The Effect of Idoxuridine (IDU) on Corneal Stromal Cells in Tissue Culture*

WALTER J. GEERAETS, GUY WONG, AND DUPONT GUERRY, III

Department of Ophthalmology, Medical College of Virginia, Richmond

An alarming increase in the severity and frequency of the stromal forms of herpetic keratitis has been reported during the last decade (Thygeson and Kimura, 1957; Thygeson, Kimura, and Hogan, 1956; Kimura and Goodner, 1963; Bianchetti, 1963). This has led to an intensive search for therapeutic means for its management. The introduction in 1962 by Kaufman, Nesburn, and Maloney of idoxuridine (IDU) as an effective agent against herpes infection of the cornea has stimulated clinical and laboratory research to evaluate its full therapeutic potential.

Initially, IDU was reported as favorably affecting the stromal forms of herpetic keratitis. Kimura and Goodner (1963) and Maxwell (1963) found this to be true in 50 to 85% of the cases reported. Subsequently, Payrau and Dohlman (1964) found that IDU adversely affected the healing of stromal wounds as evidenced by tensile strength determinations.

But other investigators (Polack and Rose, 1964; Kaufman *et al.*, 1964), basing their results upon the decreased tritiated thymidine uptake, reported that IDU only moderately inhibited the healing of stromal wounds. The degree to which the decreased tritiated thymidine uptake accurately reflected a decreased mitosis has been questioned by Pelc (1963). The same author, as well as other investigators, has expressed "reservations about assuming an invariable sequential relationship between DNA synthesis and mitotic division." Pelc found that in many organs a fewer number of cells divided than would be expected from the number of labeled cells found, thus indicating that DNA can be replaced in a cell nucleus without cell division taking place.

Since the complete effect of IDU on corneal cells is still not known, this investigation was conducted. During the experimental phase of this study, a few studies *in vivo* appeared in the literature (Kaufman *et al.*, 1962; Maxwell, 1963; Kaufman *et al.*, 1964; Ey, Hughes, and Holmes, 1964), as well as tissue culture work with various cells. However, to our knowledge, no study *in vitro* with corneal stromal cells has been reported, although it has been pointed out (Kaufman, personal communication) that the effect of IDU may vary with the cell type used.

Materials and Methods

Cell Culture

Corneal tissue cultures were used. Corneal explants were obtained from chinchilla rabbit eyes and cultured under perforated cellophane in growth medium (minimum essential medium),[1] with L-glutamine (3 mg per ml), penicillin (100 μ per ml), streptomycin (0.5 mg per ml), and 10% calf serum. The pH was 7.4 which equilibrated with 5% CO_2 at 37°C.

A cell strain of corneal stromal cells which had undergone at least eight passages was obtained and used in this experiment (fig. 1).

Idoxuridine Medium

Growth medium containing idoxuridine was prepared by dissolving powdered IDU[2] in the medium. First, a 1,000 μg per ml solution was prepared. One-tenth dilutions ranging through 0.1 μg per ml were then prepared. This material was filtered through Seitz fil-

* Supported, in part, by the Virginia Lions Organization through the Old Dominion Eye Bank and Research, Inc., Richmond.

[1] Grand Island Biological Company, Grand Island, N.Y.

[2] Nutritional Biochemicals Corporation, Cleveland, Ohio.

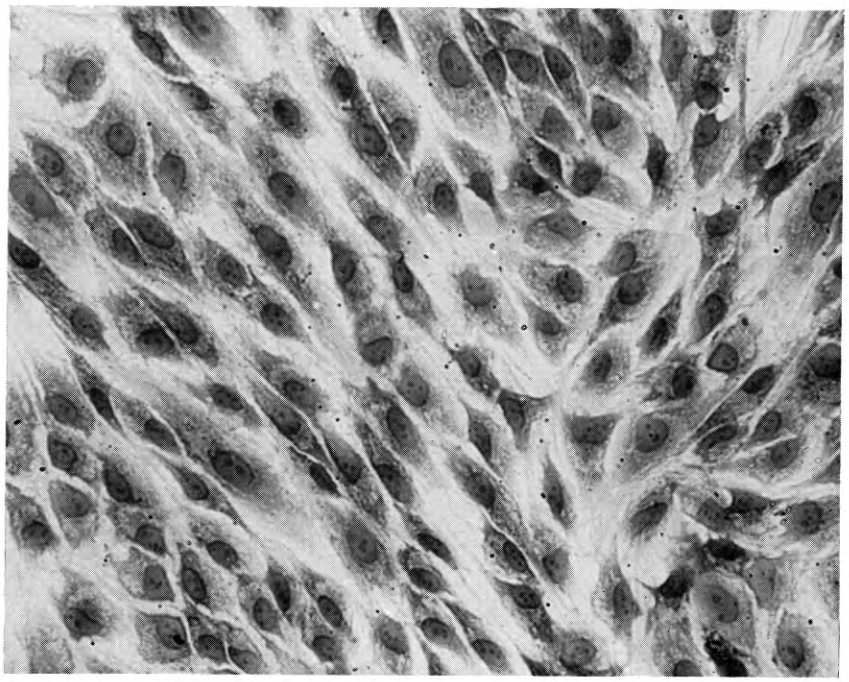

Fig. 1—Corneal stromal cells in tissue culture (220×).

ter pads before use. The medium was kept in the dark at 4°C and all had been used 2 weeks from preparation.

At the end of the study, the medium was cultured for aerobic and anaerobic organisms and fungi and was found to be sterile.

Cell Count

The cell cultures were washed twice with 4-ml portions of salt solution of the following composition: 8 gm of NaCl, 4 gm of KCl, 1 gm of glucose per 1,000 ml. To each plaque bottle 0.5 ml of 0.25% trypsin–0.2% Versene solution was then added. This solution was allowed to act for 8 minutes. The bottles were shaken every 2 minutes and examined microscopically to determine whether the cells were adequately suspended. Deactivation of the enzymatic chelating solution was achieved by adding 1.5 ml of growth medium. The cell suspension then was passed through a number 25 gauge hypodermic needle. The cells were counted at once in a hemocytometer. Six to ten samples were counted from each bottle to establish the average number of cells with the more dilute cell suspensions.

Procedure

With the foregoing materials and technics, IDU's effect on stromal tissue culture cell growth was studied. In this experiment, 72 plaque bottles (surface area of 15 cm^2) were plated with equal aliquots of a cell suspension. Twenty-four hours later, when the cells had been allowed to attach to the glass, a cell count was determined in 18 bottles selected at random. This value was

considered to be the number of cells attached to the glass at zero time (29,700). The remaining 54 bottles were divided randomly into six different groups. These cell cultures were subjected to "IDU-media" of concentrations of 0, 0.1, 1, 10, 100, and 1,000 μg per ml. The media were changed daily and cell counts were determined at the end of 72, 120, and 168 hours following exposure to IDU. The experiment was carried out in triplicate: thus, three bottles from each group were counted at the end of each of the above periods. The total experiment was repeated with a different strain of stromal cells, leaving other variables unchanged.

Results

The observations made at the end of the three different interaction periods of IDU on cultured corneal stromal cells (72, 120, 168 hours) are described separately for each period.

72 Hours

The control bottles contained an average of 92,900 cells per bottle, while the cell population treated with the lowest concentration of IDU (0.1 μg per ml) contained a mean of 51,900 cells per bottle (table 1 and fig. 2). This difference proved to be statistically significant at the 0.01 level.

The cell count of the cultures treated with 1, 10, 100, and 1,000 μg of IDU ml medium revealed 28,400, 25,000, 20,000, and 18,900 cells per bottle, respectively. These counts were of significant difference regarding the control group as well as the cells exposed to 0.1 μg of IDU per ml of medium. The level of significance was 0.05 or better. The number of cells treated with IDU-media, ranging from 1 to 1,000 μg per ml, revealed a decreasing number of cells with increasing concentration of the IDU. However, these slight differences were not significant as these counts were below those of time zero (29,700).

120 Hours

The cell count of the control bottles revealed an average of 215,000 cells per bottle; the bottles treated with 0.1, 1, 10, 100, and 1,000 μg of IDU per ml had a mean of 102,000, 42,200, 33,800, 24,800, and 18,900 cells per bottle, respectively. The number of cells in the control group compared to the treated groups was found to be significant at the 0.01 level. There was also a significant difference of the cell number of those cultures exposed to the weakest concentration of IDU (0.1 μg per ml), and those treated with higher IDU concentrations (1, 10, 100, and 1,000 μg of IDU per ml media) at a level of 0.05 or better. Similar to the 72-hour observation, the cell number decreased with the increase in IDU concentration. The difference between the cell counts in these experimental groups was of no significance statistically.

TABLE 1

Total Number of Cells in Each Bottle after Various Periods of Exposure to Different Concentrations of IDU

Exposure to IDU for	Cells per Bottle					
	0 μg IDU/ml	0.1 μg IDU/ml	1 μg IDU/ml	10 μg IDU/ml	100 μg IDU/ml	1,000 μg IDU/ml
hr						
0	29,700					
72	94,900	67,200	39,400	22,000	23,400	26,700
	106,000	38,000	25,800	22,000	20,600	14,500
	77,700	50,600	20,000	30,900	15,900	15,500
120	236,000	154,000	35,200	23,900	18,300	26,700
	194,000	101,000	65,700	41,300	21,600	15,000
	215,000	49,700	25,800	36,100	34,500	15,000
168	471,000	83,300		22,000	30,500	15,500
	436,000	67,700	50,300	35,200	35,600	17,800
	480,000	115,000	32,700	38,700	30,000	22,000

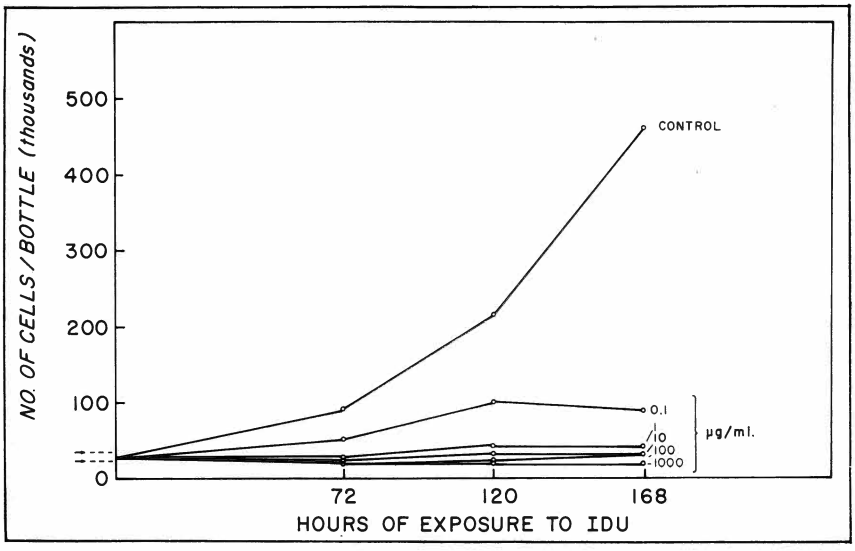

Fig. 2—Graph representing multiplication of corneal stromal cells in tissue culture without (control) and with IDU present in the culture medium (0.1 to 1000 μg per ml).

168 Hours

The difference between the number of cells in the control group compared to those of the treated groups was even more pronounced than for the time intervals of 72 and 120 hours of exposure to IDU. The control group showed a 15-fold increase in cellular population (29,700 at time zero versus 462,000 at 168 hours). In contrast, the weakest concentration of IDU in this experiment permitted only a 3-fold increase in the cellular population during the same period (29,700 at time

zero versus 88,900 at 168 hours). This 3-fold increase in the number of cells of the latter group again was significantly different from the populations treated with the greater concentrations of IDU. This was found at the 0.01 level.

As was noted in the group of shorter exposure to IDU, there was only an insignificant difference found between the number of cells in the 1, 10, 100, and 1,000 μg of IDU per ml of media-treated populations, all which showed cell counts similar to those at time zero.

Throughout this experiment, there was no significant difference in cell counts within individual groups that could have been attributed to the different periods of IDU exposure. Thus, IDU interaction upon corneal stromal cells for 72, 120, and 168 hours in equal concentrations did not result in significantly different cell counts.

When the total experiment was repeated with a different strain of stromal cells, the results obtained were very similar to those above.

Discussion

The marked inhibition of cell population increase and its relation to the concentration of IDU is consistent with recent observations by others. The growth inhibition of mouse leukemic cells in culture by IDU was reported by Mathias, Fischer, and Prusoff (1959). These authors reported that IDU, at ordinarily accepted therapeutic concentrations and less, permitted only a single doubling of the population cells in culture, and thereafter "cell death invariably commenced."

Ey, Hughes, and Holmes (1964) found that, with concentrations of IDU at less than therapeutic strength, the multiplication of primary rabbit kidney cells in culture was entirely blocked. Cheong, Rich, and Eidenoff (1960) have reported similar results with cultures of human cervical carcinoma cells.

Studies *in vivo* by Polack and Rose (1964) with therapeutic doses of IDU illustrated that there was a delay in cell repopulation after stromal injury in rabbits. Payrau and Dohlman (1964) also found that stromal wound healing was retarded in rabbits treated with IDU. As was theorized by Polack, "this inhibition should increase if IDU could be made available to all the premitotic cells." The more marked quantitative differences in cell counts, after exposures to various concentrations of IDU in this study *in vitro*, can be based on this assumption of Polack.

The arrest of cellular multiplication after exposure to a critical concentration of IDU may be explained by lysis of the cells. Mathias and Fischer (1959) found that murine leukemia cells in 10- to 100-μg IDU solutions underwent lysis even after doubling in number.

The findings reported in this study suggest that the proliferation of corneal stromal cells is inhibited by IDU. These findings *in vitro* should be considered when IDU is clinically used in cases where there is severe stromal involvement.

Summary

Corneal stromal cells were cultured *in vitro* and exposed to various concentrations of idoxuridine (IDU), ranging from 0 to 1,000 μg of IDU per ml of medium. Inhibition of cell multiplication occurred with concentrations of 0.1 μg per ml. With concentrations of 1.0 μg per ml and greater, there was no increase in cell number from the time of exposure to IDU.

Acknowledgment

We are grateful for the valuable aid extended by Dr. Robert W. Tankersley, Jr., Medical College of Virginia, and Dr. Fred Stocker, Durham, N.C.

References

BIANCHETTI, M. Herpes simplex keratitis. *Ophthalmologia* 146: 395–410, 1963.

CHEONG, L., M. A. RICH, AND M. L. EIDINOFF. Introduction of the 5-halongenated uracil moiety into deoxyribonucleic acid of mammalian cells in culture. *J. Biol. Chem.* 235: 1441–1447, 1960.

EY, R. C., W. F. HUGHES, AND A. W. HOLMES. Clinical and laboratory evaluation of idoxuridine (IDU) therapy in herpes simplex keratitis. *A. M. A. Arch. Ophthalmol.* 71: 325–331, 1964.

KAUFMAN, H. E., A. B. NESBURN, AND E. D. MALONEY. IDU therapy of herpes simplex. *A. M. A. Arch. Ophthalmol.* 67: 583–591, 1962.

KAUFMAN, H. E., J. A. CAPELLA, E. D. MALONEY, J. E. ROBBINS, G. M. COOPER, AND M. H. UOTILA. Corneal toxicity of cytosine arabinoside. *A. M. A. Arch. Ophthalmol.* 72: 535–540, 1964.

KIMURA, S. J., AND E. K. GOODNER. *Ocular Pharmacology and Therapeutics and the Problems of Medical Management.* Philadelphia: F. A. Davis Co., 1963.

MATHIAS, A. P., G. A. FISCHER, AND W. H. PRUSOFF. Inhibition of the growth of mouse leukemia cells in culture by 5-iododeoxyuridine. *Biochim. et Biophys. Acta* 36: 560–561, 1959.

MATHIAS, A. P., AND G. A. FISCHER. Studies of the inhibition of thymidine-synthesis and -utilization by mouse leukemia cells in culture. *Federation Proc.* 18: 284, 1959.

MAXWELL, E. Treatment of herpes keratis with 5-iodo-2-deoxyuridine (IDU). *Am. J. Ophthalmol.* 56: 571–573, 1963.

PAYRAU, P., AND C. H. DOHLMAN. IDU in corneal wound healing. *Am. J. Ophthalmol.* 57: 999–1002, 1964.

PELC, S. R. On the question of renewal of differentiated cells. *Exp. Cell Research* 29: 194–198, 1963.

POLACK, F. M., AND J. ROSE. The effect of 5-iodo-2-deoxyuridine (IDU) in corneal healing. *A. M. A. Arch. Ophthalmol.* 71: 520–527, 1964.

THYGESON, P., S. J. KIMURA, AND M. J. HOGAN. Observations on herpetic keratitis and keratoconjunctivitis. *A. M. A. Arch. Ophthalmol.* 56: 375–388, 1956.

THYGESON, P., AND S. J. KIMURA. Deep forms of herpetic keratitis. *Am. J. Ophthalmol.* 43: 109–113, 1957.