Ocular Penetration of (\textsuperscript{125}I)IVDU, a Radiolabeled Analogue of Bromovinyldeoxyuridine

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Following topical application of (\textsuperscript{125}I) IVDU, the radiolabeled analogue of bromovinyldeoxyuridine ([\textsuperscript{125}I]-5-[2-bromovinyl]-2'-deoxyuridine), as 0.5% or 0.3% eyedrops, to rabbits, (\textsuperscript{125}I) IVDU appeared in the anterior chamber fluid at drug levels well above the minimum concentration (0.01 \(\mu\)g/mL) required for inhibition of herpes simplex virus type 1 replication. These findings are consistent with the efficacy of 0.5% bromovinyldeoxyuridine eyedrops in the topical treatment of herpes simplex uveitis.

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Bromovinyldeoxyuridine ([\textsuperscript{125}I]-5-[2-bromovinyl]-2'-deoxyuridine) is a highly potent and selective antiviral agent, which inhibits the replication of herpes simplex virus type 1 (HSV-1)\textsuperscript{1,2} and varicella zoster virus (VZV)\textsuperscript{3,4} at a concentration of about 0.01 \(\mu\)g/mL. Since bromovinyldeoxyuridine does not affect normal cell metabolism, i.e., host cell DNA synthesis, or cell growth at concentrations up to 50-100 \(\mu\)g/mL, its selectivity index can be estimated at 5,000-10,000.

In animal experiments bromovinyldeoxyuridine has proven efficacious in the topical and systemic, i.e., oral, treatment of a wide variety of experimental HSV-1 infections, including skin lesions,\textsuperscript{5,6} orofacial lesions,\textsuperscript{7} genital lesions,\textsuperscript{8} and encephalitis.\textsuperscript{9,10} Previous studies have revealed that bromovinyldeoxyuridine is significantly better than 5-iodo-2'-deoxyuridine (IDU, idoxuridine) in the topical treatment, as either eye ointment or eyedrops, of superficial HSV-1 keratitis in rabbits.\textsuperscript{11,12} Furthermore, bromovinyldeoxyuridine is significantly better than 5-trifluoromethyl-2'-deoxyuridine (TFT, trifluridine) in promoting the healing of deep stromal keratitis.\textsuperscript{13} A significant reduction in the severity of herpetic iritis also has been observed upon topical administration of bromovinyldeoxyuridine to rabbits, and, in this regard, 0.5% bromovinyldeoxyuridine eyedrops proved again significantly more effective than 1% trifluridine eyedrops.\textsuperscript{14} Topical 3% bromovinyldeoxyuridine in petrolatum ointment also has been found efficacious in suppressing the severity of iritis due to an acyclovir-resistant HSV-1 mutant.\textsuperscript{15}

The efficacy of bromovinyldeoxyuridine in the topical treatment of herpetic keratitis has been established in a large number of patients with either dendritic corneal ulcers, geographic corneal ulcers or stromal disease.\textsuperscript{16,17} Most of these patients first had been treated with idoxuridine, trifluridine or 9-\(\beta\)-D-arabinofuranosyladenine (Ara-A, vidarabine), albeit unsuccessfully, before bromovinyldeoxyuridine treatment was started. They all responded favorably to 0.1% bromovinyldeoxyuridine eyedrops. This also was the case for the patients with stromal keratitis, where bromovinyldeoxyuridine was used alone or in combination with topical corticosteroids.

For bromovinyldeoxyuridine to be effective against stromal keratitis and iritis, it should be able to penetrate the cornea and achieve sufficiently high concentrations in the deep ocular tissues. The present study was undertaken to examine the ability of bromovinyldeoxyuridine eyedrops to penetrate to the aqueous humor. To monitor the intraocular penetration we used a radiolabeled analogue of bromovinyldeoxyuridine, namely (\textsuperscript{125}I) IVDU or (\textsuperscript{125}I) iodovinyldeoxyuridine ([\textsuperscript{125}I]-5-[2-\textsuperscript{125}I]iodovinyl]-2'-deoxyuridine). Iodovinyldeoxyuridine behaves in all aspects similarly to bromovinyldeoxyuridine.\textsuperscript{14-16} (\textsuperscript{125}I) IVDU has been used in the past to determine the mechanism of action of bromovinyldeoxyuridine, i.e., its phosphorylation in HSV-1 infected cells by the virus-encoded thymidine kinase,\textsuperscript{18} and incorporation into HSV-1 DNA,\textsuperscript{19} as opposed to the lack of incorporation of (\textsuperscript{125}I) IVDU into DNA of uninfected cells.\textsuperscript{20}
Materials and Methods

Synthesis of IVDU

(E)-5-(2-Iodovinyl)-2'-deoxyuridine (IVDU) was obtained by decarboxylative iodination of (E)-5-(2-carboxyvinyl)-2'-deoxyuridine. The latter compound was prepared directly from 5-iodo-2'-deoxyuridine in an analogous manner as described before,21 by reaction with methyl acrylate in the presence of palladium acetate as catalyst, followed by saponification of the ester group.22 Thus, (E)-5-(2-carboxyvinyl)-2'-deoxyuridine (3.75 g, 12.5 mmol), iodine (7.5 g, 30 mmol), sodium iodate (2.97 g, 15 mmol) and about 5 g of potassium carbonate were combined in 150 mL of dry dimethylformamide and the mixture was stirred for 3 hr at a temperature of 60°C until the reaction was complete as shown by TLC. The solvent was then evaporated in vacuo and the excess of iodine was removed by several co-evaporations with carbon-tetrachloride. Finally, the oily residue was taken up in ethanol, evaporated to dryness and crystallized from ethanol. Recrystallization from the same solvent afforded 3.35 g (70% yield) of pure white IVDU; mp: 142-144°C (dec); TLC: Rf 0.47 (chloroform-methanol, 8:2); NMR (DMSO-d6): δ 8.16 (s, 1H, H-6), 7.25 (d, vinylic H, J = 14.5 Hz), 7.05 (d, vinylic H, J = 14.5 Hz), 6.20 (t, 1H, J = 6.5 Hz, H-l'), 5.15 (br, 2H, OH-3', and OH-5'), 4.30 (m, 1H, H-3'), 3.86 (m, 1H, H-4'), 3.70 (m, 2H, H-5') and 2.20 (m, 2H, H-2').

Synthesis of (125I)IVDU

Two millicuries of (125I)NaI in 20 μL 0.05 N NaOH was evaporated to dryness in a water bath at 60°C under a stream of nitrogen. To the residue was added 5 mg of IVDU in 0.5 mL ethanol and 1.5 μmol HCl in 0.1 mL ethanol. The ethanolic solution was heated in a sealed tube, followed by saponification of the ester group. Finally, the oily residue was taken up in ethanol, evaporated to dryness and crystallized from ethanol. Recrystallization from the same solvent afforded 3.35 g (70% yield) of pure white IVDU; mp: 142-144°C (dec); TLC: Rf 0.47 (chloroform-methanol, 8:2); NMR (DMSO-d6): δ 8.16 (s, 1H, H-6), 7.25 (d, vinylic H, J = 14.5 Hz), 7.05 (d, vinylic H, J = 14.5 Hz), 6.20 (t, 1H, J = 6.5 Hz, H-l'), 5.15 (br, 2H, OH-3', and OH-5'), 4.30 (m, 1H, H-3'), 3.86 (m, 1H, H-4'), 3.70 (m, 2H, H-5') and 2.20 (m, 2H, H-2').

Animals

Rabbits originating from a crossbreed of California albino and Dendermonde white rabbits, weighing approximately 2-2.5 kg, were used. The use of rabbits in this investigation conforms to the ARVO Resolution on the Use of Animals in Research.

Drug Administration and Sample Collection

Using a micropipette, 5 μL of either 0.2% (125I)IVDU or 0.5% (125I)IVDU were instilled into each eye of four rabbits, at 1-hr intervals. In each medication group, one rabbit was killed 1 hr after the first, second, third, and fourth application of the compound. Before killing the rabbits, 5 mL blood was collected from the ear vein. Immediately after the death of the animals, aqueous humor samples were collected by paracentesis of the anterior chamber with a tuberculin syringe. Contamination of the aqueous fluid by the tear film thereby was avoided. The aqueous samples from each eye were analyzed separately. All experiments were repeated several times, but the data presented refer to only one representative experiment.

Determination of (125I)IVDU Content

Aliquots of the anterior chamber fluid (0.1-0.2 mL) and of the plasma (1 mL) were examined for 125I radioactivity in a 3'' NaI(Tl) well detector, coupled to a single channel analyzer. The (125I)IVDU content of the samples was determined by comparison with the radioactivity measured under the same conditions for a (125I)IVDU standard solution.

Chromatographic Analysis of the Samples

Ten microliters of the sample and 5 μL of an ethanolic solution of IVDU (1 mg per mL) were spotted on a 2 cm X 15 cm plate coated with silica gel Gf254. The plate was developed with CHCl3-MeOH (90:10) and the position of IVDU was determined in ultraviolet light at 254 nm. The distribution and relative amounts of the radioactive compounds (125I)iodide (RF 0), (125I)IVDU (RF 0.3) and 125I-IVU ([E]-5-[2-[125I]iodovinyl]uracil) (RF 0.3) also were determined.

Antiviral Activity

The antiviral activity of the anterior chamber fluid was determined by incubating serial dilutions of the sample on primary rabbit kidney cells, which had been infected with 100 CCID50 (cell culture infecting dose-50) of HSV-1 (strain KOS). Viral cytopathoge-
Table 1. Penetration of (125I)IVDU into the anterior eye chamber and plasma after its administration as 0.5% eyedrops

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Eye 1 (ng/mL)</th>
<th>Eye 2 (ng/mL)</th>
<th>Average (ng/mL)</th>
<th>Plasma (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 5 μL/eye</td>
<td>105</td>
<td>76</td>
<td>90</td>
<td>28</td>
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<td>2 x 5 μL/eye</td>
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<td>3 x 5 μL/eye</td>
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<td>152</td>
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<td>137</td>
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<td>4 x 5 μL/eye</td>
<td>210</td>
<td>234</td>
<td>222</td>
<td>176</td>
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</tbody>
</table>

* Treatment consisted of 0.5% (125I)IVDU eyedrops administered at 5 μL/eye one to four times, with 1-hr intervals.
† The drug concentrations in the anterior chamber and plasma were measured 1 hr after treatment.

The inhibition of HSV-1 replication in primary rabbit kidney cells, the drug levels achieved in the anterior eye chamber and plasma after its administration as 0.2% eyedrops were proportionally lower than those noted following application of 0.5% (125I)IVDU eyedrops and ranged from 37 ng/mL, 1 hr after a single administration of 0.2% (125I)IVDU eyedrops to 92 ng/mL, 1 hr after a set of four applications of 0.2% (125I)IVDU (Table 2). Again, (125I)IVDU was detected in the plasma, albeit at lower levels than those found in the aqueous humor.

The minimum antiviral concentration of iodovinyldeoxyuridine being 10 ng/mL (as determined by the inhibition of HSV-1 replication in primary rabbit kidney cells), the drug levels achieved in the anterior chamber fluid following topical application of 0.2% (125I)IVDU eyedrops exceeded this minimum antiviral concentration by a factor of three- to nine-fold (six-fold at an average). That the drug attained an adequate therapeutic concentration in the aqueous humor was ascertained further by measuring the antiviral activity of the anterior chamber fluid samples. All samples yielded an antiviral effect up to a dilution of 1:6 (Table 3). Thus, based on the inhibition of HSV-1 replication, the concentration of iodovinyldeoxyuridine in the aqueous humor could be estimated roughly at 60 ng/mL, which is consistent with the radioactivity data (Table 2).

The drug levels achieved in the anterior chamber fluid following the administration of 0.2% (125I)IVDU eyedrops consisted mainly of intact (125I)IVDU, with as minor components (125I)IVU and free (125I)iodide (Table 3). The release of (125I)IVU most probably results from the action of thymidine phosphorylase and free iodide must be attributed to the action of a deiodinase. Both enzyme systems (thymidine phosphorylase and deiodinase) are assumed to be present in peripheral tissues.

Table 2. Penetration of (125I)IVDU into the anterior eye chamber and plasma after its administration as 0.2% eyedrops

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Eye 1 (ng/mL)</th>
<th>Eye 2 (ng/mL)</th>
<th>Average (ng/mL)</th>
<th>Plasma (ng/mL)</th>
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<td>1 x 5 μL/eye</td>
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<td>2 x 5 μL/eye</td>
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<td>77</td>
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<td>4 x 5 μL/eye</td>
<td>96</td>
<td>88</td>
<td>92</td>
<td>43</td>
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</table>

* Treatment consisted of 0.2% (125I)IVDU eyedrops administered at 5 μL/eye one to four times, with 1-hr intervals.
† The drug concentrations in the anterior chamber and plasma were measured 1 hr after treatment.
Discussion

Several antiviral agents, currently available for the treatment of herpes simplex keratitis, ie, idoxuridine, trifluridine, vidarabine, and acyclovir, have a pronounced healing effect on the corneal epithelium infection. Bromovinyldeoxyuridine has a significantly better healing effect on experimental HSV epithelial keratitis than idoxuridine, whether the drugs are used as eye ointment or eyedrops.11,12 Dendritic and geographic corneal ulcers in a large number of patients responded promptly to topical 0.1% bromovinyldeoxyuridine eyedrops instillation.16,17 Patients with keratitis that were clinically resistant to idoxuridine, trifluridine, or vidarabine also healed quickly upon bromovinyldeoxyuridine treatment.16,17

Topically applied antiviral agents should be able to penetrate the cornea to exert a beneficial effect on HSV stromal keratitis or iritis. In this respect, idoxuridine and vidarabine are unsuitable for the therapy of deep stromal keratitis or iritis. Upon topical application of idoxuridine or vidarabine, the main products detected in the aqueous humor are their metabolites.24,25 Antiviral concentrations of trifluridine have been detected in the aqueous humor after topical application of trifluridine in keratoplasty patients before surgery.26 However, trifluridine is unable to traverse the normal cornea, since neither trifluridine nor its metabolite, 5-carboxy-2'-deoxyuridine, were detected in the aqueous humor (at the sensitivity level, ie, 2 μM or 0.5 mg/L, of the assay techniques used), upon repeated topical application to cataract patients before surgery.27 That trifluridine can penetrate the cornea of eyes suffering from HSV infection is indicated by its efficacy in the treatment of experimental HSV stromal keratitis and iritis.13,14,28,29

In our experiments, 0.5% bromovinyldeoxyuridine eyedrops had a significantly better healing effect than 1% trifluridine eyedrops on both deep stromal keratitis13 and iritis.14 Our findings show that (125I)IVDU, the radiolabeled analogue of bromovinyldeoxyuridine, administered topically as 0.5% or 0.2% eyedrops, penetrates the cornea to attain adequate antiviral concentrations in the aqueous humor. This explains our earlier observations on the efficacy of bromovinyldeoxyuridine 0.5% eyedrops in the topical treatment of deep stromal keratitis13 and iritis.14

Poirier and co-workers27 showed that acyclovir attained antiviral concentrations in the aqueous humor after four to six topical applications of 3% acyclovir ointment, before cataract operation. However, in our experiments (to be published), acyclovir was less active than trifluridine in the treatment of experimental HSV stromal keratitis and iritis. Also, Varnell and Kaufman30 found that 3% acyclovir ointment did not significantly affect the severity of stromal disease, unless 3% vidarabine ointment was administered simultaneously.

Because of their toxicity, idoxuridine and trifluridine cannot be used systemically. Subconjunctival administration of vidarabine significantly reduces the severity of iritis but causes granuloma formation.31 Intravenous administration of vidarabine requires large fluid volumes because of its poor solubility. Acyclovir and bromovinyldeoxyuridine can be administered orally or intravenously. Both of these compounds are effective in the treatment of VZV infections. Upon oral administration to patients with herpes zoster skin eruption32 or ophthalmic zoster,33 bromovinyldeoxyuridine caused rapid healing of skin lesions, keratitis, and iritis. Considering the adequate ocular penetration of bromovinyldeoxyuridine, as demonstrated here with its radiolabeled analogue (125I)IVDU applied topically as 0.5% or 0.2% eyedrops, this formulation should be pursued as a useful adjunct to oral bromovinyldeoxyuridine therapy in the management of HSV and VZV infections of deep ocular tissues.

Key words: ocular penetration, (125I)IVDU, BVDU, antihers- age

References

8. Sim IS: Oral and topical treatment of experimental HSV-1


