in reversible obstructive airway disease. Such research dictates the need to assay dyphylline concentration in body fluids.

Gas chromatography (GC) has been used to assay dyphylline in serum and saliva (Butts, et al. 1974; Shihabi and Dave, 1977) and high pressure liquid chromatography (HPLC) has been used for plasma and serum (Maijub and Stafford, 1976; Drummond, 1977). The GC method is quite time consuming and neither method was reported to have been used to assay samples of urine. In fact, one report (Simons, et al. 1977) states that intact dyphylline could not be detected in the urine using GC. It was necessary in our laboratory to analyze over 500 samples of plasma, saliva and urine for dyphylline with some concentrations in the ng/ml range which required a far more sensitive assay than those previously reported. Therefore, a rapid, specific, simple and sensitive high pressure liquid chromatographic method of analysis was developed.
Materials and Methods

A commercial liquid chromatograph\(^1\) was used at ambient temperature with a fixed wave length ultraviolet detector at 280 nm. All commercial chemicals used were reagent grade and solvents were filtered and degassed prior to use. The mobile phase contained acetonitrile (ACN) 9% V/V in distilled water and the flow rate was 2.0 ml/min. The internal standard used was beta-hydroxyethyltheophylline (BHET) which was made in a one liter flask and kept tightly sealed throughout the experiment except for the brief period each day when aliquots were used for the experiment. Thus, a single internal standard solution of each concentration was used throughout the experiment.

Sample Preparation

Recently expired whole blood was obtained from the Red Cross and different units were used to prepare plasma standard curves. Saliva and urine were obtained from volunteers not taking any drugs. All body fluids were appropriately spiked from stock solutions of dyphylline in distilled water prepared volumetrically. Plasma and saliva standard solutions were prepared to contain the concentrations shown in Table 1 and urine standards contained 5, 10,
25, 50, 75, 100, 250, and 1000 μg/ml. All standard solutions were divided into aliquots and frozen until assayed.

**Analysis Procedure**

**Scheme 1. Method for Analysis of dyphylline in body fluids.**

<table>
<thead>
<tr>
<th>Plasma or Saliva</th>
<th>Urine</th>
</tr>
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<tbody>
<tr>
<td>Thaw plasma or saliva (0.5 ml)</td>
<td>Thaw and combine 1.0 ml urine with 1.0 ml internal standard solution (BHET 10 μg/ml in distilled water)</td>
</tr>
<tr>
<td>Add 0.5 ml ACN containing internal standard (BHET 800 ng/ml)</td>
<td>Vortex</td>
</tr>
<tr>
<td>Add 0.5 ml of 0.2N NaOH Vortex x 30 seconds</td>
<td>Pass through an XAD-2 column 5 pre-washed with 50 ml methanol and then 10 ml water</td>
</tr>
<tr>
<td>Add 5.0 ml Isopropanol/Chloroform mixture (10/90 by volume) 3</td>
<td>Rinse column with 10 ml water and discard</td>
</tr>
<tr>
<td>Shake gently for 10 minutes</td>
<td>Wash columns with 15 ml isopropanol/chloroform (1:3) in two portions and collect in a centrifuge tube containing 1.0 ml of 0.2N NaOH</td>
</tr>
<tr>
<td>Centrifuge x 15 minutes</td>
<td>Shake gently 5 minutes</td>
</tr>
<tr>
<td>Suction off aqueous layer and protein precipitate</td>
<td>Centrifuge and aspirate aqueous layer</td>
</tr>
<tr>
<td>Dry organic layer with anhydrous granular sodium sulfate</td>
<td>Transfer 1.0 ml to a new tube and evaporate to dryness under nitrogen at 50°C</td>
</tr>
<tr>
<td>Evaporate to dryness under nitrogen at 50°C</td>
<td>Reconstitute with 1.0 ml methanol and inject 10-30 μl into HPLC</td>
</tr>
<tr>
<td>Reconstitute with 50 μl methanol and inject 10 to 40 μl into HPLC</td>
<td></td>
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2 Samples which are too concentrated for the procedures presented here may be diluted with water prior to assay.

3 See Shihabi and Dave, 1977.

4 Based on work of Butts, et al. 1974.

5 Applied Science Laboratories
Human Data

The usefulness of the procedure is illustrated by results of assaying saliva, plasma and urine samples from a volunteer who received a single oral dose of 1200 mg of dyphylline\(^6\) with 240 ml water. The subject fasted overnight prior to dosing and 4 hours after dosing at which time a meal was administered. Water was allowed ad libitum, whole blood (collected from a forearm vein) and saliva samples were collected just before dosing at time "zero" and at 10, 15, 20, 30 and 45 minutes, and at 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, 16, 20, 24, 28, 32, and 36 hours post dosing. Urine samples were collected at time "zero" and for the intervals 0-2, 2-4, 4-6, 6-8, 8-16, 16-24, 24-32 and 32-40 hours post dosing. A more extensive pharmacokinetic study is planned using the assay methods reported here.

Results and Discussion

Typical elution patterns for blank and subject plasma, saliva and urine samples are shown in Figure 1. The bottom right tracing (G) shows that some common xanthines do not interfere with the assay. It can also be seen that dyphylline and internal standard separate completely and there is no absorbance from blank samples in the region of the drug

\(^6\)Neothylline tablets (3 x 400 mg) lot No. 3538, Lemmon Pharmacal Co., Sellersville, PA.
Figure 1. Typical HPLC chromatograms. A, zero hour plasma; B, two hour plasma; C, zero hour saliva; D, one hour saliva; E, zero hour urine; F, six to eight hour urine; G, mixture of some column xanthines.

peaks. Calibration curves were prepared for all body fluids but in the interest of space constraints, only the plasma standard curve data is presented in Table 1. Similar tables were generated for saliva and urine standard curves. The individual graphs of PHR vs dyphylline
concentration for plasma data (Table 1) appeared linear and provided least squares linear regression $r^2$ values of 0.9998 and 0.9999. The slopes and intercepts of the curves were not significantly different ($p < 0.05$) so the data were pooled to produce a single standard curve. Least squares linear regression equations were calculated for both PHR vs. Conc. ($r^2 = 0.9998$) and ln PHR vs. ln Conc. ($r^2 = 0.9990$) from the combined data. Inverse estimations of the concentrations were obtained by inserting the original PHR or ln PHR data into the appropriate linear regression equations (see footnotes a and b, Table 1) generated from the combined standard curve data. The accuracy and precision of the equations are shown in Table 1.

The ln PHR vs ln Conc. relationship was chosen for determination of concentrations of dyphylline unknowns in plasma since this method produced mean inversely estimated concentrations of 100% of theory and did not tend to over estimate the low concentrations as did the PHR vs Conc. relationship, i.e., the 25 to 75 ng/ml concentrations averaged 99.2% of theory using the ln PHR vs ln Conc. relationship. This is similar to earlier work with Tolmetin (Ayres, et al. 1977) and it is the weighting given by use of the ln PHR vs ln Conc. which avoids inaccuracy in estimating lower concentrations.
Table 1. Inversely estimated concentrations from individual standard curve data using different pooled standard curves for dyphylline in human plasma (25-2000 ng/ml).

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>PHR vs Conc(^a)</th>
<th>Ln(PHR) vs Ln(Conc)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
</tr>
<tr>
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<td>50</td>
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<td>1500</td>
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<tr>
<td>2000</td>
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\(^a\) PHR = -0.0050447 + 0.00067691 (conc); \(r^2 = 0.9998\);
\(^b\) Ln(PHR) = -7.5669 + 1.039 (Ln conc); \(r^2 = 0.9990\);
\(^c\) Inversely estimated concentration;
\(^d\) (Inversely estimated concentration/known concentration)\((100)\) which
gives the calculated value as a % of known;
\(^e\) Average % of known. The
theoretical value is 100;
\(^f\) Standard deviation;
\(^g\) (Standard deviation/average)\((100)\).
For saliva the lowest concentrations measurable were 50 ng/ml. Again, standard curve data from different days were pooled because the slopes and intercepts were not significantly different (p < 0.05) and least squares linear regression equations were obtained for both PHR vs Conc. and ln PHR vs ln Conc. The later equation \[ \ln (\text{PHR}) = -7.8282 + 1.0658 (\ln \text{Conc.}) \], \( r^2 = 0.9990 \), was chosen for analysis of unknowns since the mean inversely estimated concentrations were 100% of theory with a lower coefficient of variation (C.V.% = 5.95). The results were similar for urine standard curve data and unknown concentrations were estimated from the equation \[ \log (\text{PHR}) = -2.04 + 0.980 (\log \text{Conc.}) \]. The mean inversely estimated urine dyphylline concentration was 100%, with standard deviation 10.1, coefficient of variation 10.1%, and \( r^2 = 0.997 \).

Typical plasma and saliva concentration curves of dyphylline are shown in Figure 2. These were obtained using the HPLC assay procedures described herein. More than 500 samples have been injected on the column with no apparent change in efficiency. Some cleaning of the column filter was necessary when occasional pressure increases were observed.

Intact dyphylline excreted in the urine was 1002 mg or 83.5% of the orally administered dose. To insure that analysis of urine samples measured dyphylline and not a metabolite, portions of the solvent containing the
dyphylline "peak" were collected after injections from urine samples indicated the presence of dyphylline. The compound in the sample was identified to be dyphylline by mass spectroscopy by comparison to an authentic dyphylline sample, thus confirming that the HPLC peaks were dyphylline and that the drug was excreted largely intact in the urine. Injection of the urine samples which did not contain added internal standard showed no peak at the internal standard retention time, which indicates that dyphylline is not
converted to the internal standard, BHET.

The curvature in the terminal portion of the semi-logarithmic plot of drug concentration in plasma or saliva vs time (Figure 1) shows that in this subject the true body half-life must be greater than has previously been assumed (Simons, et al. 1975; Drummond, 1977) based on time 0 to 8 hours data. Thus, the simple, rapid, specific and very sensitive methods presented here should prove useful in studies designed to more thoroughly define the pharmacokinetics of the potentially very useful drug, dyphylline.

References


SECTION TWO

PHARMACOKINETICS OF DYPHYLLINE ADMINISTERED ORALLY TO HUMANS

Lee G. Gisclon, Gerald H. Ewing, and James W. Ayres

Abstract

Plasma, saliva, and urine dyphylline concentrations were measured after administration of a single oral dose of 15 mg/kg to six healthy male subjects. Improved analytical methods have allowed sampling over longer time periods than have previously been reported. The resultant plasma, saliva and urine data show that dyphylline pharmacokinetics cannot be described by traditional one or two compartment open models. Model independent pharmacokinetic parameters including renal clearance, drug excreted, time to plasma peak, and peak plasma values have been determined. In contrast with a previous report, the analysis of dyphylline in urine has established that dyphylline is primarily eliminated unchanged in the urine.

The observed rapid and reproducible rise in plasma dyphylline levels is consistent with prediction of rapid onset of action following oral dosing with the tablet investigated.

A high degree of correlation was found between saliva and plasma dyphylline concentrations of individuals using
parabolic equations, the $r^2$ values ranging from 0.935 to 0.996. This relationship suggests nonlinear protein binding of dyphylline occurs within the range of plasma levels attained by the dose given in this study.

These observations should prove to be useful for designing future pharmacokinetic studies and clinically when monitoring of plasma dyphylline levels via saliva sampling is anticipated.

Dyphylline, 7-(2,3-dihydroxypropyl)-theophylline, was synthesized in 1946 in a search for new drugs which would retain the therapeutic activity of theophylline, yet would avoid the problems of solubility, incompatibility, and toxicity observed with theophylline\(^1\). Several recent reviews and articles\(^2\text{-}^9\) summarize the current extensive body of knowledge concerning the use of theophylline as a bronchodilator.

In comparison to theophylline, a relatively small amount of research has been conducted with dyphylline. This is surprising in that early investigations\(^1,^{10}\) indicated that dyphylline was less toxic in animals than either theophylline or aminophylline. Subsequent studies\(^11\text{-}^{14}\) noted the lack of observed adverse effects even when dyphylline was given in large doses to human subjects. Dyphylline has been demonstrated to produce bronchodilation\(^1,^{11}\text{-}^{13}\), however, further investigation is indicated to thoroughly define the efficacy of dyphylline as a
bronchodilator and accurately define its toxicity in humans.

Early work by Isaksson and Lindholm\textsuperscript{15} and more recently by Simons et al.\textsuperscript{13,14,16} forms the basis of what is currently known about dyphylline pharmacokinetics. Evaluation of this earlier work indicated that a more extensive examination of dyphylline pharmacokinetics was necessary. This study was undertaken to; 1) describe the pharmacokinetics of single dose orally administered dyphylline using a prolonged sample collection period (0-36 hours post dosing), 2) evaluate correlation of plasma dyphylline levels with saliva levels, and 3) to measure urinary dyphylline excretion in an attempt to clarify the metabolic fate of dyphylline in humans and to describe the renal elimination parameters of the drug.

\textbf{Methodology}

Approval for this research was obtained from the Committee for Protection of Human Subjects, Oregon State University. Informed consent was obtained from six male volunteers ranging in age from 20 to 27 years, and weight from 165 to 187 lb (75-85 kg). The subjects were interviewed by a registered pharmacist and reported no known disease or allergy and none were taking prescription medication. The subjects agreed to abstain from the use of any medication or alcohol from one week prior to the study.
until its completion. The subjects were allowed nothing by mouth from 10 PM the evening prior to the study until 4 hours post-dosing, except water ad lib. Food and beverages (excluding xanthine containing beverages and alcohol) were allowed ad lib starting 4 hours post-dosing. Each subject drank 240 ml of water 2 hours prior to dosing. All subjects were dosed orally with approximately 15 mg/kg of dyphylline\(^a\), along with 240 ml of water.

Whole blood samples of approximately 7 ml each were collected at 0 hour (just prior to dosing), 10, 15, 20, 30, and 40 minutes, and at 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, 16, 20, 24, 28, 32, and 36 hours post-dosing. The collection of blood samples was facilitated in the first 8 hours by placement of heparin locks, which were flushed with heparin in normal saline (10 U/ml). The heparin solution was removed from the lock by syringe immediately prior to sample collection and blood collected in a new syringe was immediately transferred to a heparinized Vacutainer\(^R\) (Becton-Dickinson) to prevent coagulation. Blood samples were collected in heparinized Vacutainers\(^R\) via venipuncture after removal of the heparin lock. Samples were inverted gently several times, then stored in crushed ice until centrifuged to harvest the plasma. All samples were centrifuged within 30 minutes after collection and the

\(^a\)3 x 400 mg Neothylline\(^R\) tablets, lot #3538, Lemmon Pharmaceutical Company, Sellersville, PA 18960.
plasma samples were stored without delay in tightly sealed polystyrene tubes at -20°C until analyzed.

Saliva samples were collected concurrently with each blood sample. Saliva collection began at the time blood was beginning to be drawn and continued for 1 minute. Saliva flow was stimulated by chewing a 1 x 1 inch square of Parafilm\textsuperscript{R} for 1 minute prior to sampling time. Samples ranging from 2 to 4 ml were collected in polystyrene tubes which were tightly sealed and stored in crushed ice until they could be refrigerated at -20°C (not later than 30 minutes).

A 0 hour urine sample was collected just prior to dosing. Subsequent samples were then collected for the intervals 0-2, 2-4, 4-6, 6-8, 8-16, 16-24, 24-32, and 32-40 hours post-dosing. The subjects stored urine for each collection interval in a separate container and the bladder was completely voided at the end of each interval. Samples were stored at 4°C until the end of the interval, at which time total volume was measured and an aliquot was removed and stored in a tightly sealed polystyrene container at -20°C.

Plasma, saliva, and urine samples were analyzed utilizing high pressure liquid chromatography and extraction procedures which were developed specifically for dyphylline in this laboratory\textsuperscript{17}. The analytical methods developed are specific and sensitive and allowed the measurement of
dyphylline concentrations to 28 hours post-dosing in plasma and 24 hours post-dosing in saliva.

**Results and Discussion**

Figure 1 portrays the mean plasma and saliva dyphylline concentrations (± SE of the mean) plotted on a logarithmic axis vs time. Absorption was rapid, with the plasma peak for the mean data being 18.2 mcg/ml at 40 minutes. Individual plasma peaks occurred at 15 to 60 minutes (mean 32.4 minutes) and ranged from 18.3 to 28.9 mcg/ml (mean 21.8 mcg/ml). The individual saliva peaks ranged from 7.02 to 16.9 mcg/ml (mean 11.5 mcg/ml) and occurred from 30 to 60 minutes (mean 43.3 minutes) post-dosing. The mean AUC 0-24 hours calculated by the trapezoid rule for the plasma data was 59.0 (mcg/ml x hours), individuals ranged from 43.5 to 72.9 (mcg/ml x hours).

Hudson et al.\textsuperscript{12} demonstrated significant bronchodilation in patients with confirmed reversible airway obstruction when given 1000 mg dyphylline orally. Blood levels were reported to average 16 mcg/ml from 1-1.5 hours, and 14 mcg/ml from 2-3 hours post-dosing. Simons et al.\textsuperscript{13} demonstrated significant reduction in exercise-induced

\textsuperscript{b}Three subjects had plasma dyphylline levels measurable to 28 hours, one subject to 24 hours, and two subjects to 16 hours post-dosing.
Figure 1. Semilogarithmic graph of mean plasma (●) dyphylline concentration vs time, and mean saliva (■) concentration vs time. Inset graph shows mean plasma and saliva dyphylline concentrations on an expanded scale from 0-120 minutes post-dosing. Error bars indicate SE of the mean. Solid lines connect the actual data points and have not been smoothed by curve fitting due to the nonlinear nature of the data.
bronchospasm by pre-dosing with 15 mg/kg oral dyphylline, and estimated the minimum effective therapeutic serum level to be about 12 mcg/ml. The data in Figure 1 are consistent with these reports for dyphylline peak concentrations and time to peak, indicating that 1200 mg of dyphylline administered orally should produce a rapid therapeutic response.

The semi-logarithmic plots (Figure 1) of the mean plasma and saliva data appear to be reasonably linear in the period from 5 to 8 hours post-dosing. The half life of dyphylline calculated from the mean data (via linear regression) through the 5 to 8 hour period was 2.27 hours for plasma and 2.11 hours for saliva. Both values are in close agreement with the published half life of 2.11 ± 0.36 hours, which was determined from serum concentrations measured to 8 hours post-dosing. However, the semi-logarithmic plots of individual data also reveal significant curvature throughout these time periods. The absence of a log-linear relationship in the terminal portion of the log concentration vs time curve precludes calculation of a meaningful half life for dyphylline based on the
traditional one or two compartment open models. In light of this information, the true half life of dyphylline will be found to be considerably longer than 2.11 hours.

Figure 2 shows the mean saliva concentrations (y axis) vs the corresponding mean plasma dyphylline concentrations (x axis) from 1 to 12 hours post-dosing. The mean data are curvilinear and well defined by a parabolic relationship.

Figure 3 is a plot of saliva vs. corresponding plasma dyphylline concentrations for four individuals from the respective saliva peaks to 12 hours post-dosing (the two remaining individuals closely overlapped data already graphed and were left out for sake of clarity). The curves of four subjects are described by parabolic relationships and two subjects appear linear for the concentration ranges involved. The curves of subjects 2, 4, and 6 in Figure 3 are examples of parabolic relationships, while subject 3 is an example of the apparent linear relationship. The graphs do not contain values prior to the saliva peak because saliva dyphylline concentrations peaked about 15 to 20 minutes later than plasma dyphylline concentrations for most individuals and were not highly correlated with plasma concentrations until this equilibrium point had been reached.

The use of a mean data curve such as Figure 2 to predict plasma dyphylline levels from saliva levels in a clinical setting would not result in accurate estimates
Figure 2. Graph of mean saliva dyphylline concentration vs mean plasma concentration from 1 to 12 hours post-dosing. The solid line is predicted by the parabolic equation: $[\text{saliva dyphylline}] = 0.173 ([\text{plasma dyphylline}]) + ([\text{plasma dyphylline}]^2) + 0.3291$. $r^2 = 0.994$, CV% = 9.63.
Figure 3. Graph of saliva dyphylline concentration vs plasma dyphylline concentration for 4 individuals from the saliva peak to 12 hours post-dosing. Solid lines are predicted curves, data points are observed values for each individual. Subjects 2(★), 4(●) and 6(●) are parabolas with $r^2$ values of 0.994, 0.957, and 0.996 respectively. Subject 3(▲) is described by a straight line with $r^2 = 0.989$. 
because of the variety of parabolic and apparent linear relationships for individuals as seen in Figure 3 for the concentration ranges investigated.

The curvilinear relationships in Figures 2 and 3 are interesting when examined in light of reports\textsuperscript{18,19} which correlate plasma protein binding of drugs with the concentrations in saliva and plasma. A simplified equation from the work of Dvorchik et al.\textsuperscript{18} ($F_b = 1 - \frac{K[S]}{[P]}$) takes into account plasma and saliva pH as well as the pKa of the drug. $F_b$ represents the fraction of drug bound to protein, $[S]$ and $[P]$ are drug concentrations in saliva and plasma respectively, and $K$ is a proportionality factor. For dyphylline $K=1$, thus the curves in Figures 2 and 3 should be straight lines with intercepts $= 0$ and slopes $= 1 - F_b$ if the fraction of protein bound dyphylline is a constant.

For many drugs the percent bound to plasma protein is a constant. However, the nonlinear relationships in Figures 2 and 3 show that the fraction of protein bound dyphylline for four of the six individuals may be changing over the concentration range achieved with a single dose of 15 mg/kg. The fraction of dyphylline which is protein bound for the individuals which are linear does not change over the concentration range achieved. These conflicting data may reflect differences in the protein binding capacity for dyphylline among individuals. Application of the previous equation for the mean data in Figure 2 predicts
that plasma dyphylline concentrations from 1 to 10 mcg/ml will be 55-59% protein bound, while plasma concentrations of 20 mcg/ml will be about 46% protein bound. For the upper curve in Figure 3, the change in percent protein bound would be from 58% to 19% as plasma dyphylline concentration changes from 1 to 20 mcg/ml. This nonlinear protein binding may explain the apparent nonlinear pharmacokinetics of dyphylline as evidenced by the data in Figure 1. In light of this information, prediction of plasma dyphylline concentrations following multiple dosing will be complex and will require further research including protein binding parameters of dyphylline at steady state plasma levels.

Figure 3 shows that data points for each individual are closely grouped about the particular curve describing that individual. As concentrations increase, the separation of individual curves can be clearly seen, indicating variation in the protein binding parameters among individuals. Nonlinear tissue binding, slow establishment of equilibrium between saliva and plasma, or multicompartmental pharmacokinetics are also possible explanations for the curvature of the data in Figures 1 to 4.

The analysis of urine for dyphylline indicated that the mean total unchanged drug excreted from 0 to 40 hours post-dosing was 985 mg, or 82.1% of the dose administered. These findings are in conflict with a recent publication.
in which the authors assumed that dyphylline was almost totally metabolized because unchanged dyphylline was not detected in the urine. To determine whether unchanged dyphylline or a metabolite was being measured in the present study, a series of solvent samples containing the peak considered to be dyphylline were collected from the high pressure liquid chromatograph after dyphylline peaks were detected in the urine of subjects. The solvent was evaporated and the compound was collected and identified to be dyphylline using mass spectroscopy via comparison to known dyphylline samples. Urine samples of the subjects were also analyzed without internal standard, and the absence of peaks at the internal standard retention time indicated that dyphylline was not metabolized to the internal standard.

Furthermore, no evidence was found to indicate that any metabolite of dyphylline was excreted in the urine, since no peaks other than dyphylline or internal standard were found in any urine sample, except those which could be reproduced from blank urine samples not containing dyphylline or internal standard. The data also indicate that on the average, at least 82% of the oral dose of dyphylline is absorbed intact.

The mean renal clearance for the six subjects was
found to be 299 ml/min\(^c\), therefore dyphylline appears to be eliminated by a combination of glomerular filtration and active tubular secretion.

Figure 4 is a graph of the log mean urinary dyphylline excretion rates (± SE of the mean) vs midpoint of the excretion intervals. The graph is reasonably log-linear through the 3 to 20 hour period, and the half life of 2.28 hours calculated from the regression line in this time period again agrees with the published half life of 2.11 hours. However, as in the case of plasma and saliva data, the graph does not remain log-linear. Dyphylline was measurable in the urine of 3 of the 6 subjects for the 24-32 hour collection interval, and the remaining 3 subjects for the 32-40 hour interval. Semi-logarithmic graphs of urinary excretion rates vs midpoint of collection intervals for the individual subjects were all nonlinear from 20 hours on, indicating that the data in Figure 4 is a true representation of nonlinearity and not simply a reflection of the fact that dyphylline was not measurable in the urine of 3 of the subjects at that time. The nonlinearity of the plasma, saliva, and urinary data are in agreement, indicating that first order elimination is not occurring during the time period investigated.

\[\text{Renal clearance} = \frac{\text{Amount in urine (mg) 0-t hr/AUC 0-t (mcg/mlxhrs)}}{[(1000 \text{ mcg/mg})(1/60 \text{ hr/min})]}=\text{ml/min}. \]

AUC 0-t is from time 0 to the last measurable plasma concentration using the trapezoidal rule.
Figure 4. Graph of mean dyphylline urinary excretion rate in mg/hr (± SE of the mean) vs time at mid-point of collection interval.
Dyphylline may obey multicompartmental or nonlinear tissue binding pharmacokinetics complicated by nonlinear protein binding. Further research is indicated before a compartmental model and associated pharmacokinetic parameters and micro-constants can be estimated. Model independent assumptions were used to analyze the data generated in this study and no model dependent parameters were reported. This approach should serve to minimize false impressions concerning the pharmacokinetics of dyphylline until sufficient information is obtained to establish the appropriate pharmacokinetic model.

References


