Fate of ^{14}C-3-Methylamino-1,2,3,4-tetrahydrocarbazole Hydrochloride in Rats and Dogs

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Abstract □ The fate of ^{14}C-3-methylamino-1,2,3,4-tetrahydrocarbazole hydrochloride, a potential psychotherapeutic agent, was studied in rats and dogs. Rats were given a single oral or intraperitoneal dose while dogs received the drug either orally or intravenously. Radioactivity in plasma samples and excreta was determined by liquid scintillation counting. In addition, ^{14}C-levels in various rat tissues were estimated. The drug appeared to be well absorbed in both species. ^{14}C-Levels were highest in liver and lung and lowest in plasma. Excretion was primarily urinary and was more complete within the first 96 hr in the rat than in the dog, which suggested a longer ^{14}C-half-life in the dog. No evidence was seen that the drug was demethylated.

Keyphrases □ ^{14}C-3-Methylamino-1,2,3,4-tetrahydrocarbazole hydrochloride—distribution and excretion in rats and dogs □ Distribution—^{14}C-3-methylamino-1,2,3,4-tetrahydrocarbazole hydrochloride in rats and dogs □ Excretion—^{14}C-3-methylamino-1,2,3,4-tetrahydrocarbazole hydrochloride in rats and dogs □ Psychotherapeutic agents, potential—3-methylamino-1,2,3,4-tetrahydrocarbazole hydrochloride, ^{14}C-labeled, distribution and excretion in rats and dogs

Derivatives of aminotetrahydrocarbazole possess antidepressant activity in experimental animals and humans (1) and cardiotonic activity in laboratory animals (2). One such compound, of interest for psychotherapeutic applications, is 3-methylamino-1,2,3,4-tetrahydrocarbazole hydrochloride (I). As part of the drug development process, a study of the biological fate of this compound, ^{14}C-labeled (* in Structure I), was carried out in rats and dogs.

EXPERIMENTAL

Synthesis and Dose—Compound ^{14}C-I, mp 306–309°, was prepared from *U*-^{14}C-phenylhydrazine hydrochloride using the synthesis described by Schut (3). The initial specific activity was 2.39 μCi/mg. Radiochemical purity was demonstrated by ascending paper chromatography [methanol–water–pyridine (20:5:1)].

In all experiments, specific activities and doses of I were expressed as free base equivalents. All animals received a single dose of ^{14}C-I as a freshly prepared solution in isotonic saline. Rats received 10 mg (11.95 μCi)/kg po or ip, and dogs were given 2 mg (4 μCi)/kg po or iv.

Animals—Charles River COBS(CD) male rats, 135–170 g, and male mongrel dogs, 7–14 kg, were assigned at random (4) to experimental groups. Animals dosed orally were fasted 16 hr overnight prior to dosing, and food was not reintroduced until 2 hr after dosing. Drinking water was available *ad libitum*. After drug administration, all animals were placed in individual metabolism cages.

Rat Studies—Fifteen rats with ligated bile ducts were assigned to groups of three each. The animals were decapitated by group 0.5, 1, 2, 4, and 8 hr after oral administration of ^{14}C-I. At death, the GI tracts were ligated at the distal end of the esophagus and at the ileocecal junction. Water was added to each GI tract to a final volume of 50 ml, and each mixture was homogenized in a blender.

In tissue distribution experiments, 30 rats were divided into groups of three each. After oral administration of ^{14}C-I, rats were killed by group at the following intervals: 10, 20, 30, and 45 min and 1, 1.5, 2, 4, 8, and 24 hr. Samples of adrenal, dorsal aorta, brain, perirenal fat, heart, kidney, liver, lung, plasma, and spleen were obtained immediately and stored frozen for subsequent analysis. An additional three rats were killed, and their tissues were used to provide undosed, control data.

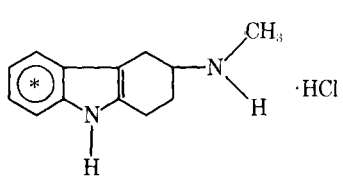

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Table I—GI Absorption of ^{14}C-I by Rats with Ligated Bile Ducts

Hours	Percent of ^{14}C-Dose Absorbed[a]
1	30.99 ± 8.12
2	47.81 ± 9.42
4	61.08 ± 9.64
8	81.44 ± 5.82

[a] Mean ± *SEM* (n = 3). The percent of dose absorbed was calculated by subtracting the disintegrations per minute remaining in the GI tract at each time interval from the disintegrations per minute in the dose, then dividing by the disintegrations per minute in the dose, and multiplying the result by 100.

Twelve rats in groups of six were given ^{14}C-I: one group orally and the other intraperitoneally. Urine and feces were collected daily through 96 hr. Excreta collected prior to dosing served as controls. After volume measurement, urine samples were stored frozen. Feces were dried, powdered, and weighed.

Dog Studies—Twelve dogs were assigned to groups of six each. One group received ^{14}C-I orally while the other group received the drug *via* the cephalic vein. Heparinized syringes were used to obtain blood samples at 10, 20, and 40 min and 1, 2, 4, 8, and 24 hr after dosing. Plasma samples derived from these blood samples were stored frozen for subsequent analysis. All urine and feces voided were collected daily during the 4-day experiment and were stored as already described. Control plasma, urine, and fecal samples were obtained from each dog prior to the study.

Radiochemical Assays—Aliquots of plasma, urine, or GI tract homogenate samples were added to liquid scintillator[1] (5) and counted directly. A sample oxidizer[2] was used to combust aliquots of fecal and rat tissue samples to $^{14}CO_2$, which was collected in a mixture of trapping reagents[3].

All samples were counted in a liquid scintillation spectrometer[4] for 10 min or 10,000 counts. Counting efficiencies were determined by external standardization, and all counts per minute were converted to disintegrations per minute.

Metabolism Studies—Metabolism experiments of a limited scope were run on 0–24-hr urine obtained during the rat and dog excretion studies. These urine samples were pooled by species; 20-ml aliquots of each, adjusted to pH 9.5, were extracted using 1.5 × 8.5-cm columns of a nonionic resin[5]. After a 10-ml water wash, radioactivity was eluted by two successive 10-ml volumes of ethyl acetate. One drop of 0.1 *N* HCl was added to ethyl acetate eluates pooled by species, which were then evaporated to dryness under a nitrogen stream. Radioactivity in the water-wash fraction was discarded. With this technique, 96 ± 2% *SEM* of ^{14}C-I added to control rat or dog urine could be recovered.

The samples were reconstituted in 0.2 ml of chloroform–methanol (1:1 v/v), 1–5-μl aliquots of which were injected into a gas chromatograph equipped with a dual flame-ionization detector system[6]. Separations were carried out on a 0.9-m glass column of 3% OV-17 on 100–120-mesh Gas Chrom Q. The column temperature was 175° (isothermal), and helium at 37 ml/min was the carrier gas. The retention time of I under these conditions was 7 min. Similarly, the retention time of 3-amino-1,2,3,4-

[1] XDC.
[2] Tri-Carb model 306, Packard Instrument Co., Downers Grove, Ill.
[3] Carbosorb and Permafluor V, Packard Instrument Co., Downers Grove, Ill.
[4] Tri-Carb model 3375, Packard Instrument Co., Downers Grove, Ill.
[5] Drug-Skreen (XAD-2), Brinkmann Instruments, Westbury, N.Y.
[6] Model 2740, Varian Instruments Co., Palo Alto, Calif.

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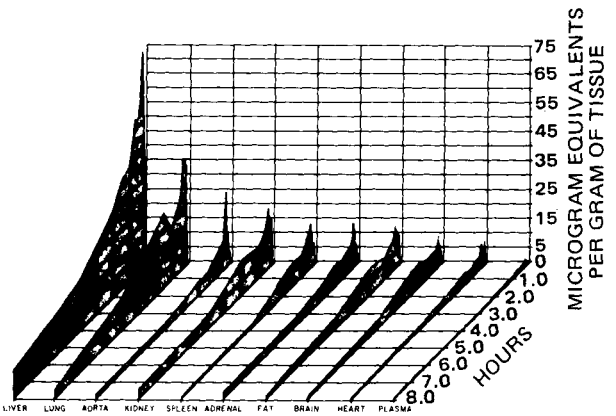

Figure 1—*Tissue levels of undifferentiated carbon-14 in the rat after a single 10-mg/kg po dose of ^{14}C-I.*

tetrahydrocarbazole was 5.9 min. A standard curve, linear from 0 to 4 mg, was obtained with I.

RESULTS AND DISCUSSION

GI absorption experiments in rats (Table I) indicate that I is well absorbed.

The distribution of carbon-14 in rat tissues after a single oral dose is illustrated (through 8 hr) in Fig. 1. While it is recognized that radiochemical measurements include metabolites as well as the original drug, for convenience the levels of undifferentiated carbon-14 in tissues are expressed as microgram equivalents of I per gram or milliliter. In general, tissue ^{14}C-levels maximized within 20–30 min after oral administration, declined for a short time, and then increased again until a second peak (lower than the first and probably representing the appearance of metabolites and/or drug redistribution). The highest ^{14}C-levels were seen in liver and lung tissues. Tissue distribution of I was typical of other strong bases, which tend to distribute in intracellular water, since tissue to plasma ^{14}C-ratios were high. Tissue ^{14}C-levels declined rapidly after 90 min in at least pseudo-first-order fashion, resulting in an average tissue ^{14}C-biological half-life estimate of about 3.1 hr.

Plasma ^{14}C-levels in dogs after oral or intravenous administration of ^{14}C-I are illustrated in Fig. 2. After oral administration, plasma levels were maximal at 4 hr (3 hr later than the rat). Although rats received a fivefold larger dose than dogs, the maximum average ^{14}C-level in the rat was only about three times greater than in the dog.

Excretion data obtained from rats and dogs dosed either orally or parenterally with ^{14}C-I are summarized in Table II. Rats excreted about 79% (orally) and 87% (intraperitoneally) of the dose within 96 hr, and 85 (orally)–92% (intraperitoneally) of the 96-hr total was passed in the first

Table II—Urinary and Fecal Excretion of Carbon-14 by Rats and Dogs after Single Oral or Parenteral Doses of ^{14}C-I

Species	Accumulated Percent Dose Excreted[a] Oral	Parenteral[b]
Rat urine		
24 hr	48.48 ± 4.76	61.61 ± 5.36
96 hr	55.39 ± 5.00	64.90 ± 4.98
Rat feces		
24 hr	19.17 ± 1.53	18.66 ± 1.48
96 hr	24.02 ± 1.57	22.33 ± 1.69
Rat total		
24 hr	67.65 ± 3.63	80.27 ± 4.52
96 hr	79.41 ± 2.78	87.23 ± 3.64
Dog urine		
24 hr	31.31 ± 7.91	37.41 ± 4.36
96 hr	44.21 ± 8.83	51.74 ± 5.49
Dog feces		
24 hr	4.70 ± 0.74	4.58 ± 1.69
96 hr	13.96 ± 0.89	13.80 ± 0.91
Dog total		
24 hr	36.01 ± 7.60	41.99 ± 4.27
96 hr	58.17 ± 9.02	65.54 ± 4.91

[a] Mean ± *SEM* (n = 6). [b] Intraperitoneal administration was used for rats and intravenous administration was used for dogs.

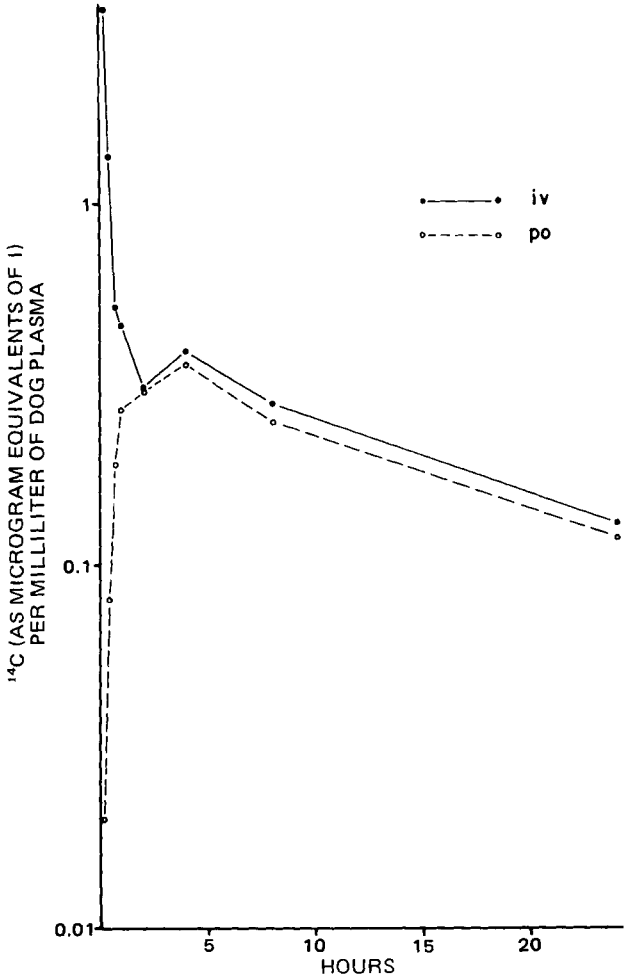

Figure 2—*Semilogarithmic plot of plasma levels of undifferentiated carbon-14 in the dog after a single oral or intravenous dose of ^{14}C-I.*

24 hr. Dogs excreted only about 58% (orally) and 66% (intravenously) of the dose within 96 hr with 62 (orally)–64% (intravenously) of the 96-hr total excreted the 1st day. These data suggest a longer whole body ^{14}C-half-life in the dog. In both species, urine was the preferred route of ^{14}C-excretion. In neither species was the quantity of carbon-14 excreted in urine after oral or parenteral administration statistically different (Student t test), which further indicates that I was well absorbed.

The metabolism of cyclindole, 3-dimethylamino-1,2,3,4-tetrahydrocarbazole, was reported previously (6). This compound, which differs from I only by having an additional N-methyl group, is metabolized primarily by demethylation and/or formation of 7-hydroxylated derivatives. Since I is a metabolite of cyclindole, their hydroxylation patterns should be similar. However, the reference hydroxylated compounds, 3-methylamino- and/or 3-amino-7-hydroxy-1,2,3,5-tetrahydrocarbazole, were not available for GLC comparison with compounds in urine extracts. Therefore, the thrust of the present metabolism experiments was toward identification of unchanged I, its demethylated analog, if present, and any other metabolites sufficiently volatile that derivatization was not required for GLC detection (*i.e.*, nonphenolic metabolites).

About 66 and 68% of the carbon-14 was extractable from rat and dog urine, respectively, using nonionic resin columns. Since the polarity of compounds affects their extraction from the nonionic resin, the 30–35% of urinary carbon-14 not retained by the resin suggests excretion of a metabolite(s) relatively more polar than I by both species, which could be the glucuronic or sulfuric acid conjugates of hydroxylated I.

From the results of radiochemical and GLC analyses, it was estimated that 32 and 28% of a single ^{14}C-I dose administered to rats and dogs, respectively, was excreted unchanged in urine within 96 hr. The remainder of the extractable carbon-14 was probably 7-hydroxylated metabolite(s). While the N-demethylated analog of I was not detected in urinary extracts from either species, the possibility that the demethylated, hydroxylated derivative of I was formed cannot be totally dismissed.

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(6) D. P. Benziger, K. G. Balfour, and J. Edelson, *Drug Metab. Dispos.*, **5**, 239 (1977).

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Radioimmunoassay for Danazol in Human and Monkey Plasma

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Received December 12, 1977, from the *Department of Drug Metabolism and Disposition and the Department of Biometrics, Sterling-Winthrop Research Institute, Rensselaer, NY 12144.* Accepted for publication February 7, 1978.

Abstract □ A sensitive method is described for the radioimmunoassay of danazol in monkey and human plasma. Antiserum was developed in rabbits, and a second antibody was used to separate bound from free danazol. The radioimmunoassay was specific for danazol, and the limit of detection ranged from 1.4 to 2.8 ng/ml. Exogenous danazol could be quantitated accurately in both monkey and human plasma. The radioimmunoassay results agreed with values obtained by inverse isotope dilution after intravenous administration of ^{14}C-danazol to monkeys. The assay was used successfully to measure danazol in plasma from human volunteers receiving 200 mg of danazol.

Keyphrases □ Danazol—radioimmunoassay in human and monkey plasma □ Radioimmunoassay—danazol in human and monkey plasma □ Anterior pituitary suppressants—danazol, radioimmunoassay in human and monkey plasma

Danazol[1] (17α-pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol), a novel steroid, lacks estrogenic or progestational activity upon oral administration but has markedly attenuated androgenic potential (an "impeded" androgen) (1). It inhibits either pituitary gonadotropin synthesis or release in rodents (1, 2), monkeys (3), and humans (4). In males, it depresses the sperm count (5); in women, danazol relieves the symptomatology of endometriosis (6) and may be valuable in the management of benign breast disorders (7).

Limited studies have appeared on plasma danazol concentrations (8, 9). The present report describes a sensitive and accurate method for the measurement of danazol in plasma of both monkeys and humans.

EXPERIMENTAL

Preparation of Antidanazol Serum—The danazol hapten[2] was conjugated with ϵ-amino groups of lysine residues in bovine serum albumin by a carbodiimide condensation (10). A trinitrobenzenesulfonic acid determination (11) indicated that approximately nine hapten molecules were conjugated to each albumin. The hapten–protein conjugate (1.0–2.0 mg) was emulsified in complete Freund's adjuvant[3] and injected intradermally at multiple sites along the dorsal surfaces of three Dutch belted rabbits (12). Booster immunizations of the conjugate in incomplete Freund's adjuvant were administered at approximately 3-week intervals. The rabbits were bled *via* the marginal ear vein.

Preparation of Second Antibody—Anti-rabbit γ-globulin was prepared by repeated subcutaneous injection at monthly intervals of rabbit γ-globulin[4], 100 μg, emulsified in complete Freund's adjuvant to an adult goat. The goat was bled 2 weeks after each immunization. The dilution of the second antibody was 1:16, as determined by a progesterone assay[5] according to the procedure of Midgley *et al.* (13).

Solutions—Iodination buffer contained 0.5 *M* Na_2HPO_4–0.5 *M* NaH_2PO_4 (4:1), adjusted to a final pH of 7.5. Dilute iodination buffer was the same buffer diluted 1:10 with water. Phosphate-buffered saline was made by dissolving 24.51 g of sodium chloride, 1.77 g of monobasic sodium phosphate monohydrate, 3.21 g of dibasic sodium phosphate, and 0.285 g of thimerosal[6] in 3 liters of water and adjusting to pH 7.0. The assay buffer solution contained 0.1% (w/v) gelatin dissolved in phosphate-buffered saline.

Radioiodination—A methyl tyrosinate derivative of danazol[7] (I) was radioiodinated at room temperature by a modification of the method of Greenwood and Hunter (14). Iodination buffer (50 μl) was mixed with 5 μl of a methanol solution of I (1 mg/ml) in a vial. Sodium ^{125}I-iodide[8] (1.0 mCi) was added, the vial was stoppered, and the contents were mixed gently. Chloramine-T[9], 30 μg in 15 μl of dilute iodination buffer, was added, and the reaction mixture was agitated for 2 min. The reaction was stopped by addition of 60 μg of sodium metabisulfite in 30 μl of dilute iodination buffer.

Transfer solution (100 μl), containing 16% (w/v) sucrose dissolved in the assay buffer solution, was added, and the contents of the vial were layered on a 0.5 × 20-cm anion exchange[10] column equilibrated with the assay buffer solution. The vial was rinsed with 70 μl of a solution containing 8% (w/v) sucrose dissolved in the assay buffer, which was then added to the column. The column eluate was collected in 1.0-ml fractions, which were counted to determine the radioactivity profile.

Extraction Procedure—A methanol solution containing 20 μg of hydrocortisone (2 mg/ml) and 40,000 dpm of 1,2-^{3}H-progesterone[11] (40 Ci/mmole) was added to a set of 15 × 85-mm disposable glass tubes, and the solvent was evaporated in a 45° water bath under a nitrogen stream. Plasma (0.5 ml), containing known or unknown amounts of danazol, and water (0.5 ml) were added to the tubes and they were mixed. The mixture was allowed to remain either at room temperature for 2 hr or at 4° overnight. Then *n*-hexane[12] (5 ml) was added to each tube, and the tubes were shaken mechanically for 5 min.

[1] Danocrine, Winthrop Laboratories, New York, N.Y.

[2] 17-Hydroxy-17α-pregn-4-en-20-yno[2,3-*d*]isoxazol-6-ylideneaminoxyacetic acid.

[3] Difco Laboratories, Detroit, Mich.

[4] Fraction II, Miles Laboratories, Kankakee, Ill.

[5] J. E. Peterson and G. D. Niswender, unpublished data.

[6] Sigma Chemical Co., St. Louis, Mo.

[7] Methyl 4-hydroxy-α-[[[[[(17α)-17-hydroxypregna-2,4-dien-20-yno[2,3-*d*]-isoxazol-6-ylidene]amino]oxy]acetyl]amino]benzenepropanoate.

[8] New England Nuclear, Boston, Mass.

[9] Eastman Kodak, Rochester, N.Y.

[10] QAE-Sephadex, Q-25-120, Sigma Chemical Co., St. Louis, Mo.

[11] New England Nuclear, Boston, Mass.

[12] Nanograde, Mallinckrodt, St. Louis, Mo.

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