ACKNOWLEDGMENTS AND ADDRESSES

Received June 1, 1976, from the *Division of Pharmaceutical Chemistry, College of Pharmacy, University of Arizona, Tucson, AZ 85721.*

Accepted for publication October 29, 1976.

Supported by Contract NOI-CM-33750 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, MD 20014, and the Elsa U. Pardee Foundation, Midland, MI 48640.

* E. Mead Johnson Memorial Fellow of the American Foundation for Pharmaceutical Education.

[x] To whom inquiries should be directed.

Perfusion and Molecular Modification of Idoxuridine to Alter Its Cerebrospinal Fluid Metabolism

PHILIP H. GUTIN *, LEON D. PROCKOP [‡], K. F. YIP, JAMES D. WEINSTEIN, and K. C. TSOU [x]

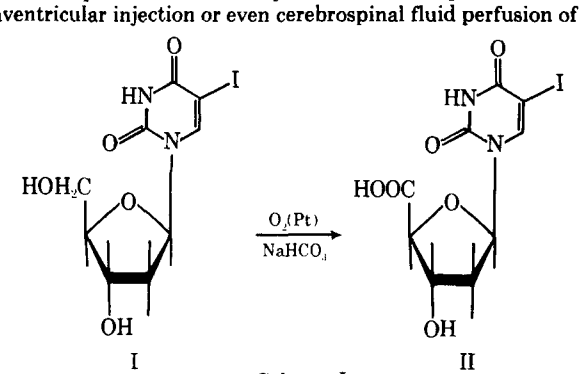
Abstract □ Two methods to deter the rapid intrathecal degradation of idoxuridine were investigated: (*a*) rapid drug perfusion through the ventricular system, and (*b*) modification of the molecule to its uronic acid derivative, 2′-deoxy-5-iodo-5′-uridinecarboxylic acid, to make it less susceptible to enzymatic digestion. Perfusion of idoxuridine through the ventricular system (ventriculocisternal) of dogs at 0.97 ml/min saturated the metabolic pathway so that the outflow solution yielded a single spot (R_f 0.76) on TLC indicative of the intact molecule. The ^{125}I-labeled uronic acid was synthesized from the ^{125}I-labeled parent compound, and the labeled compounds were compared after their individual intracisternal injection in dogs. Since there was no difference in the disappearance rates, the stability of the uronic acid was, in fact, no greater than that of the parent compound *in vivo*. Ventricular perfusion of idoxuridine, however, seems a suitable means for increasing the amount of active drug delivered to central nervous system tumors and viral infections.

Keyphrases □ Idoxuridine—metabolism in cerebrospinal fluid, effect of rapid perfusion and molecular modification, dogs □ Metabolism—idoxuridine in cerebrospinal fluid, effect of rapid perfusion and molecular modification, dogs □ Perfusion, rapid—idoxuridine through ventricular system, effect on metabolism in cerebrospinal fluid, dogs □ Molecular modification—idoxuridine to uronic acid derivative, effect on metabolism in cerebrospinal fluid, dogs □ Antiviral agents—idoxuridine, metabolism in cerebrospinal fluid, effect of rapid perfusion and molecular modification, dogs

Idoxuridine (I), an antimetabolite, is a useful topical antiviral agent in the treatment of *Herpes simplex* infections of the cornea (1, 2). This agent has also been administered systemically in cases of disseminated *H. simplex* and *H. simplex* encephalitis, but its value in this respect remains largely unproved (3–5).

BACKGROUND

The development of the Ommaya reservoir made possible the direct intraventricular injection or even cerebrospinal fluid perfusion of chemotherapeutic agents for central nervous system (CNS) viral infection or malignant disease (6). This subcutaneously implanted reservoir, with a catheter extending to the lateral ventricle, facilitates the delivery of high concentrations of potentially useful drugs to the brain. This route of administration is safe for I administration to patients with brain tumors or encephalitis (7). However, reservoir-delivered intraventricular therapy with I for progressive multifocal leukoencephalopathy in one patient (8) and for malignant brain tumors in four patients treated on this neurosurgical service was of no value[1].



I II

Scheme I

The lack of efficacy of intraventricular I therapy may be related to the very rapid metabolism of the drug by enzymes believed to be associated with the glial, ependymal, neural, or choroid plexus cells in or near the cerebral ventricles, where the drug is degraded to iodouracil and then deiodinated by the rate-limiting step to iodide and uracil (9, 10). Therefore, two methods of minimizing such enzymatic degradation were investigated: (*a*) rapid ventriculocisternal perfusion of I to maintain high levels of drug in the ventricular cerebrospinal fluid and, consequently, saturate the metabolic pathways; and (*b*) modification of the parent compound to its uronic acid derivative, 2′-deoxy-5-iodo-5′-uridinecarboxylic acid, in the hope that it would retain its antiviral and antitumor activity but be less susceptible to enzymatic breakdown. The uronic acid of floxuridine was synthesized previously and showed dramatic resistance to hydrolysis by acid or phosphorylase while remaining moderately toxic to tumor cells *in vitro* (11).

EXPERIMENTAL

Ventriculocisternal Perfusion—Mongrel dogs, 7–20 kg, were anesthetized with pentobarbital, 60 mg/kg iv, with supplements given as needed. The dog was secured in a stereotactic head frame, and an endotracheal tube was placed to ensure adequate ventilatory exchange. A skin incision was made, and a bur hole was drilled in the skull so that its center lay 22–24 mm forward from the ear bar and 7–8 mm lateral to the midline. A No. 18 needle was placed percutaneously into the cisterna magna.

With continuous pressure monitoring, a No. 19 needle in a stereotactic holder was introduced slowly through the bur hole vertically to a depth of 16–18 mm until the pulsations of the choroid plexus transmitted to the cerebrospinal fluid were noted on the pressure recording to assure ventricular placement. Cerebrospinal fluid pressure was measured on a pressure transducer[2] and transmitted for reading on a polygraph[3].

Artificial cerebrospinal fluid was prepared as a modified Krebs–Ringer bicarbonate solution that closely resembled the ionic composition of normal spinal fluid (12). Compound I[4] was diluted to 0.1 mg/ml in artificial cerebrospinal fluid and infused through the ventricular needle first at 0.39 ml/min and then at 0.97 ml/min by means of a constant-infusion pump[5].

Samples were collected from the cisternal needle over timed periods after a 30-min perfusion to ensure homogeneity in the ventricular system,

[1] J. W. Weinstein, unpublished data.
[2] Stratham, Oxnard, Calif.
[3] Grass, Quincy, Mass.
[4] Schwarz/Mann, Orangeburg, N.Y.
[5] Harvard Apparatus, Millis, Mass.

Table I—TLC Characterization of I and II

Compound	Silica Gel Solvent 1[a]	Silica Gel Solvent 2[b]	Cellulose, Solvent 3[c]
I	0.50	0.60	0.76
II	0.36	0.10	0.64

[a] Propanol–ammonium hydroxide–water (7:1:2). [b] Chloroform–propanol (2:8). [c] 1 *M* ammonium acetate–ethanol (3:7).

and pressure was checked after each perfusion. Perfusion experiments used for compilation of the final data were considered technically satisfactory if the needles were placed successfully on the first pass, outflow solutions were free of blood cells, and the pressure remained less than 200 mm H_2O throughout the experiment. Outflow samples were analyzed by TLC for the integrity of the I molecule. The inflow solutions served as controls.

Synthesis of ^{125}I-Labeled 2′-Deoxy-5-iodo-5′-uridinecarboxylic Acid (II)—The synthesis of radiolabeled II from radiolabeled Compound I (Scheme I) was similar to that cited previously for 2′-deoxy-5-fluoro-5′-uridinecarboxylic acid (11). A solution of ^{125}I-labeled Compound I[6] (100 mg containing 100 μCi), sodium carbonate (4 mg), platinum black (100 mg), and 30 ml of water (pH of the mixture was 9.0) was oxidized with gaseous oxygen at 80° for 20 hr. After the solution was cooled to room temperature, the catalyst was removed by filtration.

The filtrate was evaporated to dryness, and the residue was dissolved in 100 ml of water and poured into a Sephadex A-25 (carbonate form) ion-exchange column (1.5 × 30 cm). Unreacted ^{125}I-labeled Compound I was eluted from the column by 0.02 *M* ammonium bicarbonate (500 ml). Compound II was removed by eluting the column with 0.2 *M* ammonium bicarbonate. The eluate was combined and lyophilized to give white solid II as the ammonium salt (29 mg, yield 28.5%, 28.5 μCi); IR (KBr): ν_{max} 3050 and 1700 cm^{-1}. The results of the TLC characterization of I and II are given in Table I.

Disappearance Studies—A No. 20 spinal needle was introduced into the cisterna magna of an anesthetized, intubated dog and was left there throughout the experiment. An aliquot of cerebrospinal fluid (1.5 ml) was removed and replaced by 1.0 ml of artificial cerebrospinal fluid containing 0.5 μCi of ^{125}I-labeled Compound I in one set of experiments and 0.5 μCi of II, prepared as described, in a second set of experiments. The second set occurred 6 months later and used the same animals. Injection of the compounds was followed by vigorous barbotage, and serial spinal fluid samples were taken at 30-min intervals. Gamma radiation in aliquots of the samples was measured in a gamma spectrometer[7].

RESULTS AND DISCUSSION

Ventriculocisternal Perfusion—Compound I (0.1 mg/ml), perfused through the dog ventricular system at 0.39 ml/min, yielded chromatographic spots on cellulose plates at R_f 0.41 and 0.55, indicating that the molecule was degraded. The unperfused inflow solution, serving as a control, yielded a single spot representative of the intact I molecule at R_f 0.76. An increase in the perfusion rate of the I solution to 0.97 ml/min saturated the degradative mechanisms, so the outflow solution contained only parent molecule (R_f 0.76).

Moderate perfusion rates protect against the degradation of I in the dog ventricular system. In humans, perfusions between a lateral ventricle and the spinal subarachnoid space, the lateral ventricle, and even the brain tumor bed have been accomplished (13). Compound I delivered by this method could be of therapeutic value in CNS viral or malignant disease and deserves further study.

Disappearance Studies—In a previous study (9), the intracisternal injection of I into dogs was followed by rapid disappearance of I and the concomitant appearance of iodouracil and iodide. Furthermore, after injection of ^{125}I-labeled Compound I, radioactivity disappeared with a half-time of about 45 min. The disappearance of the ^{125}I-label reflects in part the active transport of iodide (14, 15) cleaved from iodouracil. To some extent, however, the ^{125}I-label disappearance must also reflect the bulk flow of iodouracil from the cerebrospinal fluid since iodouracil → iodide + uracil is the rate-limiting step in the drug degradation (9). In any case, the disappearances of iodide and iodouracil as detected by serial cerebrospinal fluid ^{125}I-measurements represent the exit of the metabolites of inactivated drug and, therefore, provide a good index for following the degradation of ^{125}I-labeled I and II after intracisternal injection.

[6] Amersham/Searle, Arlington Heights, Ill.
[7] Baird-Atomic, Cambridge, Mass.

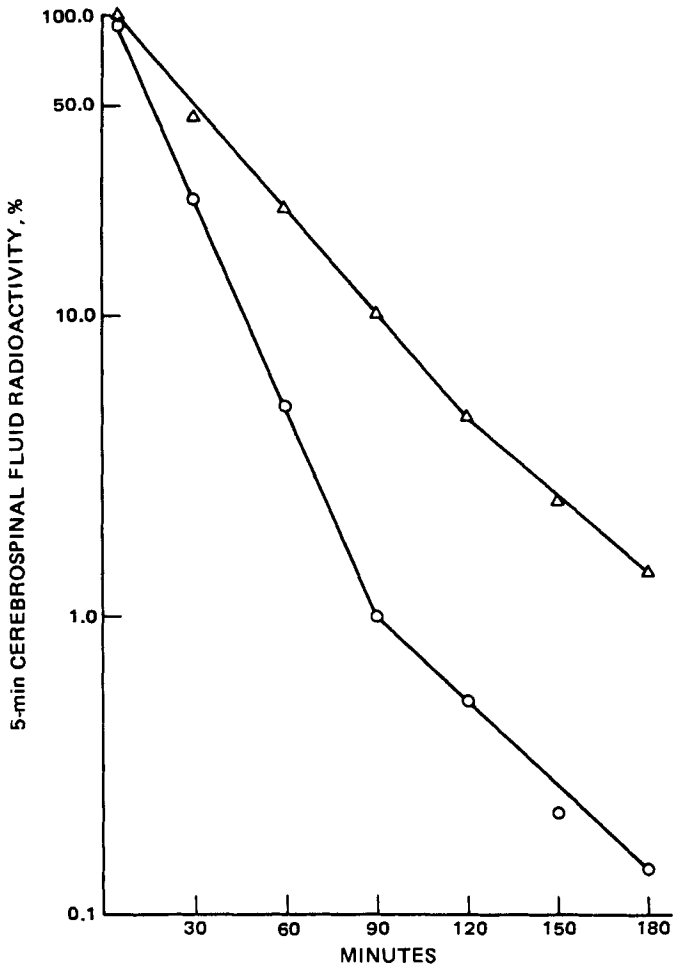


Figure 1—*Disappearance of radioactivity from cerebrospinal fluid of Dog 3 after injection of ^{125}I-labeled I (0.5 μCi) (○) or II (0.5 μCi) (△). Each curve is plotted as the percentage of the 5-min radioactivity. The initial rapid phase was due to mixing, and the slower late phase was due to drug clearance.*

Disappearance of radioactivity from cerebrospinal fluid after intracisternal injection of ^{125}I-labeled I or II was plotted on semilogarithmic paper. Half-times were determined by graphic analysis. There were two slopes to each curve: a more rapid initial phase, probably due to mixing within the cerebrospinal fluid compartment, and a slower, late phase, thought to represent primarily drug clearance (16). Data were expressed in terms of disappearance rates:

$$\text{disappearance rate (\%/min)} = \frac{\ln}{\text{half-time}} = \frac{0.693}{\text{half-time}} \quad \text{(Eq. 1)}$$

The mean of the disappearance rates of the ^{125}I-label from the cerebrospinal fluid of three dogs following ^{125}I-labeled Compound I injection was 0.024 ± 0.007 (*SD*). After II injection in the same animals, it was 0.021 ± 0.002. These numbers are not significantly different ($p > 0.40$). The disappearance plots from a typical animal are shown in Fig. 1.

The disappearances of the ^{125}I-label after injection of ^{125}I-labeled I and II were identical. This result indicates that the formation and clearance of the metabolites of these drugs occur at the same rate, so the chemical stability of uronic acids to hydrolysis by acid or phosphorylases seems to offer no advantage *in vivo*. Therefore, modification of the I molecule to its uronic acid holds little promise for discouraging the rapid intrathecal degradation of this agent.

REFERENCES

(1) H. E. Kaufman, *Proc. Soc. Exp. Biol. Med.*, **109**, 251 (1962).
(2) E. Maxwell, *Am. J. Ophthalmol.*, **56**, 571 (1963).
(3) L. W. Catalano, Jr., G. H. Safley, and M. Museles, *J. Pediatr.*, **79**, 393 (1971).
(4) C. J. Breeden, T. C. Hall, and M. R. Tyler, *Ann. Intern. Med.*, **65**, 1050 (1966).
(5) *N. Engl. J. Med.*, **292**, 599 (1975).

(6) A. K. Ommaya, R. C. Rubin, E. S. Henderson, D. P. Rall, F. G. Gieseke, E. A. Bering, and M. Bagan, *Med. Ann. D.C.*, **34**, 455 (1965).
(7) W. A. Creasey, S. Flanigan, R. W. McCollum, and P. Calabresi, *Proc. Am. Assoc. Cancer Res.*, **9**, 16 (1968).
(8) D. Tarsy, E. M. Holden, J. M. Segarra, P. Calabresi, and R. G. Feldman, *Cancer Chemother. Rep.*, **57**, 73 (1973).
(9) D. R. Clarkson, W. W. Oppelt, and P. Byvoet, *J. Pharmacol. Exp. Ther.*, **157**, 581 (1967).
(10) W. H. Prusoff, J. J. Jaffe, and H. Gunther, *Biochem. Pharmacol.*, **3**, 110 (1960).
(11) K. C. Tsou, N. J. Santora, and E. E. Miller, *J. Med. Chem.*, **12**, 173 (1969).
(12) W. W. Umbreit, R. H. Burris, and J. F. Stauffer, "Manometric Techniques," Burgess, Minneapolis, Minn., 1957, p. 149.
(13) R. C. Rubin, A. K. Ommaya, E. S. Henderson, E. A. Bering, and D. P. Rall, *Neurology*, **16**, 680 (1966).
(14) B. Becker, *Am. J. Physiol.*, **201**, 1149 (1961).
(15) D. J. Reed and D. M. Woodbury, *J. Physiol. (London)*, **169**, 816 (1963).
(16) L. D. Prockop, *Neurology*, **18**, 189 (1968).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 28, 1976, from the *Departments of Neurology and Neurosurgery and the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, and the Neurology Service, Philadelphia General Hospital, Philadelphia, PA 19104.*

Accepted for publication November 9, 1976.

Supported by U.S. Public Health Service Grant CA 07339 from the National Institutes of Health, Grant AT (30-1) 3784 (K. C. Tsou), and Grant NB 08029 (Philadelphia General Hospital).

* Present address: Department of Neurosurgery, University of California Medical Center, San Francisco, CA 94143.

‡ Present address: Neurology Section, Department of Internal Medicine, College of Medicine, University of South Florida, Tampa, FL 33612.

x To whom inquiries should be directed. Present address: Hospital of the University of Pennsylvania, Philadelphia, PA 19104.

Synthesis of 10α-Methoxy-Δ^{8,9}-lysergaldehyde from Elymoclavine

TUNG-CHUNG CHOONG, BRIAN L. THOMPSON *, and H. RICHARD SHOUGH x

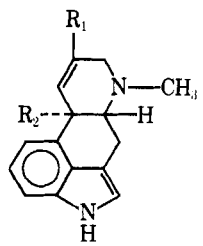
Abstract □ A new synthesis is described for 10α-methoxy-Δ^{8,9}-lysergaldehyde involving the oxidation of elymoclavine with manganese dioxide in methanol. Lysergol and agroclavine provide no reaction under the same conditions.

Keyphrases □ 10α-Methoxy-Δ^{8,9}-lysergaldehyde—synthesized by oxidation of elymoclavine with manganese dioxide in methanol □ Alkaloids, ergot—10α-methoxy-Δ^{8,9}-lysergaldehyde, synthesized by oxidation of elymoclavine with manganese dioxide in methanol □ Elymoclavine—oxidized with manganese dioxide in methanol to 10α-methoxy-Δ^{8,9}-lysergaldehyde

Elymoclavine (I) is well established as an intermediate in the biosynthesis of the lysergic acid-type ergot alkaloids (1). The oxidation of elymoclavine at C-17 is of interest because Δ^{8,9}-lysergaldehyde (II) and/or Δ^{9,10}-lysergaldehyde are possible biosynthetic intermediates and because of the well-known pharmacological properties of the ergot alkaloids. Elymoclavine is easily oxidized to the 8-hydroxy-Δ^{9,10} derivatives penniclavine and isopenniclavine (2), and chanoclavine (6,7-*seco*-elymoclavine) is readily oxidized to chanoclavine aldehyde (3), but the hydroxymethyl group of elymoclavine has proven quite resistant to common oxidants (4, 5).

Lin *et al.* (4) obtained the enol acetate of Δ^{9,10}-lysergaldehyde (6-methyl-8-acetoxymethylene-9-ergolene) by dimethyl sulfoxide–acetic anhydride oxidation of elymoclavine. Mayer and Eich (5) obtained traces of lysergic acid by Oppenauer oxidation of elymoclavine and small yields of dihydrolysergic acid from dihydroelymoclavine. Dihydrolysergaldehyde has been prepared by reduction of lysergic acid derivatives (6).

The manganese dioxide oxidation of 10α-methoxyelymoclavine (III) to 10α-methoxy-Δ^{8,9}-lysergaldehyde (IV) was reported (7). Compound III was obtained by hydride reduction of the 10α-methoxy ester (V) prepared by mercuric acetate oxidation of methyl lysergate in methanol (8). This was the first successful attempt to obtain a lysergaldehyde by oxidation of an elymoclavine derivative.



I: $R_1 = CH_2OH$, $R_2 = H$
II: $R_1 = CHO$, $R_2 = H$
III: $R_1 = CH_2OH$, $R_2 = OCH_3$
IV: $R_1 = CHO$, $R_2 = OCH_3$
V: $R_1 = COOCH_3$, $R_2 = OCH_3$
VI: $R_1 = CH_3$, $R_2 = H$

Previous attempts in this laboratory to prepare lysergaldehydes from elymoclavine also were unsuccessful. However, 10α-methoxy-Δ^{8,9}-lysergaldehyde (IV) can be prepared in good yield by the direct oxidation of elymoclavine with manganese dioxide in methanol.

EXPERIMENTAL[1]

For the preparation of 10α-methoxy-Δ^{8,9}-lysergaldehyde (IV), ely-

[1] Melting points were determined on a Thomas-Hoover Uni-Melt melting-point apparatus and are uncorrected. UV spectra were run on a Beckman model 24 spectrophotometer. IR spectra were run in potassium bromide using a Beckman IR-8 spectrophotometer. NMR spectra were obtained in deuterochloroform on a Jeol C-60H spectrometer with tetramethylsilane as the internal standard. Mass spectra were recorded on an LKB-9000S spectrometer.