Apomorphine: Bioavailability and Effect on Stereotyped Cage Climbing in Mice

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Abstract □ Plasma levels of apomorphine and its conjugates were studied following intravenous, intraperitoneal, and oral administrations to mice. Following hydrolysis, apomorphine and its conjugates were assayed by high-performance liquid chromatography. The absolute bioavailability of apomorphine was 4%. A significant first-pass effect due to extensive conjugation in the liver was postulated based on calculated bioavailabilities and comparison of plasma levels of apomorphine and its conjugates following oral, intraperitoneal, and intravenous administrations. Apomorphine-induced stereotypical cage climbing in mice was investigated following administration of apomorphine by the three routes. Analysis of time-course data obtained from the cage-climbing experiments indicated an absolute bioavailability of 16% for apomorphine.

Keyphrases □ Apomorphine—bioavailability, effect on stereotyped cage climbing, mice □ Bioavailability—apomorphine, intravenous, intraperitoneal, and oral administration □ Behavior—apomorphine-induced cage climbing in mice, intravenous, intraperitoneal, and oral administration □ Emetics—apomorphine, bioavailability, effect on stereotyped cage climbing in mice

Recently, there has been considerable interest in the pharmacology and clinical utility of apomorphine (I). Its ability as an antiparkinsonian (1, 2) was noted at least 15 years before the clinical adoption of levodopa (3). More recent studies confirmed the clinical efficacy of subcutaneously administered apomorphine in the treatment of parkinsonism (4). Activity is observed when I is given to humans orally (5); however, the necessary dosage differential between these two administration routes is reportedly greater than an order of magnitude (5).

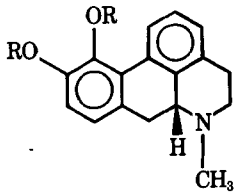
In addition to its potential use as an antiparkinsonian, I has been implicated in the treatment of Huntington's chorea (6, 7), tardive dyskinesia (8, 9), spasmodic torticollis (9), Gilles de la Tourette's syndrome (10), schizophrenia (11–13), and thalamic pain (14). The clinical application of I, however, is confounded by its apparent poor, oral absorption (5), short duration of action (4, 5), and chemical instability (15).

BACKGROUND

Prodrugs may provide a viable alternative to the use of apomorphine (I) *per se*. Its *O,O'*-diesters, II–VI, were previously prepared and tested in mice and rats (16–20). When injected intraperitoneally, these compounds showed prolonged pharmacological effects typical of I. Furthermore, the duration of I-like effects appear to follow rank-order rates of hydrolyses expected for II–VI. However, only indirect evidence has been provided to correlate the pharmacological activity of I prodrugs with circulating blood levels of I (18–20).

The disposition of I in mice following intravenous administration was also studied (21, 22). Similarly to humans, the results in mice indicate a relatively short half-life for I. Recent studies (23) confirmed these earlier data (21, 22). Significant first-pass effects were suggested from experiments with ^{3}H-labeled I and measurements of total and ethyl acetate-extracted radioactivity in mouse plasma following intravenous, intraperitoneal, and oral administrations (23).

In the present experiments, plasma analyses of I and its conjugates by high-performance liquid chromatography (HPLC) were conducted to document more carefully the bioavailability of I in mice. In addition, comparisons were made between chromatographically measured I and murine stereotyped cage climbing (24–26). The latter pharmacological test is dopaminergic in nature (27) and is possibly an effective model for predicting the antiparkinsonian activity of drugs like I (26). It was hoped that possible blood level-pharmacological correlations might be useful in future evaluations of I prodrugs.



I: R = H
II: R = CH_3CO
III: R = CH_3CH_2CO
IV: R = $(CH_3)_2CHCO$
V: R = $(CH_3)_3CCO$
VI: R = C_6H_5CO

EXPERIMENTAL

Reagents—All organic solvents used in the HPLC procedures were distilled-in-glass grade[1]. Water was deionized and double distilled from glass. *R*-(−)-Apomorphine hydrochloride hemihydrate[2] [98% pure as determined by UV spectrophotometry (28)], *N*-*n*-propylnorapomorphine[3], boldine[4], [^{3}H]apomorphine[5] (specific activity 31.3 Ci/mmole), and ascorbic acid[6] were used as obtained. β-Glucuronidase (bacterial type II; 51,300 modified Fishman units/g) was used as received[7], following activation by addition of ~0.05 ml of chloroform/ml of enzyme solution. All other solvents and reagents were analytical reagent grade.

HPLC Analyses—HPLC assays for apomorphine (I) in plasma were performed in duplicate as previously reported (29, 30) but with the following modifications: a 7-cm × 2.1-mm i.d. guard column[8] was installed before the analytical column, and the citrate–phosphate buffer (29, 30), was replaced by 0.05 *M* acetate buffer (pH 3.25) containing 0.001 *M* sodium lauryl sulfate. Analyses of I glucuronides were accomplished after enzymatic hydrolysis. In these instances, 200-μl plasma samples were mixed with 400 μl of a pH 6.8 buffer solution (0.07 *M* phosphate buffer with 8.7 m*M* ascorbic acid) and 50 μl of a β-glucuronidase solution, equivalent to 130 Fishman units.

The enzyme solution was incubated for 30 min at 37°, and the incubation conditions were chosen since a 15-min incubation time under nearly identical conditions was reported to be sufficient for the complete hydrolysis of *N*-*n*-propylnorapomorphine glucuronide at a concentration level of 45 μg/ml (31). Incubation was terminated by the addition of 1.5 ml of acetonitrile solution containing 2 μg of boldine/ml as an internal standard. This solution was vortexed for 5 sec and centrifuged for 10 min at 1230×*g*. An 800-μl portion of the supernate was then transferred to a 1-ml siliated vial[9], evaporated, and assayed according to an earlier method (29). The amount of conjugated I in a sample was determined as the difference between the assay for free I, and the total assay, which includes I glucuronides.

[1] Li Chrosolv, E. Merck, Darmstadt, West Germany, or Burdick & Jackson Laboratories, Muskegon, Mich.
[2] McFarland Smith, Edinburgh, Scotland.
[3] Sterling Winthrop Research Institute, Rensselaer, N.Y.
[4] ICN Pharmaceuticals, Cleveland, Ohio.
[5] New England Nuclear, Boston, Mass.
[6] Fisher Chemical Co., Pittsburgh, Pa.
[7] Sigma Chemical Co., St. Louis, Mo.
[8] Co-Pell ODS, Whatman, Clifton, N.J.
[9] Reactivial, Pierce Chemical Co., Rockford, Ill.

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Table I—Plasma Levels of Apomorphine and Its Conjugates following Administration to Mice

	Plasma Concentration, μg/ml[a]							
	Intraveneous							
	7.5 mg/kg		15 mg/kg		Intraperitoneal, 50 mg/kg		Oral, 50 mg/kg	
Minutes	Apomorphine	Conjugates	Apomorphine	Conjugates	Apomorphine	Conjugates	Apomorphine	Conjugates
3	2.7	0.0	4.0	0.0	3.1	7.3	0.5	4.0
5	2.3	0.0	3.4	0.7	5.0	22.9	1.0	9.5
10	0.5	2.2	2.5	2.0	3.1	22.0	0.7	12.6
15	0.0	1.0	2.0	2.8	4.2	22.7	0.0	7.0
30	0.5	0.0	0.7	2.6	0.8	14.6	0.0	5.4
45	0.0	0.9	0.0	0.7	0.0	7.4	0.0	8.8
60	0.0	0.0	0.0	1.0	0.0	1.8	0.0	3.3
90	0.7	0.0	0.0	0.9	0.0	0.8	0.0	1.9
120	0.0	0.0	0.0	0.0	0.0	2.4	0.0	2.4

[a] As determined by HPLC; average of five or six animals per data point. Values of <0.4 μg/ml (limit of assay sensitivity) were determined as zero.

Table II—Bioavailability Parameters for Apomorphine as Determined by HPLC

	Parent Drug[a]			Conjugates[a]		
Route/Dose, mg/kg	AUC_{0-90}, μg min/ml	C_{max}, μg/ml	t_{max}, min	AUC_{0-90}, μg min/ml	C_{max}, μg/ml	t_{max}, min
iv/15[b]	49	4.0	3	94	2.8	15
iv/7.5[b]	29	2.7	3	31	2.2	10
po/50[c]	7	1.0	5	369	12.6	10
ip/50[d]	70	5.0	5	610	22.9	12

[a] Obtained from HPLC analyses of pooled plasma of five or six mice at each study time (see *Experimental*). [b] Percent parent drug circulating (AUC parent drug/AUC parent drug + AUC conjugates × 100) = 34%. (iv/15) and 48% (iv/7.5). [c] Percent parent drug circulating = 2%. [d] Percent parent drug circulating = 10%; calculated from results with [^{3}H]apomorphine (ip/50), 10% (ether extraction), and 16% (ethyl acetate extraction).

Assay of [^{3}H]Apomorphine in Plasma—Two 100-μl portions of a plasma sample were each mixed with 1 ml of 0.5 *M* phosphate buffer (pH 7.0) saturated with sodium chloride. The solutions were extracted with 4.0 ml of ether or ethyl acetate by vortexing for 5 min and then were centrifuged at 1230×*g* for 20 min. A 2.0-ml portion of each organic layer was mixed with 18.0 ml of scintillation cocktail[10], and 0.5 ml of each aqueous layer was mixed with 9.5 ml of the cocktail. The solutions were counted[11] at 40% efficiency.

Bioavailability Studies—All investigations were conducted with experimentally naive male CD-1 albino mice[12], 20–36 g. Throughout the studies, access to food and water was provided *ad libitum*. Animals were maintained on a 12-hr light–dark cycle (lights on at 6 am and off at 6 pm), and all testing was done between 9 am and 5 pm. Only one plasma sample or one videotaping of stereotypic cage climbing was evaluated from each mouse after dosing; however, each data point was obtained by averaging analyses of pooled plasma samples or stereotyped behavior of five or six animals.

Dose levels of 50 mg of I/kg were administered orally and intraperitoneally, and 15 and 7.5 mg/kg were given intravenously. For the radiochemical study, a 50-mg of I/kg ip dose was given equivalent to ~100 Ci/kg of [^{3}H]apomorphine. Apomorphine was administered orally as an aqueous solution by intubation into the stomach *via* a suitable needle attached to a syringe; intraperitoneal and intravenous doses were given by injection.

Blood samples (400 μl) were taken from the infraorbital sinus (32) into heparanized tubes at 0, 3, 5, 10, 15, 30, 45, 60, 90, and 120 min following I administration and were immediately centrifuged. For chromatographic assay samples, 40 μl of a 150 m*M* ascorbic acid solution was added as an antioxidant to every 1–1.5 ml of plasma pooled from five or six mice per time interval following administration. For selected time periods, up to three replicate samples were collected by the pooled plasma method to evaluate the reproducibility of results. The samples were stored at −15° until analyzed. This temperature and the use of ascorbic acid prevents significant decomposition of I for up to 10 weeks (33). All chromatographic assays were completed within 2 weeks of sampling. For the radioactive samples, 100-μl portions of plasma from each mouse were immediately extracted and/or counted directly within 48 hr. Five [^{3}H]-apomorphine samples were collected for each time period.

Stereotyped Cage Climbing—A modification of a previous procedure (24) was used. For a 1-hr habituation period, the mice were placed into cylindrical cages, 12 cm in diameter × 14 cm high, with walls of vertical bars, 2 mm in diameter and 1 cm apart, surmounted by fine wire mesh. Immediately prior to the administration of I (2.0 and 7.5 mg/kg iv, 2.0 and 4.0 mg/kg ip, or 50 mg/kg po) or isotonic saline (intraperitoneally), behavior was videotaped to establish a baseline of activity for each animal. Beginning 3 min after drug administration, the behavior of each animal was videotaped and later rated "blind" by methods previously described (26).

Calculations—Bioavailability of oral and intraperitoneal doses compared to the intravenous dose was calculated as the ratios of the corresponding areas under the experimental curves (AUC) obtained by cutting and weighing. Areas beyond the last sampling point were initially obtained by extrapolating the apparent concentration decay phase of the individual experimental plasma concentration *versus* time curves to infinite time (34). In the final analyses (Table II), AUC values for the 0–90-min period were obtained because of negligible plasma levels of drug and conjugates for most routes at 120 min. Bioavailability was calculated by dividing AUC_{po} by AUC_{iv} after multiplication by the intravenous–oral

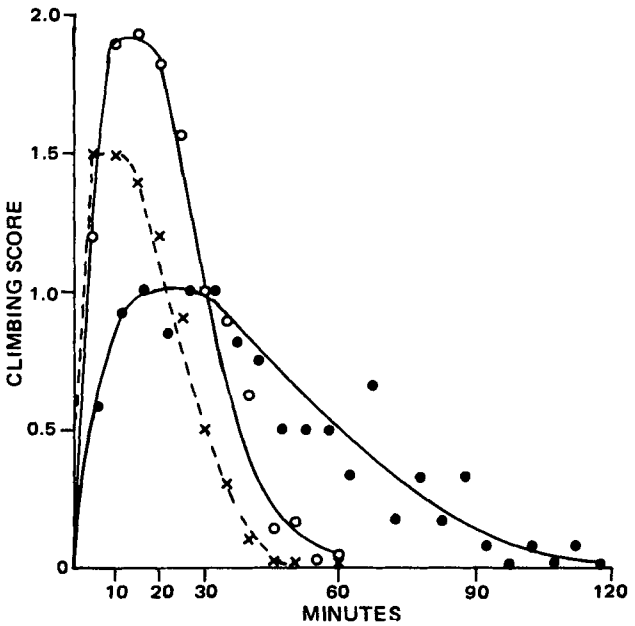


Figure 1—*Time course of murine cage climbing following administration of apomorphine. Key:* ○, *2.0 mg/kg iv;* ×, *4 mg/kg ip; and* ●, *50 mg/kg po.*

[10] Ready-Solo GP, Beckman Instruments, Fullerton, Calif.
[11] LS 8000 scintillation counter, Beckman Instruments, Fullerton, Calif.
[12] Charles River Laboratories, Wilmington, Mass.

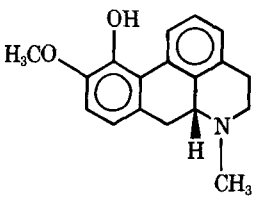
dose ratio. Peak serum concentrations (C_{max}), maximum cage-climbing scores, and times of the peak concentration or score (t_{max}) were obtained from the individual plasma level or stereotypic behavior *versus* time curves.

RESULTS AND DISCUSSION

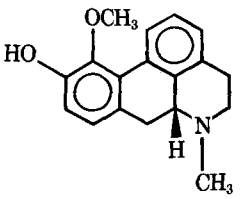
Plasma samples obtained from mice dosed with apomorphine (I) by intravenous, intraperitoneal, and oral routes were analyzed by HPLC (29, 30). Only parent drug was detected in plasma ether extracts, confirming preliminary results obtained by TLC with [^{3}H]apomorphine (23). The major metabolite(s) behaved as an *O*-glucuronide(s) from analyses of plasma fractions treated with β-glucuronidase. The importance of *O*-glucuronidation in the metabolism of I in mice was reported previously (21, 35).

The data in Tables I and II reveal that I is largely present in plasma in its conjugate form, especially after intraperitoneal and oral administrations. Furthermore, experiments utilizing [^{3}H]apomorphine and both ether and ethyl acetate extractions provided measurements of I and its apparent conjugates (following intraperitoneal administration) that were consistent with those in the HPLC determinations. In no experiments were the mono-*O*-methyl ethers of I, apocodeine (VII), and isoapocodeine (VIII) detected as metabolites.

Compounds VII and VIII are readily determined by the HPLC system utilized (36) and are formed *in vitro* when I is incubated with rat liver catechol-*O*-methyltransferase preparations fortified with *S*-adenosylmethionine (37–39). Furthermore, *in vivo* experiments (stereotyped behavior) in rats with I and catechol-*O*-methyltransferase inhibitors seem to suggest a role for VII and/or VIII in the metabolism of I (39). Indeed, Symes *et al.* (40) found elevated brain levels of I in rats pretreated with pyrogallol and tropolone, both catechol-*O*-methyltransferase inhibitors. However, these authors acknowledged that pyrogallol and tropolone may serve as inhibitors of apomorphine *O*-glucuronidation. The mono-*O*-methylation of I is perhaps species specific or is of little apparent quantitative significance in the mouse.



VII



VIII

The absolute bioavailability of I following oral administration [(*AUC* parent drug po/dose po)/(*AUC* parent drug iv/dose iv) × 100] averaged 4%, which confirms similar results obtained using [^{3}H]apomorphine (23). Thus, there appears to be a significant first-pass effect when I is administered orally to mice. The relative amount of unconjugated I found in plasma after intraperitoneal administration was higher than that observed after oral administration but was relatively lower than the levels of parent drug found after intravenous dosing. The percent of circulating parent drug followed the order intravenous > intraperitoneal > oral, and the results with intraperitoneal dosing were confirmed by experiments with [^{3}H]apomorphine (Table II). This finding probably indicates considerable portal uptake after intraperitoneal administration as suggested previously (41).

Despite the apparent rapid and extensive conjugation of I in the mouse, its bioavailability as measured by the extent of absorption of drug plus conjugates [(*AUC* parent drug po + *AUC* conjugates po/dose)/[*AUC* parent drug iv + *AUC* conjugates iv/dose) × 100] averages nearly 90%. Thus, the poor pharmacological activity observed following oral administration of I to mice is likely related to the efficient conjugation to an apparently inactive (or poorly active) conjugate. Similar conjugations and analogous first-pass effects may be the cause of reportedly poor oral activity of I in rats (16) and humans (5).

Stereotyped Cage Climbing—Apomorphine-induced stereotypical cage climbing was studied after intravenous, oral, and intraperitoneal administrations. Cage climbing in mice is an unusual, but apparently specific, response to certain dopaminergic agents (25–27). Treated mice respond by "verticalization" in which two or four paws remain clutching to the vertical bars of specially designed cylindrical cages (24). The cage climbing response is accurately and reproducibly rated by the videotape methodology previously described (26).

Table III—Time Course Measurements of Apomorphine-Induced Cage Climbing in Mice

Route/Dose, mg/kg	t_{max}	*AUC*, units/min
iv/7.5	10	240
iv/2.0	15	86
po/50	15	286
ip/4	10	32
ip/2	10	16

The time courses of I-induced cage climbing for representative doses of I are depicted in Fig. 1. Responses occurred almost immediately following administration, while maximum ratings were observed (Table III) within a few minutes of the t_{max} determined for I by HPLC. Thus, the behavioral response appears to mimic the time course–blood levels of I in mice (21–23). The potentiation of I-induced stereotypical behavior in rodents by compounds that can serve as inhibitors of *O*-glucuronidation or catechol-*O*-methyltransferase also supports this proposal[13] (39, 40).

The relative *AUC* values (Table III) observed for the time course of cage climbing after different administration routes nicely parallel the data determined radiochemically and by HPLC and also by previous radiochemical assays (23). An absolute bioavailability measurement for I can be calculated using the stereotyped cage climbing data in Table III [*i.e.*, (*AUC*-cage climb po/dose po)/(*AUC*-cage climb iv/dose iv) × 100]. When averaged over the available data, the absolute bioavailability of I was calculated as 16%, which is of a similar order of magnitude as the 4% found after HPLC analyses.

As an extension of the data presented, it appears that murine cage climbing may be useful in evaluating the *in vivo* release of I from its prodrugs. Before this evaluation, however, correlations should be sought between chemical analyses of I released *in vivo* and stereotyped cage climbing produced by prodrugs. Evaluations of this type are currently being investigated and will be the subject of a future report.

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Antitumor Agents XLII: Comparison of Antileukemic Activity of Helenalin, Brusatol, and Bruceantin and Their Esters on Different Strains of P-388 Lymphocytic Leukemic Cells

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Abstract □ Based on the fact that some known antineoplastic agents possess an ester moiety within their structure, the esters of helenalin, a sesquiterpene lactone, and of brusatol and bruceantin, quassinoids, were synthesized and tested for antileukemic activity in the P-388 screen. These agents gave different T/C % values dependent on the P-388 lymphocytic leukemia strain and the host strain of mice used. Later studies demonstrated that the agents caused different degrees of inhibition of nucleic acid and protein synthesis in the various P-388 strains. The higher the degree of inhibition of precursor incorporation into the nucleic acid or protein, the higher was the T/C % value obtained in a given P-388 strain. The study demonstrates the lack of consistency of P-388 lymphocytic leukemia cell lines used in various laboratories and indicates that the inbred strain of mice is a critical factor in the tolerance of drug toxicity and, thus, T/C % obtained.

Keyphrases □ P-388 lymphocytic leukemic cells—antileukemic activity of helenalin, brusatol, and bruceantin and their esters □ Antileukemic agents—comparison of helenalin, brusatol, and bruceantin and their esters □ Helenalin—comparison with brusatol, bruceantin, and their esters, antileukemic activity □ Brusatol—comparison with helenalin and bruceantin and their esters, antileukemic activity □ Bruceantin—comparison with helenalin and brusatol and their esters, antileukemic activity

The naturally occurring sesquiterpene lactones continue to provide numerous examples of structures exhibiting significant cytotoxic antitumor activity (1). In general, they have proven to be potent inhibitors of Walker 256 carcinosarcoma growth in rats and Ehrlich ascites carcinoma growth in mice and marginal inhibitors of P-388 lymphocytic leukemia in mice (2). For example, eupaformosanin (3), molephantinin (4), phantomolin (5), eupahyssopin (6), and helenalin (I) (7) all demonstrated highly significant inhibitory activity in the Walker 256 carcinosarcoma survival system with a T/C value of ≧300% at the low dose of 2.5 mg/kg (T/C >140% required for significant activity). However, in the P-388 murine lymphocytic leukemia screen, an *in vivo* test system currently used as a standard method by the National Cancer Institute (NCI) for evaluating compounds of natural origin, these compounds

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