Journal of Pharmacokinetics and Biopharmaceutics, Vol. 3, No. 3, 1975

Pharmacokinetics of Diphenhydramine in Man

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Received May 9, 1974—Final Jan. 28, 1975

Plasma levels and urinary excretion of diphenhydramine were measured after administration of three single 50-mg doses of diphenhydramine hydrochloride to two healthy male volunteers as an intravenous infusion, an oral solution, and a commercially available capsule. A large first-pass effect was evident from the data, with about 50% of the drug being metabolized by the liver before it reached the general circulation. The drug in solution given orally appeared to be fully available to the hepatoportal system, and the availability of diphenhydramine from the capsule was about 83% relative to the solution in one subject and 100% in the other subject. Cumulative amounts of unchanged diphenhydramine excreted in the urine were less than 4% of the administered dose. Both subjects went to sleep at the end of the 1-hr intravenous infusion, but were only drowsy following the oral treatments.

KEY WORDS: diphenhydramine; intravenous infusion; oral administration; first-pass effect; bioavailability.

INTRODUCTION

Diphenhydramine hydrochloride, 2-(diphenylmethoxy)-*N*,*N*-dimethylethylamine hydrochloride, is an effective antihistaminic agent possessing anticholinergic, antitussive, antiemetic, and sedative properties (1). Although the drug has been used clinically for many years, relatively little information

This study was performed pursuant to Contract No. FDA 69-22 with Public Health Service, Food and Drug Administration, Department of Health, Education, and Welfare, and was supported in part by Public Health Service Grant 5-P11-GM15559.

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is available on its absorption, distribution, and elimination in man. Hald (2) found that 3% of a therapeutic dose (50 or 100 mg) of the drug was excreted unchanged in human urine. Glazko *et al.* (3) administered 50-mg oral doses of diphenhydramine (as its salicylate and hydrochloride salts) twice and four times daily to five subjects for 5 consecutive days. Average peak plasma levels were 84 ± 30 and 160 ± 25 ng/ml, respectively, for the two dose levels (after accumulation had occurred during days 1–5). In addition, an apparent plasma half-life of about 18 hr was reported.

The present pilot study was undertaken to elucidate the pharmacokinetic parameters of diphenhydramine in man after administration of the drug as an intravenous infusion, an oral solution, and a commercially available capsule, and to evaluate its availability from the capsule relative to the solution.

EXPERIMENTAL

Protocol

Two healthy adult male volunteers both 27 years of age and weighing 76 and 95 kg, with normal vital signs and laboratory screening values, were selected for the study. Each subject received three single 50-mg doses of diphenhydramine hydrochloride as an intravenous infusion, an oral solution, and a commercially available capsule according to the dosage schedules shown in Table I. Treatments were separated by a 2-week period. The subjects fasted from 10 hr prior to dosing until 4 hr after administration of the drug. In order to assure hemodynamics similar to that when the drug was given orally, subjects were required to stand periodically during the infusion.

Twenty-milliliter blood samples were collected in citrated Vacutainers just prior to dosing and at the times indicated in Table II. The plasma was separated, frozen, and kept in a frozen state until just prior to assay. Urine specimens were collected in plastic bottles containing 1 ml of toluene for the following time intervals after administration: 0–6, 6–24, and 24–48 hr. The volumes were recorded, the urine was acidified with concentrated hydrochloric acid (to back-extract any diphenhydramine that may have partitioned into the toluene layer), and an aliquot was frozen until just prior to assay.

Analytical Method

Plasma and urine specimens were assayed for diphenhydramine by a GLC procedure shown to be specific for unchanged drug (4). In addition, the contents of the intravenous infusion and oral solution dosage forms were assayed for exact drug content.

Table I. Dosage Schedules and Treatments

Phase	Subject given treatment		
	A[a] (intravenous infusion)	B[b] (oral solution)	C[c] (capsule)
I	—	1	2
II	—	2	1
III	1,2	—	—

[a]Intravenous infusion: The contents of three 1-ml Benadryl ampules, 50 mg/ml (Parke, Davis and Company, Detroit), were diluted aseptically with 717 ml of 5% sterile dextrose. A 240-ml portion was infused over 1 hr at a rate of 4 ml/min using a precalibrated infusion pump.

[b]Oral solution: The contents of one Benadryl Kapseal, 50 mg, dissolved in a mixture of 90 ml of deionized water and 30 ml of Coca-Cola syrup, were swallowed; the container was rinsed with 120 ml of deionized water and the rinsings were swallowed.

[c]Capsule: One Benadryl Kapseal, 50 mg, was swallowed intact with 240 ml of deionized water.

Determination of Whole Blood/Plasma Distribution Ratio

Two-milliliter portions of citrated drug-free whole blood from each subject were incubated with tritium-labeled diphenhydramine hydrochloride (specific activity 13.4 μCi/mg, supplied by Parke, Davis and Company)[4] for 30 min at 37°C using an aqueous stock solution, 11.3 μg/ml. Final concentrations of diphenhydramine hydrochloride were 113 and 904 ng/ml. After incubation, the plasma was separated by centrifuging at 2000 rpm for 20 min. The samples were analyzed for radioactivity by adding 100 μl of each to 15 ml Unogel (Schwarz/Mann Co.) and counting for 10 min in a scintillation counter (Packard Tri-Carb liquid scintillation spectrometer). The whole blood/plasma ratio was calculated by comparing the radioactivity of whole blood samples to similarly treated plasma standards containing 113 and 904 ng/ml of tritiated diphenhydramine hydrochloride.

[4]The sample, recrystallized from a benzene–methanol solution, was shown to be homogeneous by TLC.

Table II. Plasma Levels and Urinary Excretion Data of Diphenhydramine Following Single Intravenous Infusion and Oral 50-mg Doses of Diphenhydramine Hydrochloride

	Subject 1			Subject 2		
	Intravenous infusion	Oral solution	Capsule	Intravenous infusion	Oral solution	Capsule
Dose[a] (mg)	43.7	44.1	43.8	43.7	44.7	43.8
Dose (mg/kg)	0.572	0.577	0.576	0.462	0.473	0.461
	Plasma concentration of diphenhydramine[b] (ng/ml)					
Time (hr)						
0.25	120	4.30	3.76	31.0	4.15	—
0.50	222	12.3	10.4	89.1	6.36	4.51
1	258	51.3	37.7	179	54.2	20.9
1.5	150	—		170	—	—
2	132	68.9	50.7	147	64.2	45.4
4	83.8	45.2	37.6	80.3	38.8	46.0
6	61.6	24.8	25.0	41.5	27.2	22.9
8	—	19.8	17.1	—	18.5	14.9
12	19.4	13.1	10.5	18.2	11.9	11.8
24	6.49	7.52	5.82	8.19	6.38	7.97
	Amount of diphenhydramine excreted in urine (mg)					
0–6	0.288	0.127	0.074	0.628	0.153	0.119
6–24	0.371	0.254	0.143	0.761	0.186	0.284
24–48	0.114	0.063	0.091	0.085	0.056	0.065
Cumulative amount excreted (f_e)	0.773 (1.77%)[c]	0.444 (1.01%)	0.308 (0.703%)	1.47 (3.37%)	0.395 (0.88%)	0.468 (1.07%)

[a]Dose expressed as diphenhydramine free base following assay for exact drug content.
[b]Values are averages of two determinations.
[c]Numbers in parentheses are cumulative amounts excreted expressed as percents of dose as the free base.

Determination of Plasma Protein Binding

Two-milliliter portions of drug-free plasma from each subject were incubated for 30 min at 37°C with tritium-labeled diphenhydramine hydrochloride using an aqueous stock solution, 11.3 μg/ml. Final concentrations were 113 and 1130 ng/ml.

Ultrafiltration of the plasma samples was performed at 37°C in membrane cones (Centriflo CF-50 membrane ultrafilter, Amicon Corp.) which were soaked in deionized water for at least 1 hr before use. After removal of residual water by centrifugation at 1000*g* for 5 min, 1.9 ml of plasma was pipetted into the cone, then centrifuged at 800*g* for 30 min. At the end of the centrifugation, 100-μl aliquots of ultrafiltrate were removed for scintillation counting as described above. A 100-μl portion of plasma was analyzed for radioactivity prior to centrifugation. The unbound fraction was calculated as the quotient of the radioactivity in the ultrafiltrate and in the plasma before ultrafiltration.

Data Analysis

The total area under the plasma concentration–time curve from zero to infinity was estimated for each of the six sets of data by two different methods. One method involved fitting of the concentration–time data to a polyexponential equation using the program AUTOAN (5) and an *IBM* 360/67 digital computer. The polyexponential equation was then integrated to obtain the area. The program AUTOAN interpolates one plasma concentration–time point between each pair of real points by spline and Akima methods (6). It then calculates every possible polyexponential equation from one to five exponential terms which could describe the data and provides the coefficients and exponents for the optimum number of terms. The criterion used is a moving scale percentage improvement in the sum of squared deviations depending on the value of r^2 (defined in footnote *b* to Table III). The other method involved estimating the area from 0 to 24 hr by means of the trapezoidal rule, then estimating the area from 24 hr to infinity by dividing the observed plasma concentration at 24 hr by the β value estimated from the 12- and 24-hr plasma concentrations observed following intravenous infusion of the drug.

RESULTS AND DISCUSSION

Table II summarizes both the plasma concentration and urinary excretion data for subjects 1 and 2 following the three treatments. A first-pass effect[5] was evident from the data, which showed that the area under the oral

[5]The apparent plasma clearances, estimated directly from the intravenous infusion data in Table II as dose/area, were 37.0 liters/hr [0.48 liter/(kg × hr)] for subject 1 and 41.1 liters/hr [0.44 liter/(kg × hr)] for subject 2. These high clearances suggested that diphenhydramine was subject to a first-pass effect.

solution curve was considerably less than the corresponding area following intravenous administration. The data also revealed that for one subject, but not the others, absorption of diphenhydramine from the commercial capsule was somewhat less than when the drug was administered orally in solution.

Cumulative amount of unchanged diphenhydramine excreted in the urine following the two oral treatments was about 1% of the administered dose in both subjects, while that for intravenous infusion was about 2% for subject 1 and 3% for subject 2. These low values excluded use of urinary excretion data as reliable measures of absorption efficiency.

Data Analysis

The polyexponential equations obtained by use of the program AUTOAN are given in Table III. The post-intravenous-infusion data for both subjects were described by a biexponential equation. Three of the four sets of oral data were described by a four-term exponential equation, while the fourth set was described by a five-term polyexponential equation. Although several models could be envisioned which might be appropriate, there is insufficient information in these data to make a judicious choice. Retrospectively, a more intensive plasma sampling scheme both immediately following cessation of the intravenous infusion and beyond the 24-hr time period would be necessary to permit choice of the most appropriate pharmacokinetic model.

The areas estimated by the two different methods are summarized in Table IV. The footnotes to Table IV clearly indicate how the areas were estimated.

Bioavailability Considerations

First-Pass Effect

The extent of the first-pass effect on drug bioavailability may be determined by equation 1:

$$\theta = \frac{(\text{dose})_{\text{i.v.}} \int_0^\infty C_{\text{oral}}\, dt}{(\text{dose})_{\text{oral}} \int_0^\infty C_{\text{i.v.}}\, dt} \tag{1}$$

where θ represents the fraction of the drug which is administered by the oral route that reaches the general circulation. Rowland (7) has shown that for drugs exhibiting dose-independent kinetics the bioavailability of an oral dose of drug following its first-pass through the liver may be estimated by use of equation 2:

$$\theta = 1 - \frac{f_m(\text{dose})_{\text{i.v.}}}{\dot{V}_{\text{BL}} \lambda \int_0^\infty (C_{\text{plasma}})_{\text{i.v.}}\, dt} \tag{2}$$

Table III. AUTOAN Evaluation of Plasma Concentration–Time (C, t) Data

Subject	Treatment	Polyexponential equation	r^2
1	Post intravenous infusion	$\hat{C} = 122e^{-3.32(t-1)} + 138e^{-0.166(t-1)}$	0.996[a] (0.997)[b]
	Solution, oral	$\hat{C} = -42.0e^{-10.9t} + 403e^{-3.06t} - 580e^{-1.71t} + 193e^{-0.539t} + 25.9e^{-0.0550t}$	0.994 (0.994)
	Capsule, oral	$\hat{C} = 82.8e^{-2.98t} - 254e^{-1.08t} + 155e^{-0.431t} + 17.9e^{-0.0492t}$	0.991 (0.992)
2	Post intravenous infusion	$\hat{C} = 128e^{-0.437(t-1)} + 65.5e^{-0.0996(t-1)}$	0.988 (0.986)
	Solution, oral	$\hat{C} = 73.8e^{-3.85t} - 418e^{0.887t} + 328e^{-0.538t} + 21.3e^{-0.0519t}$	0.951 (0.912)
	Capsule, oral	$\hat{C} = 82.0e^{-3.14t} - 285e^{-0.972t} + 185e^{-0.520t} + 21.3e^{-0.0432t}$	0.924 (0.887)

[a]The r^2 value not in parentheses was given by the computer and included not only the observed plasma concentrations but also the interpolated plasma concentrations.

[b]The r^2 value in parentheses was calculated only from the observed plasma concentrations without the interpolated plasma concentrations. In both cases, $r^2 = [s_{C^2} - \sum(\hat{C} - C)^2]/s_{C^2}$, where $s_{C^2} = \sum C^2 - (\sum C)^2/N$, C is the observed (or interpolated) plasma concentration, $\hat{C}$ is the predicted plasma concentration from the polyexponential equation, and N is the number of data points.

Table IV. Summary of Estimated Areas

Subject	Treatment	Estimated area 0 to ∞ (ng/ml × hr)	
		From trapezoidal rule[a]	From integration of the AUTOAN equation[b]
1	Intravenous infusion	1181	1046[c]
	Solution, oral	579.2	617.2
	Capsule, oral	468.3	516.1
2	Intravenous infusion	1062	1036[c]
	Solution, oral	557.3	568.0
	Capsule, oral	530.6	581.7

[a]Area $= \int_0^{24} C\,dt + C_{24}/\beta$, where the integral was estimated by trapezoidal rule, C_{24} is the observed plasma concentration at 24 hr, and $\beta = (\ln C_{12} - \ln C_{24})/12$ where the C_{12} and C_{24} are observed plasma concentrations at 12 and 24 hr, respectively, following intravenous infusion.

[b]Area obtained by integrating the polyexponential equation in Table III.

[c]The area obtained by trapezoidal rule in the interval 0 to 1 hr was added to the area obtained by integrating the polyexponential equation given in Table III between limits of 1 hr to ∞.

where f_m represents the fraction of the dose which is metabolized in the liver, $\dot{V}_{BL}$ is the liver blood flow rate, and λ is the ratio of the concentration of drug in blood to the concentration of drug in plasma. Rowland (7) indicated that when the value of θ obtained from equation 1 is less than that obtained from equation 2 then either absorption is incomplete or metabolism occurs in the gut or during passage of the drug across the gut wall. Alternatively, he indicated that when the value of θ obtained with equation 1 is greater than that obtained with equation 2 then the concentration of drug in the hepatic portal vein following oral administration may be sufficiently high to saturate the metabolic enzymes.

The values of θ calculated with equations 1 and 2 are given in Table V. The average value of 0.54 from equation 1 is only slightly greater than the value of 0.49 from equation 2 for subject 1. The average value of 0.51 from equation 1 is essentially the same as the average value of 0.50 from equation 2 for subject 2. Interestingly, as pointed out by one of the reviewers, a rectilinear plot of the plasma concentration data for subject 2 suggests a possible linearity for both the "during infusion" data and the postinfusion data from 1 to 4 hr. Yet for this subject the θ values obtained with equations 1 and 2 agree most closely. Also, other tests for saturation kinetics failed to support a saturation phenomenon.

The value of f_m required for use of equation 2 was obtained by means of equation 3:

$$f_m = 1 - f_e \tag{3}$$

Table V. Bioavailability of Diphenhydramine Hydrochloride

	θ				F	
	Using equation 1		Using equation 2[a]		Using equation 4	
Subject	Trapezoidal area	Polyexponential function area	Trapezoidal area	Polyexponential function area	Trapezoidal area	Polyexponential function area
1	0.49	0.59	0.52	0.46	0.81	0.84
2	0.48	0.54	0.53	0.46	0.97	1.04
Averages	0.54 (subject 1) 0.51 (subject 2)		0.49 (subject 1) 0.50 (subject 2)		0.83 (subject 1) 1.0 (subject 2)	
Overall average	0.53		0.49			

[a]Calculated using the following values: $\dot{V}_{BL} = 1.53$ liter/min (7), $\lambda = 0.82$, and $f_m = 0.98$ and 0.97 for subjects 1 and 2, respectively. The doses are listed at the top of Table II and the areas are listed in Table IV.

where f_e is the fraction of the dose of drug which is excreted unchanged in the urine. These values, in percentage units, are given in Table II. The values in Table II, divided by 100, average 0.03 and 0.02 for subjects 1 and 2, respectively. The value of λ was measured experimentally and found to average 0.82 for both subjects and to be independent of concentration.

Efficiency of Absorption

The bioavailability of diphenhydramine hydrochloride from Benadryl Kapseals relative to a solution of the drug given orally may be determined with equation 4:

$$F = \frac{(\text{dose})_{\text{soln.}} \int_0^\infty C_{\text{cap.}}\, dt}{(\text{dose})_{\text{cap.}} \int_0^\infty C_{\text{soln.}}\, dt} \tag{4}$$

Values of F calculated by means of equation 4 are given in Table V. The average values indicate that absorption from the capsule was only 83% relative to the solution in subject 1 but that absorption was complete in subject 2.

Plasma Protein Binding

Table VI gives the percent unbound diphenhydramine at two concentrations. Since the unbound fraction appeared to be independent of concentration, a direct comparison of total plasma concentration (bound plus unbound) to assess bioavailability was justified. Also given in Table VI are the experimentally determined values of λ. As indicated above, these averaged 0.82 for both subjects.

Correlation of Somnolescence with Plasma Concentration

Both subjects went to sleep shortly after the 1-hr intravenous infusion ceased, but were only drowsy following the oral treatments. From the data in Table II, one can calculate that the ratio of the peak plasma concentration of diphenhydramine following intravenous administration to that following oral administration of the drug in solution was 3.7 for subject 1 and 2.8

Table VI. Diphenhydramine Plasma Binding and Blood/Plasma Distribution in Two Subjects

	Percent unbound		Blood/plasma	
Subject	113 ng/ml	1130 ng/ml	113 ng/ml	904 ng/ml
1	1.5	1.3	0.83	0.82
2	1.8	1.7	0.81	0.83

for subject 2. These results suggest that when the plasma concentration of diphenhydramine exceeds about 70 ng/ml sleep may occur.

Elimination Half-Life

Data collected failed to delineate useful elimination half-lives of diphenhydramine in man. Apparent elimination half-lives are summarized in Table VII. These were calculated (a) from the observed plasma concentrations at 12 and 24 hr and (b) by dividing the smallest rate constant of the polyexponential equations (given in Table III) into the natural logarithm of 2. The average of the eight half-lives obtained from the oral data is 15 hr, which agrees reasonably well with the value of 18 hr reported by Glazko *et al.* (3). However, comparison with the values obtained from the two sets of intravenous infusion data indicates that these values derived from oral data are overestimates of the "true" elimination half-life, and probably indicate prolonged absorption of diphenhydramine following oral administration. It should also be noted from Table VII that the half-lives estimated from the smallest rate constants of the polyexponential equations fitted to the intravenous infusion data are smaller than those derived from the 12- and 24-hr plasma concentrations for both subjects. This suggests that the log linear phase did not begin until beyond 12 hr. Thus this analysis of admittedly inadequate data suggests that in these two subjects the "true" elimination half-life of diphenhydramine was probably in the 4- to 7-hr range. These data also suggest that estimation of elimination half-lives of diphenhydramine following either oral or intravenous administration requires measurement of plasma concentrations lower than 5 ng/ml and beyond 24 hr after dosing.

Table VII. Apparent Elimination Half-Lives

Subject	Treatment	Apparent elimination half-life (hr)	
		From 12- and 24-hr plasma levels	From smallest rate constant of polyexponential equations
1	Intravenous infusion	7.59	4.17
	Solution, oral	15.0	12.6
	Capsule, oral	14.1	14.1
2	Intravenous infusion	10.4	6.96
	Solution, oral	13.3	13.4
	Capsule, oral	21.2	16.0

CONCLUSIONS

Diphenhydramine exhibits a large first-pass effect, with about 50% metabolism occurring before the drug reaches the general circulation following oral administration. The drug administered in solution orally is apparently fully available to the hepatoportal system. When administered in capsule form orally, the drug was completely absorbed in one subject and about 83% absorbed in the other subject. Diphenhydramine is almost entirely biotransformed since less than 4% of the dose was excreted unchanged in the urine. Future pharmacokinetic investigations with this drug will require more intensive and more prolonged sampling than used in this pilot study and an assay method sensitive to at least 1 ng/ml of plasma.

ACKNOWLEDGMENTS

The authors are indebted to Anthony Glazko of Parke, Davis and Company for ^{3}H-diphenhydramine hydrochloride and Allen J. Sedman of the University of Michigan for use of the Program AUTOAN.

REFERENCES

1. T. Sollman. *A Manual of Pharmacology and Its Application to Therapeutics and Toxicology*, 8th ed., Saunders, Philadelphia, 1957, p. 559.
2. J. Hald. The excretion of diphenhydramine hydrochloride (dimethylaminoethylbenzhydryl ether hydrochloride) in urine of rabbits and man. *Acta Pharmacol.* **3:** 296–302 (1947).
3. A. J. Glazko, W. A. Dill, J. C. Drach, and T. Chang. *Compilation of Symposia Papers*, American Pharmaceutical Association, Washington D.C., 1970, pp. 339–340.
4. K. S. Albert, E. Sakmar, J. A. Morais, M. R. Hallmark, and J. G. Wagner. Determination of diphenhydramine in plasma by gas chromatography. *Res. Commun. Chem. Pathol. Pharmacol.* **7:** 95–103 (1974).
5. AUTOAN, a time-sharing digital computer program, available from Publication Distribution Service, 615 East University Avenue, Ann Arbor, Michigan 48106.
6. J. Fried and S. Zeitz. Curve fitting by spline and Akima methods: Possibility of interpolation errors and its suppression. *Phys. Biol. Med.* **18:** 550–558 (1973).
7. M. Rowland. Influence of route of administration on drug availability. *J. Pharm. Sci.* **67:** 70–74 (1972).
8. M. Gibaldi and S. Feldman. Route of administration and drug metabolism. *Eur. J. Pharmacol.* **19:** 323–329 (1972).
9. G. Levy and R. Nagashima. Comparative pharmacokinetics of coumarin anticoagulants. VI. Effect of plasma protein binding on the distribution and elimination of bishydroxycoumarin by rats. *J. Pharm. Sci.* **58:** 1001–1004 (1969).