Ocular Penetration and Efficacy of Chloroethyldeoxyuridine Against Herpetic Keratouveitis

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Topical administration of 0.1% or 1% chloroethyldeoxyuridine eyedrops caused a significant reduction in the severity of experimental herpes simplex iritis and stromal keratitis in rabbits. The healing effect caused by chloroethyldeoxyuridine was comparable to that obtained with 0.1% and 0.5% bromovinyldeoxyuridine eyedrops. The drug levels achieved in the anterior chamber fluid following topical application of 1% eyedrops of [2-14C]CEDU, a radiolabelled analogue of chloroethyldeoxyuridine, were well above the minimum concentration (0.1 μg/mL) required for inhibition of herpes simplex virus type 1 replication. The beneficial effects of chloroethyldeoxyuridine on stromal keratitis and iritis appear consistent with its efficient penetration through the cornea. Invest Ophthalmol Vis Sci 27:1453–1458, 1986

Chloroethyldeoxyuridine [5-(2-chloroethyl)-2'-deoxyuridine, CEDU] belongs to a new class of 5-substituted pyrimidine nucleoside analogues, i.e., with a 2-haloalkyl group as the C-5 substituent, which have recently been synthesized and evaluated for their antiviral properties. In vitro (primary rabbit kidney cells), chloroethyldeoxyuridine inhibits the replication of herpes simplex virus type 1 (HSV-1) at a concentration of 0.15 μg/ml, while not being toxic for the host cells at concentrations up to 200 μg/ml. In vivo (mice), chloroethyldeoxyuridine prevents the development of cutaneous HSV-1 lesions, and associated mortality, when applied topically at a concentration as low as 0.1%, and for the treatment of systemic HSV-1 infection in mice, a single oral dose of 5 mg chloroethyldeoxyuridine per kg per day suffices to achieve a significant reduction in the mortality rate.

In a previous study we found that, when chloroethyldeoxyuridine was used as 0.1% or 1% eyedrops, it suppressed the development of epithelial HSV-1 keratitis in rabbits. In its healing effect on herpes simplex keratitis, chloroethyldeoxyuridine was as effective as bromovinyldeoxyuridine, a highly potent and selective antitherpetic agent which has been recognized several years ago as a promising drug for the topical treatment of herpetic eye infections.

Because of its potential in the treatment of ocular HSV-1 infections, chloroethyldeoxyuridine has been further examined in rabbits for its capacity to penetrate the cornea and its efficacy to suppress stromal keratitis and iritis. Corneal penetration (of uninfected eyes) was assessed with radiolabelled chloroethyldeoxyuridine ([2-14C]CEDU). The effect of chloroethyldeoxyuridine on stromal keratitis and iritis was compared with that of bromovinyldeoxyuridine. Treatment was begun 24 hr after virus inoculation, at a time when stromal keratitis and iritis had appeared. Our previous studies have shown that bromovinyldeoxyuridine is efficacious in the topical treatment of stromal keratitis and iritis. When applied topically to normal rabbit eyes, [125I]IVDU, a radiolabelled analogue of bromovinyldeoxyuridine, achieves drug levels in the anterior chamber fluid which are well above its minimum antiviral concentration.

Materials and Methods

Animals

Rabbits 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 conformed to the ARVO Resolution on the Use of Animals in Research.

Drugs

Chloroethyldeoxyuridine and radiolabelled chloroethyldeoxyuridine ([2-14C]CEDU; specific radioactiv-
Corneal Penetration

[2-14C]CEDU eyedrops at 1% were prepared by dissolving the compound in physiological saline on a w/v basis. Using a micropipette, 5 μL of the solution were instilled into both eyes of each rabbit, at 1-hr intervals. One rabbit was killed 1 hr after the first, second, third, and fourth application of the compound. Blood samples (5 mL) were collected from the ear vein before killing the rabbits, and aqueous humor samples were collected immediately after the death of animals. The aqueous humor samples were aspirated in a tuberculine syringe upon paracentesis of the anterior chamber. Before paracentesis, the tear film was dried by an absorbant paper to avoid contamination of the aqueous samples. Samples from each eye were examined separately. The whole experiment was performed twice.

Analysis of [2-14C]Radioactivity

[2-14C]Radioactivity was determined in 10 μL samples by liquid scintillation spectrometry and compared to the [14C] content of 10 μL of the 1% stock [2-14C]CEDU solution measured under the same conditions.

Thin Layer Chromatography (TLC)

Twenty microliters of each sample were spotted on silica gel thin layer plates (Merck 60F 254) and chromatographed in FINK 8 [upper phase from a mixture of ethyl acetate/water/formic acid (40:15:5)]. The plates were dried and cut into pieces of 0.5 cm, and radioactivity was measured for each fraction.

Antiviral Activity

Confluent primary rabbit kidney cells in 96-well microtrays were infected with 100 CCID50 (1 CCID50 being the 50% cell culture infective dose) of HSV-1 (strain KOS). After a 1 hr adsorption period, the virus was removed and serial dilutions of the samples (plasma or anterior chamber fluid) were applied to the cells. Virus-infected cells, incubated with various known concentrations of chloroethyldeoxyuridine, served as a reference. ID50 values (i.e., sample dilution or compound concentration at which viral cytopathogenicity was reduced by 50%) were determined as soon as viral cytopathogenicity reached 100% in the untreated virus-infected cells.

Stromal Keratitis and Iritis

The effects of the antiviral compounds on stromal keratitis and iritis were evaluated in two separate experiments. Rabbits were anesthetized by a subcutaneous injection of fentanyl citrate at 1 mL/kg. Stromal keratitis was produced in 40 rabbits by injecting 0.2 mL virus suspension, containing 10^4.5 plaque-forming units (PFU) of HSV-1 (strain McIntyre), into the central corneal stroma of both eyes. Iritis was produced in another group of 40 rabbits by injecting 0.2 mL of the same virus suspension into the anterior chamber. The infected rabbits were housed in separate cages and numbered serially. In each experiment, the animals were allocated at random to four treatment groups of ten rabbits each. This allocation was not known to the person who evaluated the severity of the ocular infection.

Drugs and Treatment Protocol

Bromovinyldeoxyuridine 0.1% and 0.5% eyedrops and chloroethyldeoxyuridine 0.1% and 1% eyedrops were prepared on a w/v basis in an isotonic borate buffer solution containing 1.52 g boric acid, 0.008 g sodium borate, and 0.01 g of benzalkonium chloride. The pH of the buffer solution was 5.7. The borate buffer solution, without the antiviral drug, was used as placebo.

In the stromal keratitis experiment, the effect of treatment with chloroethyldeoxyuridine 0.1% and 1% eyedrops was compared with the effect of bromovinyldeoxyuridine 0.1% treatment. In the iritis experiment, bromovinyldeoxyuridine was used at 0.5%, since our previous studies have shown that bromovinyldeoxyuridine 0.5% eyedrops have a more pronounced healing effect on HSV-1 iritis than 0.1% eyedrops. The eyedrops were dispensed in coded bottles that looked similar in appearance. The code was not known to the person who instilled the eyedrops, nor to the observer who evaluated the keratitis and iritis.

Treatment was started 1 day after virus inoculation and continued for 5 consecutive days. One drop of each drug was instilled into the eye nine times a day at 1-hr intervals. Both eyes of each rabbit received the same medication.

Keratitis and Iritis Evaluation

Keratitis and iritis were evaluated daily by the same observer using a slit lamp. The corneal stroma and
anterior chamber structures were examined with white illumination. Fluorescein sodium 1% eyedrops and a cobalt blue filter were employed to detect any epithelial disease. The severity of keratitis was evaluated on a grade 0–5 scale, where grade 0 represented a normal transparent cornea, and grade 5 denoted an opaque cornea. Iris scores ranged from grade 0–4, depending upon the severity of iris hyperemia and exudation in the anterior chamber. Grade 0 referred to a normal iris, without any hyperemia or inflammatory reaction, and grade 4 represented severe edema and congestion of the iris with associated protein exudate in the anterior chamber.

### Statistical Analysis

The differences in the severity of keratitis and iritis scores between different treatment groups were subjected to a non-parametric, two-way analysis of variance. *P* values less than 0.05 were considered significant.

### Results

#### Corneal Penetration of [2-14C]CEDU

The drug levels achieved in the anterior chamber fluid following administration of 1% [2-14C]CEDU eyedrops are shown in Table 1. These concentrations ranged from about 1.6 μg/mL, 1 hr after a single administration of the eyedrops, to about 4.7 μg/mL, 1 hr after the last of four consecutive applications of the eyedrops (given at 1-hr intervals). The [2-14C]CEDU levels attained in the aqueous humor increased gradually with the number of applications. So did the drug concentrations achieved in the plasma, although the latter were considerably lower than those detected in the aqueous humor.

The minimum antiviral concentration of chloroethyldeoxyuridine being 0.1 μg/mL, as determined by the inhibition of HSV-1 cytopathogenicity in primary rabbit kidney cells, the radioactive drug levels attained in the anterior chamber fluid following topical application of 1% [2-14C]CEDU eyedrops exceeded this minimum antiviral concentration by a factor of 16- to 47-fold. It was ascertained that the drug attained an adequate antivirally active concentration in the aqueous humor by directly measuring the antiviral activity of the anterior chamber fluid samples. As shown in Table 2, the activity of the radioactive drug closely paralleled the antiviral activity of the drug in aqueous humor.

#### Table 1. Penetration of [2-14C]CEDU into the anterior eye chamber and plasma after its administration as 1% eyedrops

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Eye 1</th>
<th>Eye 2</th>
<th>Average</th>
<th>Plasma</th>
</tr>
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<tbody>
<tr>
<td><strong>Anterior Chamber</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 X 5 μL/eye</td>
<td>1797</td>
<td>1419</td>
<td>1608</td>
<td>53</td>
</tr>
<tr>
<td>2 X 5 μL/eye</td>
<td>3425</td>
<td>2496</td>
<td>2960</td>
<td>256</td>
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<tr>
<td>3 X 5 μL/eye</td>
<td>4041</td>
<td>4324</td>
<td>4182</td>
<td>310</td>
</tr>
<tr>
<td>4 X 5 μL/eye</td>
<td>4029</td>
<td>5024</td>
<td>4526</td>
<td>384</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 X 5 μL/eye</td>
<td>1945</td>
<td>1667</td>
<td>1806</td>
<td>15</td>
</tr>
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<td>2 X 5 μL/eye</td>
<td>2889</td>
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<tr>
<td>4 X 5 μL/eye</td>
<td>4924</td>
<td>4463</td>
<td>4693</td>
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</table>

* Treatment consisted of 1% [2-14C]CEDU eyedrops administered at 5 μL/eye one-four times, with 1-hr intervals.

#### Table 2. Analysis of anterior chamber fluid† after administration of 1% [2-14C]CEDU eyedrops

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Antiviral Activity Detectable up to Dilution‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>[2-14C]CEDU</strong></td>
<td><strong>[2-14C]CEU</strong></td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
</tr>
<tr>
<td>1 X 5 μL/eye</td>
<td>1/5</td>
</tr>
<tr>
<td>2 X 5 μL/eye</td>
<td>1/5</td>
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<tr>
<td>3 X 5 μL/eye</td>
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<tr>
<td>4 X 5 μL/eye</td>
<td>1/15</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
</tr>
<tr>
<td>1 X 5 μL/eye</td>
<td>1/6</td>
</tr>
<tr>
<td>2 X 5 μL/eye</td>
<td>1/11</td>
</tr>
<tr>
<td>3 X 5 μL/eye</td>
<td>1/17</td>
</tr>
<tr>
<td>4 X 5 μL/eye</td>
<td>1/30</td>
</tr>
</tbody>
</table>

* Treatment consisted of 1% [2-14C]CEDU eyedrops administered at 5 μL/eye one-four times, with 1-hr intervals.

† The anterior chamber fluid was analyzed 1 hr after treatment.
‡ Dilution required to reduce cytopathogenicity of HSV-1 (strain KOS) in primary rabbit kidney cells by 50% (average values for three separate determinations). When assayed in parallel, CEDU caused a 50% reduction of viral cytopathogenicity at a concentration of 0.1 μg/mL.

ND: Not determined.

The anterior chamber fluid was analyzed 1 hr after treatment.

The data in Table 1 demonstrate the efficacy of [2-14C]CEDU in providing antiviral activity in the anterior chamber and plasma.
Post infection

**Fig. 1.** Effects of bromovinyldeoxyuridine (BVDU) 0.1%, chlo-roethyldeoxyuridine (CEDU) 0.1% and 1%, and placebo eyedrops on the development of stromal HSV-1 keratitis in rabbits. Treatment was started 1 day after virus inoculation into the corneal stroma.

in Table 2, all samples yielded an antiviral effect up to a dilution that varied from 1/5-1/30. Thus, based on the concentration of chloroethyldeoxyuridine required to inhibit HSV-1 cytopathogenicity, the antiviral drug concentrations in the aqueous humor could be estimated roughly at 0.5-3 μg/mL; i.e., somewhat lower than the radioactive drug concentrations (1.6-4.7 μg/mL).

The radioactive material detected in the anterior chamber fluid following administration of 1% [2-14C]CEDU eyedrops consisted mainly of intact [2-14C]CEDU, with as secondary products, [2-14C]CEU ([2-14C]5-(2-chloroethyl)-uracil) and a yet unknown compound (Table 2). The appearance of [2-14C]CEU most probably results from the action of thymidine phosphorylase (or pyrimidine nucleoside phosphorylases in general), since chloroethyldeoxyuridine, like other 5-substituted 2'-deoxyuridines,10 is an efficient substrate for the enzyme.11 The unknown compound (X) does not correspond to a phosphorylated derivative of chloroethyldeoxyuridine, i.e., CEDU 5'-monophosphate, since its position on TLC does not move to the position of CEDU upon treatment with calf intestinal alkaline phosphatase.

The content of compound X in the aqueous humor sample augmented, upon storage of the samples at 4°C for 2 months, from about 10-20% (Table 2) to approximately 60%. While present at only 0.09% of the total radioactivity in the stock solution of [2-14C]CEDU (1% in physiological saline), the concentration of compound X raised to 5.5% of the total radioactivity after the stock solution has been stored at 4°C for 2 months. Hence, compound X may be a degradation product of CEDU, formed chemically during storage. The related

5-vinyl-2'-deoxyuridine (vinyldeoxyuridine) also gives rise to a degradation product with an RF value on TLC that is identical to that of compound X. Although the exact nature of compound X remains to be identified, it may well represent a polymerization product of vinyldeoxyuridine.

**Stromal Keratitis and Iritis**

Bromovinyldeoxyuridine 0.1% eyedrops and chloroethyldeoxyuridine 0.1% and 1% eyedrops, when applied nine times a day, significantly suppressed the development of HSV-1 stromal keratitis in comparison with placebo eyedrops (P < 0.005) (Fig. 1). In the placebo group, the severity of keratitis increased steadily until the last treatment day (day 5), whereas the antiviral drugs caused an almost complete suppression of the disease. In this respect, chloroethyldeoxyuridine 1% eyedrops appeared more efficient than chloroethyldeoxyuridine 0.1% eyedrops and bromovinyldeoxyuridine 0.1% eyedrops. However, the difference between the effect of the 1% chloroethyldeoxyuridine eyedrops and the effects of the two other treatment regimens was not statistically significant (P > 0.05). The rabbits were observed for another 3 days after cessation of therapy. The severity of keratitis did not further increase in any group during this period.

In the iritis study, chloroethyldeoxyuridine 0.1% and 1% eyedrops and bromovinyldeoxyuridine 0.5% eyedrops again exerted a significant healing effect on the severity of inflammation, as compared to placebo treatment (P < 0.01) (Fig. 2). As in the stromal keratitis experiment, chloroethyldeoxyuridine 1% eyedrops appeared to have a more beneficial effect on iritis than either chloroethyldeoxyuridine 0.1% eyedrops or bromovinyldeoxyuridine 0.5% eyedrops. However, the differences between the effects of the three treatment regimens were not statistically significant (P > 0.05).

Toxic side effects of the drugs were not observed during the observation periods (up to 5 days post-infection in the iritis study, and up to 8 days post-infection in the stromal keratitis experiment).

**Discussion**

When applied topically as 1% eyedrops in physiological saline, [2-14C]CEDU, the radiolabelled analogue of chloroethyldeoxyuridine, readily penetrates to the aqueous humor, where it achieves concentrations that, based on both radioactivity and antiviral activity measurements, are in excess of the minimum concentration (0.1 μg/mL) required for inhibition of HSV-1 replication. On the other hand, chloroethyldeoxyuridine causes a significant reduction in the severity of HSV-
cally applied antiherpes agents should possess good
titrogen production.\textsuperscript{19,20} To be useful in the clinic, topi-
which is associated with soluble and insoluble viral an-
may represent an abortive form of virus replication,
rarely found. Formation of incomplete virus particles
stromal herpes, whereas complete herpes virions are
formation of incomplete virus particles
virions are
inhibits virus replication.

For chloroethyldeoxyuridine to be effective against
stromal keratitis and iritis, it should be able to penetrate
the cornea and achieve sufficiently high concentrations
in the aqueous humor, as has been previously demonstrated
with \( [\text{125I}]\text{IVDU} \), a radiolabelled analogue of bromo-
vinyldeoxyuridine.\textsuperscript{4} Although additional toxico-
logical examinations are required to fully assess the clinical
potential of chloroethyldeoxyuridine, the compound
appears to be a promising candidate drug for the topical

treatment of HSV-1 eye infections, not only for epi-
theal keratitis, as indicated by our previous studies,\textsuperscript{4}
but also for stromal keratitis and iritis, as shown here.
Penetration of chloroethyldeoxyuridine in diseased
corneas with altered permeability characteristics may
even be greater, as trifluridine does not penetrate the
normal corneas but easily traverses the diseased cor-
neas.\textsuperscript{13}

In the present experiments, treatment of herpes sim-
ples virus-induced stromal keratitis and iritis was
commenced 24 hr after virus inoculation, when im-
une mechanisms could not have developed. It is not
known to what extent immune reactions contribute to the
clinical manifestation of herpetic iritis, or whether
virus replication is involved at all; yet, immune reac-
tions are assumed to maintain the inflammatory re-
sponse of deep ocular tissues in clinical herpes virus
disease.\textsuperscript{14-19} Incomplete, noninfectious virus particles
have been detected in abundance\textsuperscript{18,19} in recurrent
stromal herpes, whereas complete herpes virions are
rarely found. Formation of incomplete virus particles
may represent an abortive form of virus replication,
which is associated with soluble and insoluble viral an-
tigen production.\textsuperscript{19,20} To be useful in the clinic, topi-
cally applied antiherpes agents should possess good
corneal permeability, inhibit virus replication in the
deep ocular structures, and consequently arrest the vi-
rus-associated antigen synthesis.

\textbf{Key words:} chloroethyldeoxyuridine (CEDU), ocular pene-
tration, herpes simplex iritis, herpes simplex stromal keratitis

\textbf{Acknowledgments}

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