In Vitro Inhibition of Ocular Cell Proliferation With Ara-C: Blockage of the Antiproliferative Effect With 2'-Deoxycytidine

Anthony S. Hojek, Richard K. Parrish II, Kim S. Mallick,* and Michael Gressel†

Cytosine arabinoside (ara-C), a cell cycle-phase specific antimetabolite, was tested in a tissue culture model of human scleral fibroblasts (HSCF), and human retinal pigmented epithelium cells (HRPE), to investigate possible applications in the modulation of wound healing after glaucoma filtering surgery and in the postoperative management of proliferative vitreoretinopathy. Cell proliferation after a 72 hr incubation in the presence of ara-C was inhibited to 50% of the control growth rate (ID₅₀) with calculated doses of 60 ng/ml in HSCF and 4 and 5 ng/ml in HRPE cultures. 2'-Deoxycytidine, a competitive inhibitor of ara-C action, completely eliminated the cell growth retardation of both cell types produced by ara-C after 72 hr incubation. Invest Ophthalmol Vis Sci 27: 1010-1012, 1986

The use of antimetabolites in the treatment of ocular disorders characterized by cellular proliferation, such as proliferative vitreoretinopathy (PVR), and postoperative scarring of filtering blebs has been reported.1-4 Ara-C (1-beta-D-arabinofuranosylcytosine), an analog of the naturally occurring pyrimidine nucleoside 2'-deoxycytidine, inhibits cell division by impairment of DNA synthesis,5 and has been used as a topical antiviral agent for treatment of Herpes simplex keratitis.6-8 These studies demonstrating ara-C-related decrease in thymidine uptake and DNA synthesis suggest possible therapeutic use of ara-C to limit proliferation associated with PVR and filtering surgery failure. Clinical application, however, has been limited by corneal epithelial toxicity,9 similar to that produced by systemic ara-C.10

Prevention of ara-C-related corneal epithelial toxicity by concurrent administration of topical 2'-deoxycytidine9 indicated that this side effect might be overcome in the treatment of other ocular disorders as well. However, the dose relationship between ara-C concentration and its antiproliferative effect on human ocular cells has not been established.

Tissue culture methods offer the advantages of enabling one to evaluate effects of antiproliferative agents on a single cell type and providing for precise control of the drug doses applied to the cells. We employed tissue culture to evaluate the antiproliferative effect of ara-C on human scleral fibroblasts (HSCF); cells which rapidly proliferate after glaucoma filtering surgery; and human retinal pigmented epithelium (HRPE); cells which are thought to proliferate under conditions of PVR and traction retinal detachment. The effect of 2'-deoxycytidine added concomitantly to cell cultures containing ara-C was also evaluated to assess blockage of ara-C-induced inhibition of cell division.

Materials and Methods. HSCF cultures were initiated as explants and propagated in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 15% fetal calf serum, penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin-B (1.25 µg/ml), and were employed between the third and sixth passages.

HRPE cultures were initiated as follows: whole human eyes provided by the Florida Lions Eye Bank were refrigerated for 20-30 hr after enucleation. The anterior segment, vitreous, and neural retina were removed. The exposed pigmented epithelium was rinsed with calcium and magnesium-free saline G (CMFSG), incubated with 0.05% trypsin, 0.5 mM EDTA in CMFSG at 37°C for 3 hr, and removed by gentle pipetting. The cell suspension was centrifuged and resuspended in F-10 medium supplemented with 20% fetal bovine serum, 50 µg/ml gentamicin, 1.25 µg/ml amphotericin-B, and 30 mM of additional glucose. HRPE cells were used between the fourth and seventh passages.

Both cell types were incubated in a humidified atmosphere of 5% CO₂ in air at 37°C. Cells were enzymatically detached by removing the medium, rinsing with CMFSG, and adding 0.05% trypsin, 0.05 mM EDTA in CMFSG at 37°C for 10 min. Cells were resuspended in medium and centrifuged at 800 g for 10 min and counted with a hemacytometer or cell counter (Coulter Model ZM, Coulter Electronics Inc., Hialeah, FL).

Experiments with both cell types were carried out by seeding 5 × 10⁴ cells in 2 ml of medium per 35 mm plastic tissue culture dish (Falcon #3003), or 1.25 × 10⁴ cells per 16 mm well in 1 ml of medium (24 well tissue culture cluster-Costar #3524) and allowed to attach. Cell number in quadruplicate wells was determined 24 hr after plating (day 0). The remaining wells were treated, in quadruplicate with a complete medium change containing varying concentrations of ara-C (0-1000 ng/ml). After 5 days, control cultures approached confluence, and the cells were enzymatically detached and counted. Cell growth was calculated by subtracting the initial cell number (day 0) from the final cell number (day 5). Cell growth in the test media was expressed as a percentage of the growth in the control medium.

To investigate possible 2'-deoxycytidine blockage of the ara-C inhibitory effect, cells were incubated in DMEM with either 2'-deoxycytidine alone (10⁻⁷ M-10⁻³ M), or in combination with ara-C (100 and 1000 ng/ml).

Results. Ara-C produced a dose-related growth retardation in both cell types (Figs. 1, 2). Inhibition to
50% of the control growth rate (ID$_{50}$), was produced with calculated doses of 60 ng/ml with HSCF (Fig. 1) and 4 and 5 ng/ml with the HRPE cells (Fig. 2). Results of different experiments for each cell type are plotted together to illustrate the reproducibility of the ara-C effect (Figs. 1, 2). The effect of 1 and 72 hr of exposure of HSCF cultures to ara-C is demonstrated in Figure 3. An ID$_{50}$ of 800 ng/ml was produced after 1 hr exposure, compared to 8 ng/ml after continuous exposure for 72 hr. Cell proliferation was reduced to 5% of control growth with 100,000 ng/ml ara-C after 1 hr of exposure and 100 ng/ml after 3 days of exposure.

Blockage of the ara-C induced growth retardation, and the relative lack of toxicity of 2'-deoxycytidine was demonstrated in both cell types (Figs. 3, 4). Blockage of the inhibitory effect of 100 ng/ml ara-C was produced with $10^{-5}$ M 2'-deoxycytidine in the HSCF and HRPE cultures (Figs. 3, 4). The inhibitory effect of 1000 ng/ml of ara-C was also blocked with larger doses of 2'-deoxycytidine in HRPE cultures (Fig. 4).

**Discussion.** Selection of an ideal antiproliferative drug is based on relative cellular specificity and lack of toxicity to uninvolved ocular tissues when given at a therapeutically effective dose. The potential advantage of ara-C over other types of drugs is that, as a cell cycle phase-specific antimetabolite, the toxic effects are generally limited to actively replicating cells in the cell cycle. Mitotically quiescent tissue, such as the lenticular and ciliary body epithelium, is less likely to experience toxic effects.

We have quantitated the inhibitory effect of ara-C on the in vitro proliferation of HSCF and HRPE cultures. These cells are critically involved in scleral and retinal wound healing. Presently, there are no specific drugs which are known to selectively inhibit cellular growth in ocular tissues. We did note a tenfold greater sensitivity to the inhibitory effects of ara-C in HRPE compared to HSCF cultures. The mechanism of this differential cell sensitivity is not clearly understood.

The antiproliferative effect of ara-C on both cell types was blocked by the addition of 2'-deoxycytidine to the test medium, presumably through competition for the binding site of DNA-polymerase. This finding and the observation that 2'-deoxycytidine alone produced little toxicity suggests that this nucleoside could possibly be used to prevent ara-C-related toxicity when given concomitantly. However it is possible that simultaneous
administration of these might also prevent or limit the desired therapeutic effect. Treatment of proliferative vitreoretinopathy and filtering surgery in animal models may answer these questions.

Key words: cytosine arabinoside, 2'-deoxycytidine, scleral fibroblasts, retinal pigmented epithelial cells

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* Present addresses: Mt. Sinai Medical Center, Department of Ophthalmology, Cleveland, Ohio, and the Department of Ophthalmology, Lorain Community Hospital, Lorain, Ohio. Submitted for publication: April 12, 1985. Reprint requests: Anthony S. Hajek, PhD, Bascom Palmer Eye Institute, University of Miami, School of Medicine, PO Box 016880, Miami, FL 33101.

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