Antagonism and toxicity of IDU by its degradation products

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IDU, which stands at room temperature, although decomposing extremely slowly, loses antiviral activity fairly rapidly as measured by tissue culture assay. Similarly, miniscule amounts of its major degradation product, iodouracil, inhibit the antiviral activity of IDU, and iodouracil appears toxic to the human cornea. This loss of effectiveness of IDU with age, although documented in tissue culture, was not observed in vivo in experimental keratitis in the rabbit. Although the tissue culture results and the finding of minimal toxicity in man support our clinical impression of biologic instability of IDU, the clinical significance of these findings is not yet certain.

The drug 5-iodo-2'-deoxyuridine (IDU) has been found to be effective in the eradication of herpes simplex virus from the corneas of rabbits and man. In initial studies it was our clinical impression that the drug was biologically unstable. Because of this, fresh powder was made into drops each week, the drops were refrigerated both for experimental and patient use, and all drops were discarded after one week. With this procedure, results were consistent and no toxicity was noted. Since these initial studies, other investigators have noted punctate keratitis and perhaps other toxicity to the corneal epithelium, and the medication, although clearly beneficial, has not always been consistent in its effect. It appeared to us and independently to other physicians that often when inconsistency in results developed, and when toxicity to the cornea was seen, the drops had been kept at room temperature for some time. This strengthened the initial impression of biologic lability and prompted further study.

Chemically, IDU is relatively stable. The breakdown is exceedingly slow, being only about 1 to 2 per cent a month depending on conditions of formulation and storage. Since the loss of IDU was slight and should not be important, it appeared that either the impression of biologic instability was incorrect or the chemical tests were inadequate. Further consideration suggested that although there might be little loss of active IDU, the products of degradation might be toxic and might even antagonize the therapeutic antiviral effects of the parent compound. Studies of this possibility are reported below.

Methods and results

Since it has been our impression that IDU is less effective as the solution ages, IDU was left at room temperature in a clear bottle for 2 months. (The expiration period for unrefrigerated material

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suggested by some companies is 6 months.) Rabbit kidney cell cultures were infected with $10^8$ infective doses of herpes virus and incubated until definite cytopathogenic changes were seen. Medium containing 0.1 or 0.01 mg. per milliliter of "aged" IDU was added to the cultures. All medium was changed daily and the cultures were observed to determine whether the cytopathogenic effects of the virus were halted. At the finish of the study, the "aged" IDU was cultured for aerobic and anaerobic bacteria and fungi and was found to be sterile. The solution was crystal clear and colorless.

Cultures were treated with fresh IDU in concentrations of only 0.01 mg. per milliliter; after 5 days of therapy virus was no longer detectable by culture either in the supernatant fluid or sonically disrupted cells and the cytopathogenic change was halted. The cytopathogenic effect of the virus in cultures treated with concentrations of 0.01 mg. per milliliter of IDU and 0.1 mg. per milliliter of IDU that had been at room temperature for 2 months advanced, and cultures were destroyed 3 days after treatment started as virus synthesis resumed (Fig. 1). Uninfected cultures treated with this IDU also showed some toxic changes. Aged IDU, therefore, had become ineffective in halting the progression of virus damage even in a concentration 10 times the minimal effective dose and was toxic.

Since iodouracil (IU) is a major breakdown product of IDU, and deoxyuridine is also formed, normal cell cultures were treated with 5-iodouracil, deoxyuridine, and IDU to determine the concentrations that were cytotoxic. The medium was changed daily for 2 weeks if the cell sheet remained intact, but the toxicity of the other compounds slowly developed, becoming apparent in 5 to 7 days.

Fresh IDU in concentrations as high as 1 mg. per milliliter showed no apparent toxicity in cell cultures of rabbit kidney, monkey kidney, mouse embryo, and primary human amnion, while both iodouracil and deoxyuridine were toxic in concentrations of only 0.001 mg. per milliliter. Cultures treated with IDU were kept for 2 weeks and remained intact.

In order to determine whether nontoxic concentrations of iodouracil could be responsible for inhibition of the antiviral properties of IDU, rabbit kidney cell cultures were infected as above. These cultures were treated with medium containing 1 mg. per milliliter of IDU or medium containing 1 mg. per milliliter of IDU to which 0.0001 mg. per milliliter of iodouracil was added. The medium was changed daily, as was the medium in nontreated controls. At intervals cultures were washed to remove any cell-free virus, subjected to ultrasound, and the virus concentrations in both the medium and the cells were titered.

When iodouracil in a concentration of only 0.0001 mg. per milliliter was added to medium containing 1 mg. per milliliter of IDU, the antiviral effect of the IDU was antagonized and virus could be recovered from the cells as long as 5 days after treatment was started (Fig. 1). The cytopathogenic changes advanced and the cultures were completely destroyed by virus 6 days after treatment was started. This iodouracil concentration antagonizing the antiviral activity of IDU would represent only 0.01 per cent of the parent compound. Similarly when cultures were treated with therapeutic concentrations of fresh IDU, but the media was not changed daily, the results were similar to those observed when IU was added to IDU and cytopathic changes progressed. This lack of IDU effect is probably due to hydrolytic production of IU by the culture cells. It must be emphasized that this reversal of antiviral effect is demonstrated on cultures with definite changes of virus disease already present and that considerations applying to the cure of established infection are different from those applying to prevention of infection or prevention of cytopathogenic change in which this reversal may not occur.

Although in tissue culture this effect of iodouracil in the treatment of experimental herpetic keratitis

<table>
<thead>
<tr>
<th>Culture</th>
<th>IDU 1 mg./ml.</th>
<th>IDU + 1 mg./ml. = 1 IU 0.1 mg./ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>36</td>
<td>28</td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>14</td>
</tr>
</tbody>
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Severity of ulcers* 1.3 1.4

*Double-blinded evaluation of ulcers graded from 0 to 4, 4 being the most severe.
Biologic instability of IDU

... (Table I).

Possible toxicity to the cornea was first studied in rabbits. When rabbits were treated every 2 hours with drops containing either 1 mg. per milliliter of IDU or drops containing 1 mg. per milliliter of IDU plus 0.1 mg. per milliliter of iodouracil, a double-blind evaluation after 2 days revealed no difference. Since normal rabbits have considerable corneal staining which might mask toxicity, and in vitro toxicity had been demonstrated, human studies were undertaken.

Five normal human volunteers without any signs of keratitis were given drops of 1 mg. per milliliter of IDU in one eye and 1 mg. per milliliter of IDU plus 0.1 mg. per milliliter of iodouracil in the other eye every hour during the day and every 2 hours during the night for 3 days. Drops were randomly assigned in a double-blind manner so that neither the subjects nor the investigator knew which eye received the medication with iodouracil.

Of the 5 volunteers, 4 experienced irritation in the eye receiving the IDU plus IU. Two reported pain on instillation of the drops but had no objective signs of corneal damage. Two had punctate keratitis as well as pain. No symptoms or signs of keratitis occurred in the eyes receiving the IDU alone. One person had no abnormal symptoms or signs in either eye.

Discussion

Although clinical impression is often fallible, it prompted this search for confirmatory evidence of the biologic instability of IDU. Not all studies were conclusive; however, there is no question but that IDU which stands at room temperature, although decomposing extremely slowly, loses therapeutic antiviral activity fairly rapidly as measured by this particular tissue culture assay. Similarly, miniscule amounts of its major degradation product, iodouracil, inhibit the antiviral activity of IDU, and iodouracil appears irritating or toxic to the human cornea.

It would be unjustified on the basis of this evidence to assume that iodouracil is the only substance responsible for these observations or that this instability accounts for failures of therapy. Although the mechanism of inhibition of IDU action by IU is not clear, other pyrimidines also inhibit IDU action and the inhibition may well be caused by a relatively nonspecific inhibition of the IDU membrane transport system. The clinical importance of these findings is not clear. Clinical impressions of minimal toxicity in man with "aged" IDU, and tissue culture results support the suggestion that the presence of small amounts of the products of IDU degradation may well be important in determining the effectiveness of IDU therapy. On the other hand, results in experimental keratitis do not confirm these impressions and they may well be false. Until preparations in general are reliably pure, however, it is probably worth while to use reasonably fresh medication that has been stored in a dark bottle under refrigeration and to obtain a new supply of IDU if symptoms of burning occur.

REFERENCES