THE CYTOTOXIC ACTIVITY OF OPHTHALMIC PRESERVATIVES

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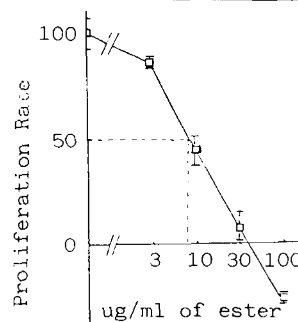
The toxicity testing of ophthalmic preservatives usually involves whole animal experiments, the Eye Irritancy Test introduced by Draize et al (1944) being the best known. Current alternative systems include specular and scanning electron microscopical techniques or slit-lamp examination to assess corneal damage. All require exposure of the eye in toto to the preservative or use a perfusion technique of freshly excised corneas. As cell damage is one feature of corneal irritation, a cell culture system might be used to show cytotoxicity of antimicrobial preservatives. Isolated corneal cells may provide a more stringent test despite offering no parallel to the protective tear fluid and mucin layer of the eye.

SIRC rabbit corneal cells (ATCC CCL60, Flow Laboratories, Irvine) were cultured as a monolayer in Dulbecco's Modified Eagle's Medium containing foetal calf serum (10%), glutamine (4mM), benzylpenicillin (100 units/ml) and streptomycin (100 µg/ml) with bicarbonate as buffer (pH 7.0 - 7.4) in a 5% CO_2/air atmosphere at 37°C. After three days, when the cells were in the logarithmic growth phase, the medium was replace with fresh medium containing a known concentration of ophthalmic preservative and incubated for a further three days. Four replicate cultures were tested at each preservative concentration. The cytotoxicity was assessed by comparing the cell proliferation of 'treated' cultures and controls between days three and six of incubation. The cells were counted by removing the medium from the culture, washing in phosphate buffered saline, adding trypsin-EDTA solution at 37°C for five minutes to form a cell suspension, which was transferred quantitatively, using an electrolyte diluent, to cuvettes in which duplicate counts were made electronically in a Coulter Counter (Model DN). The results were expressed as a percentage of the proliferation rate in untreated control cultures.

Preservative	ID_{50}	Eye-Drop Conc$^{\underline{n}}$
Methyl Hydroxybenzoate	68.0	2000
Propyl Hydroxybenzoate	8.5	300
Benzalkonium Chloride	1.4	100
Chlorhexidine Acetate	0.8	100
Thiomersal	0.04	100

Table 1. Comparison of ID_{50} and Eye-Drop Concentrations (µg/ml)

Effect of Propyl Hydroxybenzoate on Growth of SIRC cells



Under these experimental conditions, thiomersal was the most cytotoxic or cytostatic of the ophthalmic preservatives. The ID_{50} doses were considerably less than the concentrations used in eye preparations, this sensitivity suggests that the technique could be developed for the toxicity screening of such preparations.

Draize, J.H. et al (1944) J.Pharmacol. Exp. Ther. 82: 377 - 390