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Report

Prodrugs of 5-Iodo-2′-Deoxyuridine for Enhanced Ocular Transport

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Problems associated with the use of 5-iodo-2′-deoxyuridine (IDU) in the treatment of herpes simplex keratitis can be attributed largely to the polar nature of IDU resulting in its poor permeability across the lipoidal epithelial layer of the corneal membrane. Five aliphatic 5′-esters of IDU were synthesized and evaluated as prodrugs for potential use in the treatment of deep ocular infections such as stromal keratitis, iritis, and even retinitis. A parabolic relationship between *in vitro* corneal membrane permeability and carbon chain length of prodrugs is evident. For a given prodrug, enzymatic hydrolysis proceeded most readily in iris–ciliary body, followed by cornea and aqueous humor. An increase in carbon chain length made the prodrugs more enzymatically labile but more resistant to chemical hydrolysis at pH 7.4 and 34°C. The 5′-butyryl ester of IDU exhibited an approximately fourfold increase in aqueous humor IDU concentration relative to IDU at 25 min following instillation of 25-μl 5 m*M* solutions.

KEY WORDS: 5-iodo-2′-deoxyuridine; 5′-ester prodrugs; corneal membrane permeability; enzymatic hydrolysis; aqueous humor concentration.

INTRODUCTION

5-Iodo-2′-deoxyuridine (Idoxuridine; IDU) is a halogenated pyrimidine derivative currently indicated in the topical treatment of herpes simplex keratitis (1). An earlier report from this laboratory addressed the problems associated with existing IDU therapy and explained the rationale behind the development of transient lipophilic esters of IDU as a means of improving the ocular absorption efficiency of the drug (2). Also described was the general procedure for synthesis of those aliphatic esters and their physicochemical properties. In an effort to identify IDU prodrugs for improved ocular delivery, we now report on the *in vitro* corneal transport, hydrolysis kinetics, and *in vivo* ocular absorption of 5′-ester prodrugs of IDU. A lowering of the total topical dose and a sustained drug delivery can be considered a definite improvement over existing IDU therapy.

MATERIALS AND METHODS

IDU was obtained from Sigma Chemical, St. Louis, Mo., and was used without further purification. Dibasic sodium phosphate and sodium chloride were purchased from Mallinckrodt, Paris, Ky. All chemicals and reagents were either analytical or reagent grade and were used as received. Distilled deionized water was used in the preparation of buffer solutions and mobile phases. Adult male albino rabbits (New Zealand Strain) weighing 5–6 lb were used as the animal model. No particular animal pretreatment procedures regarding water, diet, or environment were followed. The analytical methods have been reported earlier (2).

In Vitro Corneal Transport Experiments

A schematic diagram of the diffusion apparatus and the procedure for the isolation and mounting of cornea has been previously described (3). After the cornea was securely mounted, 10 ml of bicarbonated Ringer solution (pH 7.4) was first added to the receptor cell. Likewise, 10 ml of a saturated drug or prodrug solution was added to the donor half-cell. The solutions were preequilibrated to the temperature of the study (34°C). The cell compartments were suitably stirred with magnetic stirrers and the temperature of the cell compartments was maintained at 34 ± 0.5°C. One hundred-microliter aliquots were withdrawn from the receptor cell at predetermined time points over a 3-hr period and subjected to HPLC analysis. Each sample was replaced with an equivalent amount of buffer. Upon completion of an experiment, the cornea was removed, rinsed, and blotted dry. The water content of the cornea was determined by weighing it before and after drying overnight in an oven at 60°C. Using the criteria of Schoenwald and Huang (4), corneas with a hydration level of 83% or greater were considered damaged, and those experiments were disregarded.

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Enzymatic Hydrolysis of Prodrugs in Ocular Tissue Homogenates

Animals were sacrificed by injecting an overdose of sodium pentobarbital into the marginal ear vein. Each eye was immediately enucleated and rinsed with cold saline to remove any traces of blood. About 150 μl of aqueous humor was aspirated using a 27-gauge × 1.27-cm needle attached to a 1-ml tuberculin syringe. Once the aqueous humor was removed, the cornea and iris–ciliary body were removed in sequence using a surgical scalpel. Immediately following their removal the tissues and aqueous humor were transferred to homogenization vessels placed in an ice bath. The tissues and aqueous humor were homogenized in 10 ml of ice-cold isotonic phosphate buffered saline for 2 min using a motor-driven tissue grinder (Brinkmann). The homogenate was centrifuged for 5 min at 10,000 rpm and the supernatant was saved for enzymatic hydrolysis studies. The protein content of each supernatant was then determined using a protein-dye binding assay (Bio-Rad Laboratories, Richmond, Calif.), with bovine serum albumin as the standard.

The supernatant was transferred to screw-capped glass test tubes and equilibrated at 34°C in a water bath. Reaction was initiated by adding 200 μl of a 1 m*M* stock solution of a given prodrug to the supernatant. At various time points, 50-μl aliquots were removed and treated with an equal volume of methanol containing 6% (v/v) perchloric acid. Twenty-five-microliter aliquots were then analyzed by HPLC for the loss of prodrug. The pseudo-first-order rate constants for the hydrolysis of the derivative were determined by linear regression of the logarithm of peak height ratios versus time plots. Triplicate samples were analyzed, and the mean value of rate constant was calculated.

Hydrolysis Kinetics in Aqueous Solution

Reactions were initiated by adding 100 μl of a 1 m*M* stock solution of a particular prodrug to 10 ml of the buffered media in screw-capped test tubes preequilibrated at 34°C. A constant ionic strength of 0.5 *M* was maintained for each buffer by adding a calculated amount of sodium chloride. The reaction was monitored by HPLC analysis of an aliquot of the sample solution removed at various times. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual prodrug concentration versus time.

In Vivo Ocular Absorption of IDU and Its Prodrugs

Drug and prodrug solutions at a concentration of 5 m*M* were prepared in isotonic phosphate-buffered solution, pH 7.4. No attempt was made to induce or to maintain sterility of the solutions, which were freshly prepared. Rabbits were placed in polycarbonate restraining boxes with no restriction in head or eye movement. Fifty microliters of the drug solution was instilled onto the corneal surface and collected in the lower cul-de-sac. During instillation, the lower eyelid was pulled slightly away from the globe to avoid any loss of drug solution but was immediately returned to its normal position following instillation. Both eyes of the animal received the same compound. However, the doses were administered 10 min apart, to aid in sample procurement. Rabbits were sacrificed by rapid injection of an overdose of pentobarbital sodium into the marginal ear vein, immediately prior to aspiration of aqueous humor. The eyes were rinsed with saline, then blotted dry, and about 150 μl of aqueous humor was aspirated from the anterior chamber with a 27-gauge, 1.25-cm needle attached to a 1-ml tuberculin syringe, at 25 min postinstillation of 5 m*M* solutions of IDU and its prodrugs. The aqueous humor sample was mixed with an equal volume of methanol containing 6% perchloric acid. After centrifugation, 30 μl of the supernatant was injected onto the HPLC.

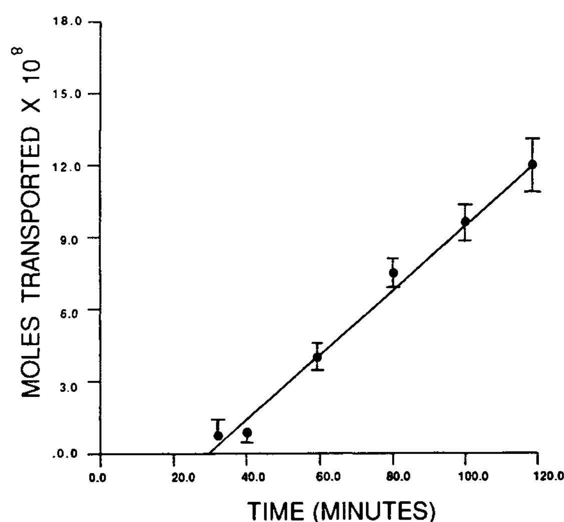

Fig. 1. *In vitro* corneal transport of IDU.

RESULTS AND DISCUSSION

In Vitro Corneal Transport Experiments

Interpretation of *in vitro* corneal transport experiments was accomplished by plotting the amount of drug and/or

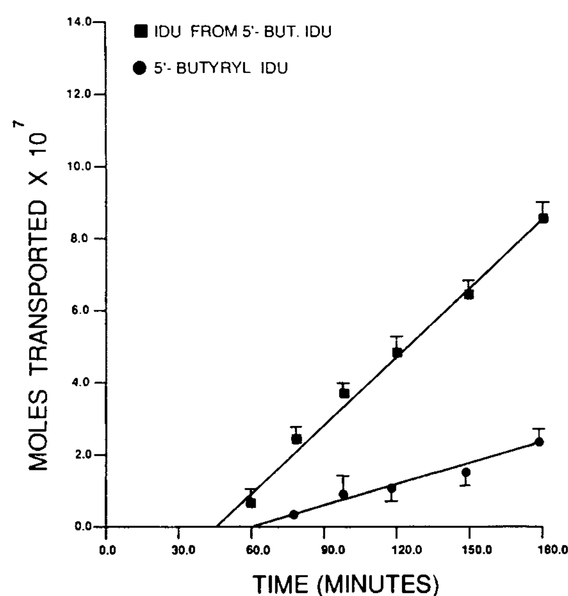

Fig. 2. *In vitro* simultaneous corneal transport and bioconversion of 5′-butyryl IDU.

Table I. Total Steady-State Flux (SSF) and Corneal Membrane Permeability Values from Transport of All Species at 34°C

Compound	SSF × 10^{11a} (M cm^{-2} sec^{-1})	CMP × 10^{6a} (cm sec^{-1})	Ratio of average CMP between 5′-ester and IDU
IDU	2.14 (0.25)	1.71 (0.20)	
Propionyl IDU	9.20 (0.85)	2.71 (0.25)	1.58
Butyryl IDU	17.40 (2.05)	5.36 (0.63)	3.13
I. butyryl IDU	18.97 (1.73)	5.00 (0.46)	2.92
Valeryl IDU	8.20 (0.50)	3.30 (0.20)	1.93
Pivaloyl IDU	5.00 (0.30)	2.62 (0.16)	1.53

[a] Mean (SD) for triplicate determinations.

Table II. First-Order Rate Constants for the Hydrolysis of 0.02 mM IDU Esters in Homogenates of Iris–Ciliary Body, Cornea, and Aqueous Humor of Albino Rabbits at 34°C and pH 7.4[a]

Compound	First-order rate constant (10^3/min^{-1})[b]		
	Iris–ciliary body	Cornea	Aqueous humor
Propionyl IDU	2.45 (0.10)	1.49 (0.15)	1.30 (0.01)
Butyryl IDU	4.49 (0.15)	3.18 (0.09)	2.95 (0.10)
I. butyryl IDU	3.39 (0.09)	2.48 (0.10)	2.30 (0.09)
Valeryl IDU	7.16 (0.25)	6.70 (0.20)	4.24 (0.16)
Pivaloyl IDU	2.12 (0.10)	1.29 (0.02)	1.05 (0.10)

[a] The protein concentration was 7.10 μg/ml.
[b] Mean (SD) for triplicate determinations.

intact prodrug appearing in the receptor phase as a function of time. Figure 1 shows the amount of IDU transported as a function of time. Simultaneous corneal transport and bioconversion was observed for all prodrugs. The amount of intact 5′-butyryl IDU and IDU resulting from hydrolysis of the prodrug during its transit through the membrane is shown in Fig. 2. The individual slope values of the linear steady-state portion of these plots were determined by linear regression and then averaged. This value was used to calculate steady-state flux (SSF) and corneal permeability values (CMP) according to a method reported earlier (5). For the calculation of total corneal permeability of a particular prodrug, individual resistances of intact prodrug and hydrolyzed drug were determined. The reciprocal of the sum of the two individual resistances results in an expression of the total CMP.

Total SSF and CMP values of the permeants along with the ratio of CMP between each prodrug and IDU are summarized in Table I. The total SSF was found to increase gradually with lipophilicity until the C-4 homologues and then decrease with further increases in lipophilicity. The total flux is dependent on the concentration (saturation solubility) of the permeating species and, hence, shows a parabolic relationship with carbon chain length, due to the exponential drop in aqueous solubility with an increase in the methylene-group contribution. The contribution of hydrolyzed prodrug to the total flux was observed to increase as the series was ascended, except for the branched-chain derivatives. Both the 5′-isobutyryl and the 5′-pivaloyl esters were hydrolyzed to a lesser extent than their normal-chain counterparts, during their transit through the corneal membrane, exhibiting the effect of steric hindrance. Figure 3 shows the observed parabolic relationship between *in vitro* CMP and carbon chain length of the permeants. Similar data have been reported for a series of steroids and n-alkyl p-aminobenzoate esters (4,5). A possible explanation for such behavior has been proposed previously (6,7) and is interpreted as resulting from a changeover in the rate-limiting mass transfer step in the transport process. The epithelium and the endothelium layers of the corneal membrane are known to have a greater lipid content per unit weight of tissue than the stroma. Consequently, the epithelial layer offers significant resistance to the transport of ionic and hydrophilic permeants, while the stroma is the principal barrier to the transport of relatively hydrophobic compounds. Large intercellular spaces in the endothelium render it a comparatively insignificant barrier. Since a recent report suggests that the thickness of the stroma-aqueous layer is expected to be much greater than that of the aqueous diffusion layer on the donor side, diffusion layer control can be interpreted as control by diffusion through the stroma. The parabolic relationship witnessed in Fig. 3 suggests that overzealous pursuit of lipophilicity may actually decrease drug availability. *In vitro* corneal penetration of all prodrugs was higher than IDU, with 5′-butyryl and 5′-isobutyryl derivatives exhibiting a threefold increase in CMP relative to IDU.

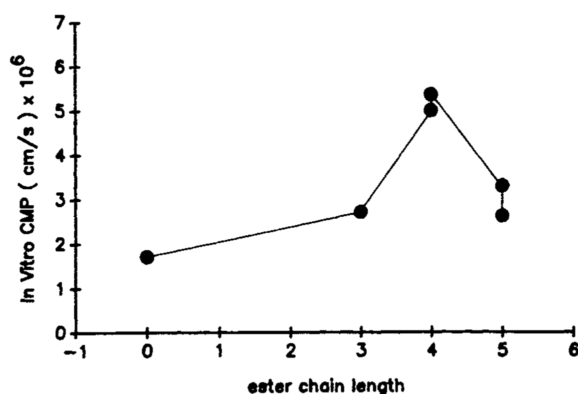

Fig. 3. A plot depicting the parabolic relationship between *in vitro* CMP and ester chain length.

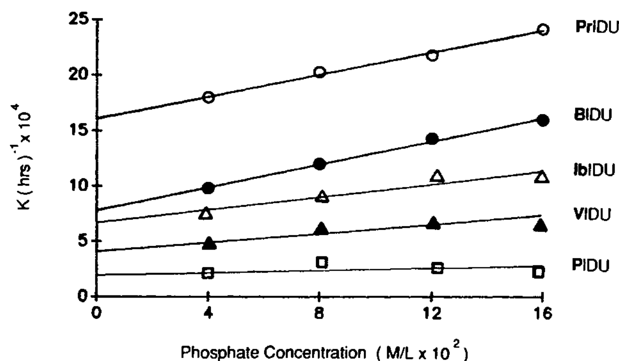

Fig. 4. Chemical hydrolysis of IDU prodrugs at pH 7.4, 34°C (μ = 0.5 M).

Table III. Chemical Hydrolysis of 0.01 mM IDU Esters at 34°C and pH 7.4 (μ = 0.5 M)

Compound	Buffer-independent first-order rate constant ($hr^{-1} \times 10^4$)	Half-life (months)
Propionyl IDU	16.18	0.59
Butyryl IDU	7.79	1.12
I. butyryl IDU	6.76	1.41
Valeryl IDU	3.97	2.39
Pivaloyl IDU	1.91	4.97

Enzymatic Hydrolysis of Prodrugs

Acetyl cholinesterase (AChE) and butyrylcholinesterase (BuChE) appear to be the principal esterases in the eye (8–10). Table II shows the first-order rate constants for hydrolysis of IDU esters in homogenates of the cornea, iris–ciliary body, and aqueous humor of the albino rabbit at a protein concentration of 7.10 μg/ml. All five IDU prodrugs were most readily hydrolyzed in homogenates of iris–ciliary body, followed by cornea and aqueous humor. Considering that the uveal tissues, such as the iris and ciliary body, have the largest relative blood flow of any tissue in the body, it seems reasonable that these tissues would have the best-developed drug metabolizing system found in the eye (11). A significant increase in enzymatic hydrolysis rate was observed with an increase in linear carbon chain length of the substrate in all tissue homogenates, except with the branched-chain esters. Esterases responsible for the hydrolysis of ester prodrugs are believed to carry a hydrophobic pocket on their active site (12,13). An increase in the lipophilicity of the substrate may facilitate its binding to the active site of the enzyme, resulting in its being more enzymatically labile. Branched-chain esters, particularly the 5′-pivaloyl ester of IDU, are relatively stable in ocular tissue homogenates, due to steric bulk hindering the attack by the esterases (14).

Chemical Hydrolysis of IDU Esters

At constant pH, temperature, and ionic strength, strict first-order kinetics were observed for the hydrolysis of all

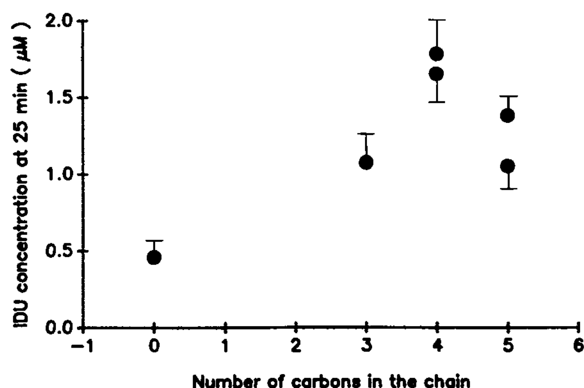

Fig. 5. A plot showing aqueous humor IDU concentrations at 25 min postinstillation of 5 mM colutions of IDU and its prodrugs.

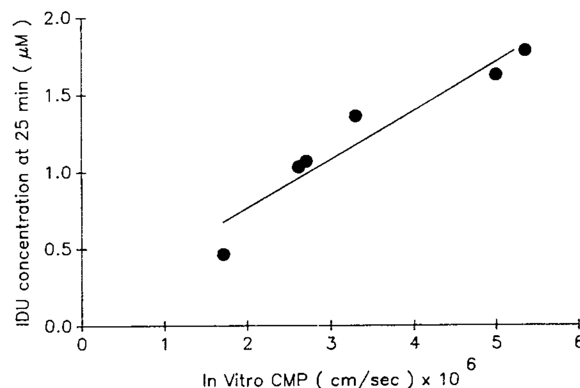

Fig. 6. A plot depicting the correlation between *in vitro* CMP and corresponding *in vivo* aqueous humor concentrations of IDU and its prodrugs.

IDU esters for four to five half-lives. As seen in Fig. 4, phosphate buffer catalysis was observed for the hydrolysis of short-chain esters. Also, an increase in carbon chain length appears to make esters less labile to chemical hydrolysis. Branched-chain esters appear to be more stable than their normal-chain counterparts at pH 7.4. Individual rate constants for hydrolysis and respective half-lives of the prodrugs are listed in Table III.

In Vivo Ocular Absorption of IDU and Its Prodrugs

Since the cornea is the rate-limiting barrier to the productive absorption of ophthalmic drugs, the amount of drug that penetrates the corneal membrane and gains access to the anterior chamber of the eye is a good measure of ocular availability. Aqueous humor concentration–time profiles have traditionally been generated for a variety of drugs to get a measure of their ophthalmic availabilities. This study was designed to detect IDU and prodrug concentration in aqueous humor at 25 min postinstillation of a 5 mM solution.

Figure 5 denotes IDU concentrations in aqueous humor at 25 min postinstillation of 5 mM solutions of IDU and its prodrugs. Hydrolysis of all prodrugs was essentially complete as indicated by lack of detection of intact prodrug in the aqueous humor. An approximately fourfold increase in aqueous humor IDU concentration was observed with the butyryl derivative, relative to IDU. (A parabolic relationship similar to one seen with *in vitro* CMP is also evident between concentration of drug in aqueous humor and carbon chain length.) Figure 6 illustrates the linear relationship observed between *in vitro* and *in vivo* data suggesting that the low corneal membrane permeability of IDU is probably responsible for its inefficient absorption and that the prodrug approach can lead to the rational development of a more cornea-permeable derivative.

Overall, these findings suggest that due to improved corneal absorption, the instilled dose of IDU can be reduced at least twofold, thereby reducing the total drug load. Also, enzymatic cleavage of the prodrug in various ocular tissues may provide a sustained release of the drug at the infected ocular sites. Therapeutic activity of the 5′-butyryl and 5′-isobutyryl esters relative to IDU in the treatment of stro-

mal keratitis will be evaluated in HSV-1-infected rabbit corneas and will be reported later.

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REFERENCES

1. *Physicians' Desk Reference for Ophthalmology,* Medical Economics, N.J., 1987, pp. 96, 151.
2. M. M. Narurkar and A. K. Mitra. *Pharm. Res.* 5:734–737 (1988).
3. A. K. Mitra and T. J. Mikkelson. *J. Pharm. Sci.* 77:771–775 (1988).
4. R. D. Schoenwald and H. S. Huang. *J. Pharm. Sci.* 72:1266–1271 (1983).
5. G. L. Mosher and T. J. Mikkelson. *Int. J. Pharm.* 2:239–243 (1979).
6. G. L. Flynn and S. H. Yalkowsky. *J. Pharm. Sci.* 61:838–853 (1972).
7. G. M. Grass and J. R. Robinson. *J. Pharm. Sci.* 77:3–14 (1988).
8. V. H. L. Lee, R. E. Stratford, Jr., and K. W. Morimoto. *Int. J. Pharm.* 13:183–195 (1983).
9. V. H. L. Lee. *J. Pharm. Sci.* 72:239–244 (1983).
10. R. S. Holmes and C. J. Masters. *Biochim. Biophys. Acta* 132:379–381 (1967).
11. N. D. Das and H. Shichi. *Exp. Eye Res.* 33:525–528 (1981).
12. M. Dixon and E. C. Webb. *Enzymes,* 3rd ed., Academic Press, New York, 1979, pp. 231–270.
13. B. H. J. Hofstee. *J. Biol. Chem.* 207:219–222 (1956).
14. V. H. L. Lee, S. C. Chang, C. M. Oshiro, and R. E. Smith. *Curr. Eye Res.* 4:1117–1125 (1985).