Florenal in treatment of herpesvirus and adenovirus eye diseases

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Florenal, a new antiviral preparation, has shown inhibitory action on the cytopathic effect of herpesvirus in tissue culture. Florenal had a marked therapeutic effect in mice challenged intracerebrally with the herpesvirus. Clinical evaluation of herpetic keratitis, adenovirus conjunctivitis, and epidemic keratoconjunctivitis patients treated with Florenal ophthalmic ointment revealed a good therapeutic response.

Key words: hepatic keratitis, herpes virus, adenovirus conjunctivitis, Florenal, keratoconjunctivitis.

In recent years a number of preparations have been developed which possess antiviral activity and are effective to some degree in the therapy of experimental herpetic keratitis; these are IDU (5-iodo-2'-deoxyuridine, F$_3$TDR (trifluorothymidine), MADU (5-methylamino-2'-deoxyuridine, oxoline, EDU (5-ethyl-2'-deoxyuridine, morgalin (N-1anhydro-bis-beta hydroxylethyl biguanide hydrochloride), gossipol, tebrophen, and bromouridine. The first attempts are being made to use interferons and the interferon inducers in the therapy of herpetic and adenovirus eye disease. However, very few effective antiviral agents are available in ophthalmologic practice. This communication concerns the experimental evaluation of Florenal, a new antiviral which may be valuable.

Materials and methods

Preparation. Florenal* is a yellow crystalline powder, soluble in water, but insoluble in alcohol and ether. The drug exhibits a high neutralizing activity with respect to influenza virus in tissue cultures and embryonated eggs. The drug was used as a powder formulation dissolved in water and as an eye ointment at concentrations of 0.25, 0.5, and 1 per cent.

Viruses. For inoculation of tissue cultures in order to study the inhibiting effect of the drug and for reproduction of experimental herpetic keratitis in rabbits, herpes simplex virus was used (Strain 1-c. MONIKI) isolated from a patient and passed over several years (R. I. Abramishvile). For intracerebral infection of mice herpes simplex virus was used (Strain Beliakov) passaged in the laboratory (N. S. Zaitseva).

Tissue culture. To study the inhibiting effect of Florenal on reproduction of herpes simplex virus, cell cultures of chicken fibroblasts were used. These cells were cultured in test tubes which contained Medium 199 with ten per cent bovine serum.

*The drug was kindly supplied by G. N. Pershin and M. S. Bogdanova.
Mice. For intracerebral inoculation of herpesvirus mongrel albino mice, weighing 6 to 8 grams each, were used. Following mouse infection the drug was introduced at varying intervals, and the survivors were compared with controls.

Rabbits. A total of 116 chinchilla rabbits, weighing 1.5 to 3.5 kilograms each, were used. In 20 chinchilla rabbits the tolerance of eye tissues to Florenal ointment was assayed. In 96 animals experimental herpetic keratitis was induced; 48 rabbits were treated with Florenal ointment and 48 animals were left untreated (control).

Experimental herpetic keratitis. Prior to inoculation into the conjunctival sac 1 per cent Dicaine solution was instilled; the central portion of the cornea was superficially scratched with a sharp lancet in a cross-hatch manner, the size of the traumatized zone not exceeding 5 to 6 mm. The viral inoculum (0.05 ml.) was placed on the injured portion and gently rubbed into the cornea. The time of onset of first signs of the disease varied from one experiment to another, being dependent on the virulence of the strain used. The first signs of the disease could be detected in 12 to 14 hours; a well-defined clinical picture of herpetic keratitis was observed 48 to 72 hours after infection. During this period of clinically apparent infection, Florenal ointment was applied and compared with controls. The rabbits were randomly assigned to treated and control groups. The severity of the clinical course was evaluated on a scale of 0 to 3. The following signs were taken into consideration: edema and hyperemia of eyelids, conjunctival hyperemia (infiltration), excretion from the conjunctival sac, size of the induration and erosion of the cornea, development of corneal vessels, cicatricial cloudiness of the cornea, and signs of iritis. The mean index of the severity of the clinical course was calculated on the basis of the sum total of individual signs.

Clinical observations

Florenal was applied as an ointment formulation for treatment of 103 human subjects with herpetic keratitis and adenovirus eye diseases.

Of 73 patients with herpetic eye diseases, 36 were suffering from superficial forms of herpetic keratitis (vesicular keratitis, marginal keratitis, and dendritic keratitis of varying severity) and 37 had deep keratitis (geographical, methaherpetic, disciform, focal, and diffuse stromal keratitis, or keratouveitis). Herpes zoster with manifestations of keratitis and keratouveitis was present in four subjects.

In 56 patients the herpetic nature of the disease was confirmed by cytological studies of scrapings as well as by detection of the herpes antigen by immunofluorescent antibody test of scrapings of the affected conjunctiva.

Clinical observation included 30 subjects with adenovirus conjunctivitis and keratoconjunctivitis. In 18 patients the process was unilateral and in 12 it was bilateral. On the average the patients sought medical advice at the Institute on the seventh day after the onset of the disease. Eye infection was as a rule, preceded by a general disease with a febrile reaction. Adenopathy was found in 28 subjects, in eight of whom the gland was very enlarged. Marked conjunctival hyperemia was present in 20 patients and moderate hyperemia in 10. Pronounced edema of the conjunctiva occurred in more than 50 per cent of patients. Follicles found in 29 patients were few, small, and commonly superficial. Excretion was meager and mucilaginous in seven patients and moderately purulent in five. Corneal disease was found in 15 subjects. In 28 of 30 patients diagnosis was confirmed by cytological studies of scrapings which showed a cell reaction and degenerative changes in the epithelium.

Results

Tolerance of eye tissues to Florenal. In experiments on rabbits the finding was that application of 0.5 and 1.0 per cent Florenal ointment five times daily during 12 days does not irritate rabbit eyes and evinces no side effects. Toxic reactions were not observed in any group of treated infected rabbits.

Antiviral activity in tissue culture. For appraising activity of the drug in tissue culture a technique of "checkerboard" (alternate) titration was used, according to which aliquots of the assayed drug were tested against 10, 100, and 1,000 TCD50 of herpes simplex virus; mixed lots were left to stand for 1 hour at room temperature, and thereupon inoculated (0.2 ml.) into each of four tubes containing cell culture. Results of the reaction were recorded.
Table I. The inhibiting effect of Florenal recorded in experiments on tissue culture inoculated with herpes simplex virus

<table>
<thead>
<tr>
<th>Florenal concentration (μg/mL)</th>
<th>Herpes simplex virus (TCD&lt;sub&gt;50&lt;/sub&gt;/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>2.0</td>
</tr>
<tr>
<td>50</td>
<td>2.8</td>
</tr>
<tr>
<td>30</td>
<td>4.0</td>
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<tr>
<td>20</td>
<td>3.7</td>
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<tr>
<td>10</td>
<td>5.0</td>
</tr>
<tr>
<td>0 (control)</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Table II. Therapeutic activity of Florenal in experimental herpetic encephalitis of mice

<table>
<thead>
<tr>
<th>Method and time of drug introduction</th>
<th>Percentage of mice surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Florenal treated</td>
</tr>
<tr>
<td>Intraperitoneally, 18 hr. prior to challenge</td>
<td>76</td>
</tr>
<tr>
<td>Subcutaneously, 3 administrations daily following challenge</td>
<td>61</td>
</tr>
</tbody>
</table>

In terms of the inhibition of cytopathic effect of the virus. The reduction of the virus titer is correlated with the drug dose (Table I). At a concentration of 100 μg per milliliter of Florenal the virus titer dropped to 4.5 log units; at 10 μg per milliliter the reduction was 1.5 log units; the virus titer in control cultures was 6.5 log units.

**Efficacy in mice inoculated with herpes virus.** The virus was inoculated intracerebrally, in dilutions from 10<sup>-1</sup> to 10<sup>-5</sup> (0.03 mL). Intramuscular injection of Florenal (0.2 mL) was given three times daily after the challenge (Table II). The experiments demonstrated that Florenal appreciably contributed to survival; intramuscular injections of the drug appeared to be more efficient than subcutaneous administrations.

**Therapeutic efficacy in treatment of experimental herpetic keratitis in rabbits.** Herpetic keratitis was induced in 96 rabbits; 48 animals (96 eyes) were allotted to control and 48 rabbits (96 eyes) were treated with Florenal ointment at different concentrations. Nineteen animals were treated with 1 per cent Florenal ointment, 10 rabbits were given 0.5 per cent ointment, and 19 animals received 0.25 per cent ointment—five applications daily from 9 A.M. to 9 P.M. Observations included nine experimental series; in each series the proportion of rabbits to which Florenal ointment and placebo was administered was equal.

In all nine series of experiments the disease in the control group was more severe; epithelization of the cornea began later or was not completed by the end of the observations. Corneal clouding was more extensive and denser while manifestation of iritis was more frequent.

In both the first and second series of experiments the severity of corneal changes was reduced twofold as early as the second day after treatment with Florenal ointment, while the time necessary for epithelization of the denuded surface of the cornea was reduced more than twofold. The final cicatricial cloudness in the treated rabbits was considerably less pronounced, and in three of eight cases was hardly perceptible (Fig. 1).

In the third series of experiments the clinical picture of the disease was more obvious and treatment was started 48 hours after infection. The eyes in the control rabbits continued to worsen while the treated animals improved after the first days of treatment with Florenal ointment.

In the fourth series of experiments the disease was fulminating, presumably due to the fact that a newly passed herpesvirus strain was particularly virulent. The treatment with 1 per cent ointment was begun 24 hours after infection. In the control group of rabbits the infection became more severe, whereas in the treated group an improvement was noted on the very first days of Florenal ointment application.

In the fifth and sixth series of experiments 0.5 per cent Florenal ointment was applied three times daily (the treatment was given 1 hour and 72 hours after challenge). The severity of corneal changes was reduced nearly twofold, as was the
Florenal treatment of eye diseases

Fig. 1. Mean index of severity of the clinical course of experimental herpetic keratitis in rabbits observed during treatment with 0.5 per cent Florenal ointment five times a day.

Fig. 2. Those treated with 0.5 per cent Florenal ointment three times a day in comparison with administration of 0.1 per cent IDU solution five times a day.

time necessary for epithelization of the denuded surface. Corneal cloudiness in the treated rabbits was appreciably less pronounced.

In the seventh, eighth, and ninth series of experiments, apart from rabbits treated with Florenal and placebo (control), rabbits were included which received treatment with 0.25 per cent oxoline ointment (five rabbits, 10 eyes), 0.01 per cent IDU (five rabbits, 10 eyes) and DN-aSe (five rabbits, 10 eyes). Comparative observations demonstrated that in the model of experimental herpetic keratitis of the rabbit therapeutic efficacy of Florenal is approximately the same as that of idoxuridine (Fig. 2) but exceeds the effect of DN-aSe. It must be emphasized, however, that the IDU drops were administered five times a day.

Treatment of 73 patients with herpetic keratitis. Florenal ointment was applied in 0.1, 0.25, 0.5, and 1.0 per cent concentrations but after the first trials the 0.1 and 1.0 per cent concentrations were discon-
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Fig. 3. Dendritic herpetic keratitis with stromal affection. Patient A, age 38. Condition prior to (a) and following (b) treatment with 0.5 per cent Florenal ointment.

continued, the former because of lower efficacy and the latter because of a slight irritation. The 0.25 per cent Florenal ointment was applied three to five times a day, the 0.5 per cent ointment was administered thrice daily, and toward the end of the treatment course the regimen was one to two applications daily. Though the study was not a controlled or double-blind one, the effects appeared dramatic.

A positive therapeutic effect was recorded in 35 of 36 patients with superficial forms of herpetic keratitis (Fig. 3). Subjective improvement occurred during the first days of treatment, while objective reduction of infiltration and the beginning of corneal epithelization were observed on day two to four.

Florenal appeared to be therapeutically effective even in severe stromal forms of herpetic keratitis and herpes zoster with manifestations of keratitis and keratouveitis (Figs. 4 and 5).

Of 36 patients suffering from stromal forms of herpetic keratitis all had some therapeutic benefit; one patient did not respond to 0.25 per cent Florenal ointment. Visual acuity improved as did the clinical disease.

Florenal ointment did not irritate the eye, but seven subjects complained that they had a cutting pain and burning sensation in the eye after 0.5 per cent and especially 1.0 per cent ointment had been applied.

In the presence of concurrent bacterial infection of the conjunctiva the antiviral treatment was supplemented by instillation of antibacterial drugs (e.g., sulfapyridazine, tetracycline) for three to five days. Twenty-eight patients also received corticosteroid drops (prednisolone, dexamethasone).

Treatment of 30 patients with adenovirus eye diseases. The 0.25 per cent Florenal ointment was applied in 13 cases and the 0.5 per cent concentration in 17. An improvement was recorded as early as the second or third day of treatment. The average duration of treatment until complete quiescence of all conjunctival manifestations and clinical recovery was
Fig. 5. Herpetic keratouveitis with superficial ulceration and hypopyon. A female patient P., age 60. Condition prior to (a) and following (b) treatment with 0.5 per cent Florenal ointment.

10.9 days (from 6 to 15). Florenal ointment did not irritate the eye and was well tolerated by patients. Side effects (a cutting pain in the eye) were recorded in one patient. Antiviral treatment included the topical application of corticosteroids.

Discussion

A new synthetic preparation, Florenal, is capable of inhibiting herpesvirus reproduction in tissue culture. The inhibiting effect of Florenal was more marked than that of tebrophen or oxoline and approximately the same as that of IDU. In experiments on mice challenged intracerebrally with herpesvirus Florenal displays a definite protection.

In a model of experimental herpetic keratitis induced by herpes simplex virus, application of 0.25 to 1.0 per cent Florenal ointment produces a marked therapeutic effect. In comparative experiments Florenal ointment is therapeutically similar to IDU (0.1 per cent drops given five times a day) and was superior to DN-aSe. Long-term application of 0.25 to 1.0 per cent Florenal ointment in rabbits had no toxic effect on infected eyes.

Florenal eye ointment applied to 73 patients with herpetic keratitis proved to be effective in 71. In the presence of superficial forms of herpetic keratitis the time necessary for recovery was generally six to 14 days. Deep stromal forms of herpetic keratitis and herpetic keratouveitis needed a longer treatment period, but therapy was successful in 36 of 37 patients. Since the 0.1 per cent Florenal ointment is less effective and the 1.0 per cent concentration is liable to produce discomfort and burning we used the 0.25 per cent ointment three to five times a day in milder cases or 0.5 per cent Florenal ointment three times a day in more severe cases.

Clinical observations indicate therapeutic effectiveness of Florenal ointment in treatment of 30 patients with adenovirus conjunctivitis and keratoconjunctivitis. The duration of treatment with Florenal ointment was 10.9 days. In the past we have used other drugs and found greater treatment periods required: 0.5 per cent tebrophen ointment, 12 days; interferon inducer SFV, 13.4 to 17 days; tetracycline, geocrotion, and DN-aSe, 20 to 25 days.

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