Systemic Absorption of Δ^9-Tetrahydrocannabinol after Ophthalmic Administration to the Rabbit

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Abstract □ Δ^9-Tetrahydrocannabinol was given by ophthalmic administration to the rabbit. Plasma concentrations were measured for two strengths of ophthalmic solution and compared with intravenous data to establish bioavailability. Absorption was variable, while maximum plasma levels were sustained for several hours.

Keyphrases □ Absorption—systemic, Δ^9-tetrahydrocannabinol after ophthalmic administration, rabbits □ Bioavailability—systemic absorption of Δ^9-tetrahydrocannabinol after ophthalmic administration, rabbits □ Pharmacokinetics—intravenous administration, half-lives systemic absorption of Δ^9-tetrahydrocannabinol after ophthalmic administration, rabbits □ Δ^9-Tetrahydrocannabinol—systemic absorption after ophthalmic administration, rabbits

Marijuana has been shown to reduce intraocular pressure in humans when smoked in the form of a cigarette (1). Δ^9-Tetrahydrocannabinol (I), the primary psychoactive ingredient of marijuana, has also been shown to reduce intraocular pressure in clinical studies when given either by the intravenous (2) or oral (3) route of administration. Although efficacy for topical application in clinical studies has not been established (4), dosage form development should be undertaken as this route of administration is expected to minimize the undesired psychotropic side effects.

Studies with the rabbit indicate that I in light mineral oil is an efficient dosage form to allow penetration of I into the eye (5). Related studies have also demonstrated that I applied topically to one eye lowers intraocular pressure in both eyes (6). Since the rabbit has become one of the animal models used to evaluate the therapeutic potential of I for treatment of glaucoma, studies have been carried out to determine the systemic absorption of I after topical ophthalmic application.

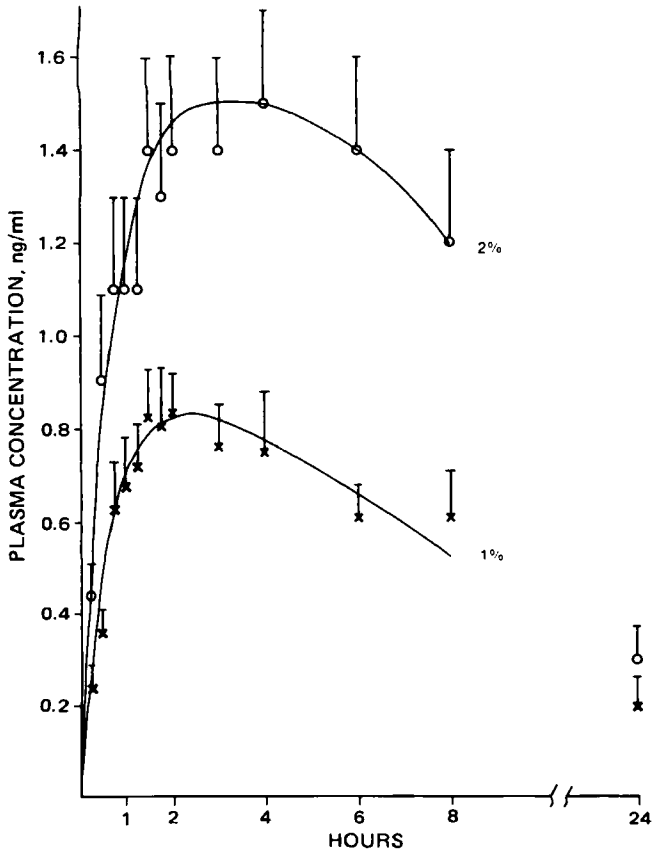

Figure 1—*Plasma concentration of I after ophthalmic administration to rabbits.*

EXPERIMENTAL

Adult albino rabbits weighing 3.4–4.8 kg were given [1′,2′-$^3H_2$]-I, which was used for intravenous and ophthalmic dosage forms. The intravenous solution, containing 170 μCi/mg of [^{3}H]-I, was prepared by dissolving [^{3}H]-I in ethanol, which was then added to a solution of 25% rabbit serum albumin. The solution was sterilized by filtering through a 0.22-μm membrane filter[1] before administration. An intravenous dose of a 0.2-ml solution contained 15 μg of I and 2 μl of ethanol.

Ophthalmic solutions were prepared in light mineral oil NF[2] viscosity of 9.5 centistokes at 21.5° (50–60 Saybolt Universal Seconds) to a strength of 1% I, 138 μCi/mg, and 2% I, 165 μCi/mg. The animals were dosed by ophthalmic topical application with 25 μl of I solution, which was applied to both eyes simultaneously to ensure measurable plasma levels of I. Each animal was dosed with 1 and 2% solutions on *separate occasions*, and four of the animals were also given a dose of 15 μg iv of I through an ear vein. A washout period of 1 month was allowed between all studies.

Blood samples were collected from an ear notch at 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 360, 480, and 1440 min after ophthalmic dosing and at 1.5, 5, 10, 20, 30, 45, 60, 90, 120, 240, 360, and 480 min after intravenous dosing. Blood samples of 2 ml were drawn, centrifuged, separated, and the plasma was immediately frozen until the assay was carried out. The ophthalmic administration, carried out with a syringe[3], yielded such small losses (≤7% of the dose) that no corrections were made.

Quantitative analysis of plasma for the concentration of I was carried out by TLC according to a previously described method (7, 8). Plasma samples were extracted twice with petroleum ether containing 1.5% isoamyl alcohol. The extracts were then concentrated and chromatographed on silica gel plates[4]. Developing solvents were acetone–*tert*-butyl alcohol–chloroform (3:5:92). Zones of I were scraped and counted. The TLC method was shown to correlate well with electron-capture GLC and with GLC–MS methods of analysis.

RESULTS

Plasma concentrations of I after ophthalmic administration of 1 and 2% solutions are reported as average values for eight animals in Fig. 1. The concentration increases to maximum values over the first 1–2 hr and then declines very slowly up to 24 hr. Variability in the data is indicated by the standard errors which are also given in the figure. Data for individual animals are presented in Table I for the maximum plasma concentration, C_{max}, which is actually a limited range of values, the time of these peak values t_{peak}, the time interval over which the C_{max} range extends, and the area under the plasma curve from t = 0 to 24 hr,

[1] Millipore Corp., Bedford, Mass.
[2] Klearol, Witco Co., New York, N.Y.
[3] Wiretrel, Bolab Inc., Derry, N.H.
[4] Brinkmann Inc., Westbury, N.Y.

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Table I—Pharmacokinetic Data Following Ophthalmic Administration of I to Rabbits

Animal No.	C_{max} ng/ml		t_{peak}, min		$AUC_{0-24\ hr}$ hr ng/ml		F, %	
	1%	2%	1%	2%	1%	2%	1%	2%
1	1.0–1.3	1.8–2.3	60–240	240–480	14	30	33	36
2	0.8–1.5	1.2–1.6	60–480	90–480	24	23	—	—
3	0.7–1.2	1.0–1.5	45–180	30–240	14	17	—	—
4	0.4–0.5	0.4–0.6	60–480	30–480	7	11	13	10
5	0.7–1.3	1.4–2.3	45–480	45–480	17	33	40	38
6	0.3–0.6	1.2–1.7	45–480	30–360	6	18	6	8
7	0.3–0.9	0.4–0.7(1.4)[a]	15–480	30–480(180)[a]	13	11	—	—
8	1.6–1.2(2.2)[a]	—	30–180(105)[a]	—	8	—	—	—
9	—	1.9–2.6	—	30–480	—	37	—	—

[a] The values in parentheses are single points above the C_{max} range which occurred within the t_{peak} range.

Table II—Pharmacokinetic Parameters of I Following Intravenous Administration[a] to Rabbits[b]

Animal No.	k_{12} hr^{-1}	k_{21} hr^{-1}	k_{10} hr^{-1}	V_1^{-1}, liter^{-1}	$t_{1/2\alpha}$ min	$t_{1/2\beta}$ hr	AUC, ng/ml hr
1	6.12(48)	1.50(25)	2.76(57)	0.27(65)	4	1.7	1.25
4	0.85(15)	0.47(31)	1.46(13)	0.15(10)	17	2.6	1.65
5	0.97(20)	0.82(21)	1.61(9)	0.13(9)	14	1.5	1.30
6	3.55(18)	1.08(10)	1.65(13)	0.31(14)	7	2.3	3.27

[a] Administration of a 15-μg dose. [b] The percent coefficient of variation of each parameter is given in parentheses. The half-life values are calculated from α and β which are calculated from: $1/2[(k_{12} + k_{21} + k_{10}) \pm (k_{12} + k_{21} + k_{10})^2 - 4\,k_{21}k_{10})^{1/2}]$.

$AUC_{0-24\ hr}$. Inspection of the individual data shows that the plasma concentration increases relatively rapidly to a maximum value, which then varies very little for the next several hours as a plasma plateau exists over an extended period of time. Animal 1 in the 1% study had a C_{max} which varied in a range from 1.0 to 1.3 ng/ml during the time t_{peak} from 60 to 240 min postdose. Similarly, the 2% solution for the same animal gave a C_{max} range of 1.8–2.3 ng/ml for the plasma plateau, which existed during the 240–480-min period, and showed higher plasma levels as compared with the 1% data during the plateau. The dose-normalized AUC values for animal 1 were approximately the same for both doses. Table I shows that the plasma maxima were higher for the 2% dose but were usually not double the lower values. The time plateau, which always extended over several hours, usually showed no difference between doses for most animals. The AUC ratio for the 2:1% solutions was >1 for the four of the animals and ~1 for the other animals.

For the intravenous studies, the average plasma data for four animals is shown in Fig. 2. The curve is biphasic for each animal in agreement with work reported earlier (9). The pharmacokinetic parameters were obtained by nonlinear regression analysis to solve the standard two-compartment open model (10) in terms of:

$$dC_1/dt = -(k_{10} + k_{12})C_1 + k_{21}C_2 \quad \text{(Eq. 1)}$$

$$dC_2/dt = k_{12}C_1 - k_{21}C_2 \quad \text{(Eq. 2)}$$

with the initial condition:

$$V_1^{-1} = C_{1(t=0)}/\text{Dose} \quad \text{(Eq. 3)}$$

Table II presents the micro rate constants k_{12}, k_{21}, k_{10}, the reciprocal volume V_1^{-1}, and the respective coefficients of variation (CV) for the four parameters. For animal 1 the CV values are so large as to render the computer fit questionable, but for the remaining three animals the CVs indicate a good computer estimate of the pharmacokinetic parameters. For the three animals the value for k_{12} has a range of 1.0–3.6 hr^{-1}, while the value for k_{21} is in closer agreement (0.5–1.1 hr^{-1}). The elimination rate constant k_{10} was well estimated with a small range, 1.5–1.7 hr^{-1}. The computer estimate of V_1^{-1} ranges from 0.1 to 0.3 liters^{-1}. The calculated half-lives are 4–17 min for $t_{1/2\alpha}$ and 1.5–2.6 hr for $t_{1/2\beta}$.

The bioavailability for the two ophthalmic solutions relative to the intravenous dose calculated by AUC ratios reported for the four animals

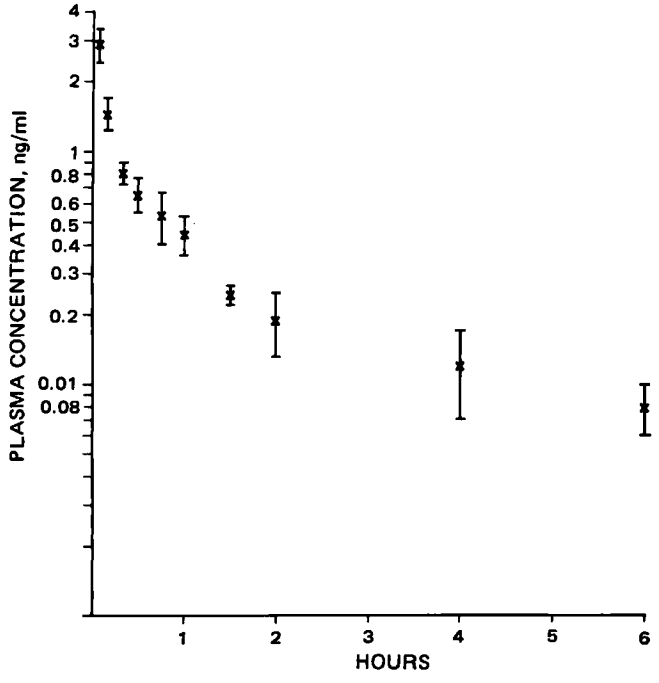

Figure 2—*Plasma concentration of I after intravenous administration of a 15-μg dose to rabbits.*

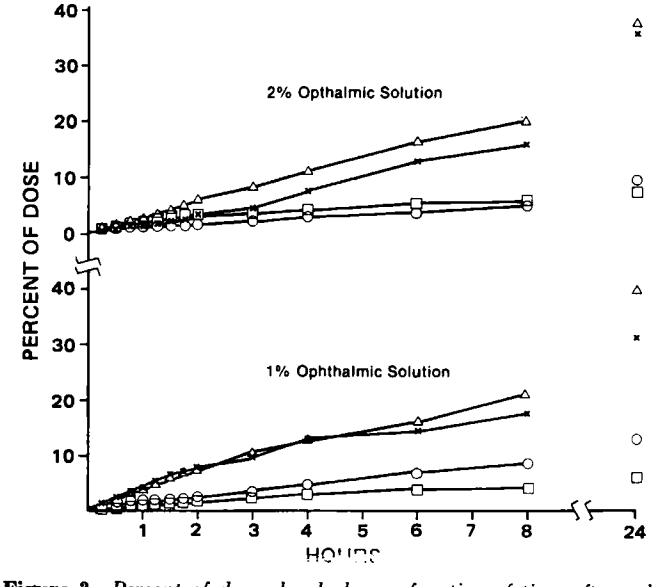

Figure 3—*Percent of dose absorbed as a function of time after ophthalmic administration to rabbits. Key: (X) rabbit 1; (○) rabbit 4; (△) rabbit 5; (□) rabbit 6.*

in Table I is approximately constant. An estimate of the fraction of dose absorbed as a function of time after opthalmic administration can be obtained from (10):

$$A_t/\text{Dose} = [C_t + k_1 AUC_{(0-t)} + (X_t)V_1^{-1}] \times V_1/\text{Dose} \quad \text{(Eq. 4)}$$

This equation was applied to the experimental data from t = 0–8 hr only, and the 24-hr points were determined separately from the dose-normalized AUC ratios. The fraction of dose absorbed as a function of time is presented in Fig. 3 for the 1 and 2% ophthalmic doses for the four animals using the parameters reported in Table II. From Fig. 3 it can be seen that absorption continues until at least 8 hr after administration for all animals at both doses. Furthermore, the fraction absorbed as a function of time does not appear to change for the two doses. The curves for animals 4 and 6 indicate a slower rate of absorption and appear to be flattening out at 8 hr, suggesting that absorption is essentially complete. The curves for animals 1 and 5 suggest that both the rate and extent of absorption is considerably greater.

The present study indicates that systemic absorption of I in the rabbit is slow and highly variable after ophthalmic administration. Prior work with rabbits demonstrates the possibility that both local and systemic mechanisms are involved in ocular pressure lowering (5, 6, 11). It would be desirable to have more detailed effect–time data together with plasma–time data in order to investigate possible I plasma–effect relationships. Clinical studies with glaucoma patients using both oral I and smoking marijuana cigarettes also show effective lowering of intraocular pressure. The pharmacodynamics is consistent with the clinical pharmacokinetics for both routes of administration, since the effect has a much slower onset and considerably longer duration for the oral study than occurs in the smoking study. The studies reported here do not differentiate local and systemic mechanisms. For ophthalmic administration, which should minimize systemic side effects, further work is needed to develop an effective dosage form (4).

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Use of Metzler's NONLIN Program for Fitting Discontinuous Absorption Profiles

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Received July 13, 1981, from the *Department of Pharmaceutics, School of Pharmacy, Temple University, Philadelphia, PA 19140.* Accepted for publication March 17, 1982. Present address: Clinical Research Department, Stuart Pharmaceuticals, Wilmington, DE 19897.

Abstract □ An alternative to the use of integral hybrid flow/compartmental model (HFCM) equations in fitting cases I and II discontinuous absorption profiles is presented. It is proposed that HFCM-integral equations be replaced by a system of differential equations in which sequential sets of equations describe the absorption profile from time zero to infinity. The required sets of differential equations for these two cases are presented as they apply to a two-compartment drug, potentially undergoing multiple absorption steps. It was shown that the use of the NONLIN program in the differential equation mode provides good fits for some unusually shaped absorption profiles of buformin, sulfisoxazole, and griseofulvin. The values of the parameter estimates and the sum of squared deviations, ΣSD, obtained with NONLIN were almost identical to those obtained with the FITSI2 program utilizing HFCM equations. While HFCM-integral equations required less computer time, they introduced the potential for negative absorption times. This problem is avoided by use of the differential equations method.

Keyphrases □ Absorption—fitting discontinuous profiles, use of NONLIN program □ NONLIN program—use for fitting discontinuous absorption profiles

Recently, discontinuous absorption processes in relation to linear pharmacokinetic models were reported (1). Integral equations for hybrid flow/compartmental models (HFCM) were derived for application to single- and multicompartment drugs exhibiting two special cases of discontinuous absorption.

In case I, absorption is assumed to begin at time t_1 and end at time t_2. The mathematical treatment of this case allows for a negative, zero, or positive value of t_1. Zero and positive values of t_1 have been used universally up to the present time in fitting data to equations for the extravascular administration of drugs exhibiting continuous absorption profiles (2). A positive t_1-value indicates the presence of an absorption lag time, while $t_1 = 0$ indicates the absence of a lag time. Negative values of t_1, however, have not been used previously and are difficult to rationalize. It was suggested that negative t_1 values obtained from computer fits using HFCM equations signify a rapid initial absorption phase (1). The absorption rate constant for this phase, however, is not estimable from these equations. Values of t_2 are always positive. When t_2 approximates the time to peak concentration, the absorption profile exhibits a discontinuity or sharp break, at which point the subsequent shape of the profile is governed only by the dispo-

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