Urinary Excretion of Dyphylline in Humans

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Abstract □ The pharmacokinetics and urinary excretion of a single dyphylline dose were studied in five normal volunteers. The mean dyphylline half-life was 1.8 ± 0.2 hr; the mean total body clearance rate and mean renal clearance rate were 333 ± 62 and 276 ± 52 ml/min, respectively; and the mean volume of distribution was 0.8 ± 0.2 liter/kg. In the urine, 83 ± 5% of the dose was excreted as unchanged drug, and theophylline was not detected. Dyphylline doses of 19–27 mg/kg, resulting in peak serum dyphylline concentrations of 19.3–23.5 μg/ml, were tolerated well by four subjects. One subject had a severe headache following a 28-mg/kg dose, associated with a peak serum dyphylline concentration of 36.4 μg/ml. This study confirms speculation that dyphylline is not metabolized to theophylline *in vivo*.

Keyphrases □ Dyphylline—urinary excretion, pharmacokinetics, humans □ Muscle relaxants, smooth—dyphylline, urinary excretion, pharmacokinetics, humans □ Pharmacokinetics—dyphylline, urinary excretion, humans

Dyphylline (dihydroxypropyltheophylline) is 70% theophylline by molecular weight ratio (1) and is said to be stable *in vitro* and *in vivo* (2). Whether it is eliminated by metabolism, excretion, or both is unknown.

Dyphylline has a mean half-life of 2.1 hr in humans (3) and, therefore, might be expected to be clinically useful only if given very frequently or in very high doses. Although doses of 15 mg/kg (4) and 1000 mg (5), resulting in mean peak serum dyphylline concentrations of 12 and 16 μg/ml, respectively, have some bronchodilator effects, *in vitro* studies indicated that much higher doses may be required for optimal bronchodilation (6).

The tolerance to high single dyphylline doses in normal volunteers was assessed, and an attempt was made to determine whether the drug is metabolized *in vivo* or is excreted unchanged.

EXPERIMENTAL

Materials—All solvents and reagents were analytical grade[1].

Dyphylline Assay—Dyphylline concentrations in aqueous solution, serum, urine, and feces were measured by reversed-phase, high-performance liquid chromatography (HPLC) using a modified method of Orcutt *et al.* (7).

To 50 μl of water or serum in a 10 × 75-mm test tube was added 100 μl of aqueous β-hydroxyethyltheophylline[2] (7.5 μg/ml) as the internal standard. A 25-μl aliquot of 20% trichloroacetic acid was added, and the solution was mixed and centrifuged. The supernate was transferred to a clean 13 × 100-mm test tube. After buffering with 300 μl of 2.5 *M*, pH 6.4 acetate buffer, the solution was extracted with 2 ml of 20% isopropanol in chloroform by mixing and centrifugation. The aqueous supernate was aspirated, and the organic layer was evaporated to dryness using low heat and a dry nitrogen stream. The sample was redissolved in 50–100 μl of mobile phase, and 25 μl was injected directly into the chromatograph.

To 50 μl of urine or fecal slurry were added 50 μl of aqueous β-hydroxyethyltheophylline (15 μg/ml) and 300 μl of 2.5 *M*, pH 6.4 acetate buffer. This sample was extracted directly with 2 ml of 20% isopropanol in chloroform and then treated similarly to the serum samples.

Instrument—A component-system high-performance liquid chromatograph[3] was used with a 30-cm × 3.9-mm i.d. stainless steel column[4] containing reversed-phase packing. The solvent was 6.5% acetonitrile in 0.01 *M*, pH 4.0 sodium acetate buffer. The flow rate was 2 ml/min at an operating pressure of 1500–2000 psi. Absorbance was measured at 280 nm. Under these conditions, dyphylline and the internal standard eluted at 9.0 and 10.5 min, respectively.

Dyphylline Conjugation in Urine—Pooled 24-hr urine samples from four subjects were analyzed for dyphylline conjugates. To 0.5-ml aliquots of urine were added 100 μl of water, 100 μl of concentrated hydrochloric acid, or 100 μl of β-glucuronidase[5] solution, 250 U/100 μl in 0.15 *M*, pH 6.9 phosphate buffer. Samples were incubated at 37° for 24 hr and analyzed for dyphylline.

Dosage Form—The dyphylline content in five tablets[6] was determined by crushing the tablets and dissolving the dyphylline in water.

Kinetic Study—Dyphylline pharmacokinetics were studied in five volunteers, ages 20–35 years, after informed consent was obtained. All were healthy, nonobese, nonsmokers who were not taking any medication. They abstained from methylxanthine-containing foods and beverages for 15 hr before and during the study.

Each fasting subject received a single dose of 1400 or 1600 mg of dyphylline[6] by mouth (range 19–28 mg/kg) with 180 ml of water. A light lunch was permitted 3 hr later. Venipuncture was performed before drug ingestions and then every 15 min until 1.5 hr and hourly from 2 to 8 hr. Urine samples were collected before and for 24 hr after drug ingestion. For four volunteers, the urine collected after drug ingestion was pooled and measured, and an aliquot was analyzed for dyphylline. For the fifth volunteer, timed urine specimens and fecal samples were collected for 24 hr, and the dyphylline content of each was analyzed.

Data Analysis (8)—The log serum dyphylline concentration *versus* time data were plotted, and the terminal linear portion of the graph was fitted to Eq. 1 by linear regression using a programmable pocket calculator[7]:

$$\ln C_p = \ln C_p^0 - Kt \qquad \text{(Eq. 1)}$$

where C_p is the serum dyphylline concentration at any time (t), C_p^0 is the serum dyphylline concentration extrapolated to zero time, and K is the first-order elimination rate constant.

Table I—Serum Dyphylline Concentrations (Micrograms per Milliliter) and Amount of Dyphylline Excreted in Urine

Hours	Subject 1	2	3	4	5
0.25	6.7	8.3	1.4	0	0
0.5	12.5	34.7	10.9	1.0	16.3
0.75	19.3	33.9	22.2	6.2	23.3
1.0	18.1	36.4	23.5	13.7	21.0
1.25	17.4	33.7	22.1	19.3	20.0
1.5	16.6	29.7	21.5	14.4	19.7
1.75	—	—	20.2	14.0	16.6
2.0	14.2	23.2	18.1	12.0	16.0
3.0	9.8	17.7	11.8	9.9	10.4
4.0	7.4	9.4	7.9	7.0	7.8
5.0	4.7	6.3	4.7	4.5	5.3
6.0	3.8	4.0	3.0	2.6	3.3
7.0	2.3	2.9	1.9	2.4	2.0
8.0	1.4	1.9	1.2	1.9	1.8
AUC, μg/ml/hr	67.68	110.97	74.31	57.29	74.57
A_e^∞, mg	1227	1301	1230	1150	1222

[1] Fisher Scientific Co., Fair Lawn, NJ 07410.
[2] Pierce Chemical Co., Rockford, IL 61105.
[3] Model UK injector, model 6000A high-pressure pump, and model 440 absorbance detector, Waters Associates, Milford, MA 01757.
[4] μBondapak C_{18}, Waters Associates, Milford, MA 01757.
[5] β-Glucuronidase type I crude bacterial powder, Sigma Chemical Co., St. Louis, MO 63178.
[6] Airet 400-mg tablets, Baylor Laboratories, Hurst, TX 76053.
[7] H-P 67, Hewlett-Packard, Corvallis, OR 97330.

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Table II—Dyphylline Pharmacokinetic Constants

Parameter	Subject 1	2	3	4	5	Mean ± *SD*
Weight, kg	73	57	60	68	68	65 ± 7
Dose, mg/kg	19	28	27	21	21	23 ± 4
K, hr^{-1}	0.38	0.43	0.45	0.32	0.38	0.39 ± 0.05
$t_{1/2}$, hr	1.8	1.6	1.5	2.1	1.8	1.8 ± 0.2
$C_{p_{max}}$, μg/ml	19.3	36.4	23.5	19.3	23.3	24.4 ± 7.0
t_{max}, hr	0.75	1.0	1.0	1.25	0.75	1.0 ± 0.2
V_d, liters/kg	0.75	0.59	0.80	1.11	0.73	0.80 ± 0.19
Cl, ml/min	345	240	360	407	313	333 ± 62
f, %	88	81	77	82	87	83 ± 5
Cl_R, ml/min	303	195	276	334	272	276 ± 52

Total body clearance (Cl) was calculated using:

$$Cl = \frac{AUC}{D} \qquad \text{(Eq. 2)}$$

where AUC is the area under the serum dyphylline concentration–time curve calculated using the trapezoid rule. The apparent volume of distribution (V_d) was determined using:

$$V_d = \frac{Cl}{K} \qquad \text{(Eq. 3)}$$

In one subject from whom timed urine samples were collected, renal clearance (Cl_R) was calculated using:

$$Cl_R = \frac{\left(\frac{dA_e}{dt}\right)}{C_p} \qquad \text{(Eq. 4)}$$

where dA_e/dt is the excretion rate calculated at the same time that C_p was determined. In this study, dA_e/dt was calculated as $\Delta A_e/\Delta t$ at 2-hr intervals. In the other four subjects in which pooled 24-hr urine was collected, renal clearance was calculated using:

$$Cl_R = (f)(Cl) \qquad \text{(Eq. 5)}$$

where f is the dose fraction excreted in the urine as unchanged drug.

RESULTS AND DISCUSSION

Dyphylline content in five tablets of 400-mg label strength was 393.8 ± 11.2 mg. Although the range of tablet content was ±3%, within the usual USP range of ±7% of label strength, the doses used in this study were probably less than calculated.

Serum dyphylline concentrations in the five subjects following 19–28-mg/kg doses are shown in Table I, and the pharmacokinetic parameters are listed in Table II. A typical log serum dyphylline concentration *versus* time plot is shown in Fig. 1.

In the five subjects, peak serum dyphylline concentrations occurred within 1 hr (Table II), confirming a previous report (3). The maximum dyphylline concentration in the present study was 36.4 μg/ml in a subject who received 28 mg/kg. The mean dyphylline half-life was 1.8 ± 0.2 hr, similar to the previously reported value of 2.1 ± 0.4 hr (3). The mean dyphylline clearance rate was 333 ± 62 ml/min, while the mean apparent volume of distribution was 0.8 ± 0.2 liter/kg.

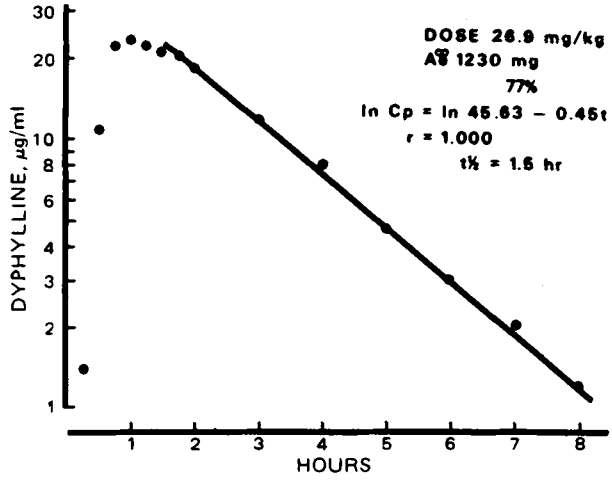

Figure 1—*Log serum dyphylline concentration* versus *time for Subject 3.*

It has been speculated that dyphylline is not metabolized *in vivo*, although unchanged dyphylline has not been detected in the urine by GLC (9). The dyphylline elimination mechanism has not been reported previously. In these five subjects, 83 ± 5% of the dyphylline dose was excreted in the urine in the unchanged form. Theophylline was not identified in serum or urine. There was no evidence of glucuronide or other types of conjugation; pooled urine samples treated with acid or β-glucuronidase showed no change in the dyphylline concentration (10).

In Subject 1, the renal clearance of dyphylline calculated using Eq. 4 was 311 ml/min, consistent with 303 ml/min calculated from Eq. 5. The mean renal clearance in the five subjects was 276 ± 52 ml/min. These results suggest that dyphylline is actively secreted as well as filtered by the glomerulus.

Dyphylline bioavailability from oral tablets is ~100% of availability from intramuscular administration (3). Analysis of feces collected at 12 and 23 hr in Subject 1 accounted for only a further 9.4 mg (0.7%) of the dose in addition to the 88% found in urine. The small amount of drug in feces may have been secreted by the bile or may not have been absorbed.

Presently, the recommended dyphylline dose in adults is 400 mg tid, although effective single doses are at least 15 mg/kg (4) or 1000 mg (5). The range of serum levels associated with optimal bronchodilation without toxicity is unknown, although dyphylline is said to have a wider therapeutic–toxic range than theophylline and to cause fewer adverse effects in the GI and central nervous systems (11, 12). In the present study, only one subject experienced adverse effects, namely severe headache, after a single 28-mg/kg dyphylline dose, associated with a peak serum dyphylline concentration of 36.4 μg/ml. The other four subjects tolerated doses of 19–27 mg/kg and peak serum dyphylline concentrations of 19.3–23.5 μg/ml without symptoms.

The adverse effects following a single high dyphylline dose in one subject and the short drug half-life remain major obstacles to its effective use in clinical practice, even with sustained-release formulations (9).

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Stability of Amitriptyline Hydrochloride in a Commercial Aqueous Solution

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Abstract □ A commercial amitriptyline hydrochloride solution was stored at 80° for up to 3 months. High-performance liquid chromatography showed no evidence of amitriptyline hydrochloride degradation. The method also indicated that two reported degradates, 3-(propa-1,3-dienyl)-1,2:4,5-dibenzocyclohepta-1,4-diene and dibenzosuberone, were present at levels less than 0.1% (the detection limit of the method) under the storage conditions. The stability of the commercial solutions is attributed to their relatively low ratio of headspace oxygen to amitriptyline hydrochloride.

Keyphrases □ Amitriptyline hydrochloride—stability, commercial aqueous solutions, high-performance liquid chromatography □ Antidepressants—amitriptyline hydrochloride, stability, commercial aqueous solutions, high-performance liquid chromatography □ High-performance liquid chromatography—analysis, amitriptyline hydrochloride in commercial aqueous solutions, stability

Amitriptyline hydrochloride decomposition products in aqueous solution were identified by Enever *et al.* (1). More recently, the same investigators reported a number of factors that influence the drug's decomposition rate (2). The latter study indicated that aqueous amitriptyline hydrochloride solutions could undergo appreciable decomposition after even a few days of storage at 80°.

Neither study reported data for amitriptyline hydrochloride stability in marketed parenteral solutions. The purpose of this investigation was to assess the stability of an aqueous amitriptyline hydrochloride solution in one such formulation[1].

EXPERIMENTAL

Materials—Amitriptyline hydrochloride[2], dibenzosuberone[3], and methanesulfonic acid[4] were used without further purification. All other chemicals were reagent grade.

High-Performance Liquid Chromatography (HPLC)—*Apparatus*—The liquid chromatograph[5] was equipped with a fixed-wavelength detector (254 nm), an oven, and an integrator.

Column—A 30 × 0.39-cm (i.d.) column containing a nitrile-bonded phase packing[6] was used at 30° with a mobile phase flow of 2 ml/min.

Mobile Phase—For amitriptyline hydrochloride analysis, acetonitrile–0.02 *M* ammonium acetate plus 0.01 *M* methanesulfonic acid in distilled water (90:10) was used. For degradate detection, the ratio was 50:50.

Sample Preparation—The sample was prepared by diluting a 2.0-ml aliquot to 25.0 ml with distilled water. The sample was filtered prior to analysis, and 10 μl was injected onto the column (attenuation 0.0256 absorbance unit/cm). Samples containing degradates were prepared in the same manner and injected at an attenuation of 0.0032 absorbance unit/cm.

Quantitation—Quantitation was achieved using the ratio of the sample peak area to that of an amitriptyline hydrochloride reference standard[2].

Storage of Amitriptyline Hydrochloride Solutions—Multiple-dose vials[1] (10 ml) were stored in a forced-air oven[7] maintained at 80°. Vials were withdrawn at predetermined times and stored at 5° prior to analysis at the completion of the study (3 months).

Synthesis of 3-(Propa-1,3-dienyl)-1,2:4,5-dibenzocyclohepta-1,4-diene (I)—An authentic sample of I was prepared from amitriptyline *N*-oxide dihydrate (3) by Cope elimination (125°/2 hr). The product was isolated by ether extraction and purified by column chromatography on silica gel with carbon tetrachloride elution. The purified sample was characterized by TLC and spectra (UV, NMR, and mass) and was stored at 5°.

Table I—Amitriptyline Hydrochloride in Aqueous Solution Stored at 80°

Days	Found, mg/ml	Percent of Initial
Initial	10.21	
4	10.32	101
8	10.25	100.4
11	10.24	100.3
16	10.20	99.9
20	10.10	98.9
24	10.15	99.4
28	10.16	99.5
35	10.19	99.8
90	10.08	98.7

RESULTS AND DISCUSSION

The results (Table I) showed no detectable amitriptyline hydrochloride loss in aqueous solutions stored at 80° for up to 90 days. The results are unusual only in that the solutions studied by Enever *et al.* (2) showed amitriptyline hydrochloride losses from 5 to 90% after storage at 80° for 30 days. The solutions used in the earlier study contained 2 mg of amitriptyline hydrochloride/ml buffered at pH 3.0 or 5.0 and were sealed in ampuls with a 4:1 ratio of headspace to solution (2). The commercial formulation contained 10 mg of amitriptyline hydrochloride/ml in an unbuffered solution that also included 44 mg of dextrose/ml, 1.5 mg of

[1] Elavil, 10 mg/ml in a 10-ml multidose vial, Merck Sharp & Dohme, West Point, Pa.
[2] Merck Sharp & Dohme, West Point, Pa.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Eastman Kodak Co., Rochester, N.Y.
[5] Hewlett-Packard model 1084, Avondale, Pa.
[6] μBondapak CN, Waters Associates, Milford, Mass.
[7] Model OV-490A-2, Blue M Electric Co., Blue Island, Ill.

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