Evaluation of cytotoxicity of some common ophthalmic drugs

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Abstract. – OBJECTIVE: The study was aimed at evaluating the in vitro cytotoxicity of some commonly used drugs in ophthalmology. Hydrocortisone sodium succinate, Dexamethasone sodium phosphate, 5-Fluorouracil, Tobramycin and Pilocarpine nitrate are frequently used in various indications involving eye care, and the aim was to test the safety of these in cell culture.

MATERIALS AND METHODS: The in vitro cytotoxicity was carried out on the NIH 3T3 cell line by the Sulforhodamine B (SRB) assay.

RESULTS: With the exception of 5-Fluorouracil, none of the other drugs demonstrated appreciable cytotoxicity up to high concentrations of 200 µg/ml at 48 hours of drug exposure.

CONCLUSIONS: Hydrocortisone sodium succinate, Dexamethasone sodium phosphate, Tobramycin and Pilocarpine nitrate were confirmed to be non-cytotoxic while 5-Fluorouracil was highly cytotoxic especially at very low concentrations.

Key Words: Cytotoxicity, NIH 3T3, SRB, Hydrocortisone sodium succinate, Dexamethasone sodium phosphate, 5-Fluorouracil, Tobramycin, Pilocarpine nitrate.

Introduction

Cytotoxicity studies form an invaluable facet of safety analyses in order to verify whether the investigational compound being used as a pharmaceutical or cosmetic are nontoxic, or whether they should be used as therapeutic agents for which cytotoxicity would play a fundamental role. New chemical entities, food additives and many active ingredients are subjected to comprehensive cytotoxicity testing before they are made available for use. A large number of cytotoxicity tests have been developed that can help us determine the effects of drugs on a particular cell line1. Today a majority of the cytotoxicity experiments are carried out in microtiter plates (96-well format). This approach permits high throughput and is economically feasible. An ELISA plate reader is used for experiments based on colorimetric as well as luminescence assays. Cytotoxicity assays measure different parameters like cell membrane integrity, metabolic activity2,3 as well as morphological4 and reproductive characteristics5 of viable cells. We used the Sulforhodamine B (SRB) assay for evaluation of cytotoxicity.

Hydrocortisone Sodium Succinate which is naturally secreted by the adrenal cortex is a glycogenic steroid. It is used as an anti-rheumatic drug, which is required in smaller dosage and is more effective when compared to cortisone6. For the past five decades, hydrocortisone has been used as a potent therapeutic tool. It reduces the inflammatory reactions in traumas and allergic symptoms in the eye7. Hydrocortisone has also been used in treatment of prostate cancer8.

Dexamethasone Sodium Phosphate, a glucocorticoid, is a drug most commonly used to reduce postoperative nausea and vomiting9. It is an anti-inflammatory drug that functions by inhibiting the action of inflammatory mediators10. It has also been used to prevent postoperative swelling and pain11.

5-Fluorouracil is one of the oldest chemotherapy drugs in use12 and is a pyrimidine analogue used in the treatment of cancer. Since 1984, it has been used as sub-conjunctival injections after filtering surgery to control high-risk glaucoma although with several side effects. Thus, it is judicious to use this only in the case of eyes with a high risk of scarring rather than for primary normal glaucoma surgeries13.

Tobramycin an aminoglycoside has been widely used as an antibiotic. Obtained from Streptomyces tenebrarius it has been found to be effective against Gram negative bacteria and few Pseudomonas infections14.

Pilocarpine Nitrate is produced from the leaves of a shrub belonging to the genus Pilocar-
The results are expressed as mean ± standard deviation (S.D.) and the IC50 (inhibitory concentration) values were calculated from the dose-response curves.

**Statistical Analysis**

The data was subjected to the single factor analysis of variance (ANOVA) across groups and dose variants where $p < 0.05$ was considered statistically significant.

**Results**

SRB assay: Pilocarpine Nitrate, Hydrocortisone Sodium Succinate and Tobramycin did not show any significant cytotoxicity up to 48 hrs on the 3T3 cell line and no IC50 could be obtained [Figures 1(a) and 2(a)]. For Dexamethasone Sodium Phosphate the 24 hour IC50 could not be obtained [Figure 1(a)] while the 48 hour IC50 was 180 µg/ml [Figure 2(a)]. 5-Fluorouracil was found to be highly cytotoxic on the 3T3 in a time and dose dependent manner with 24 and 48 hour IC50 values at 100 and 0.09 µg/ml respectively [Figures 1(b) and 2(b)].

**Discussion**

Apart from treating the particular eye condition, it is important to establish the safety of commonly used ophthalmic drugs. Most of the drugs available in the market today have already undergone extensive safety and efficacy testing. Nevertheless, we wanted to confirm the same on a simple 3T3-SRB cell cytotoxicity platform. Under the present experimental conditions, Pilocarpine Nitrate, Hydrocortisone Sodium Succinate and Tobramycin were confirmed as safe due to the absence of the IC50 values up to 48 hours and at high concentrations of 200 µg/ml. Dexamethasone Sodium Phosphate followed a comparable trend at similar concentrations; the 48 hour IC50 was at a very high dose and can thus be considered as non-cytotoxic. Among all the drugs tested, 5-Fluorouracil was the most cytotoxic and it is in keeping with the nature of the drug as it is also used as an anti-neoplastic agent.
dothelial cells and observed that at a concentration of $10^{-4}$ and $10^{-3}$M the cell growth was significantly reduced accompanied with low levels of DNA synthesis. However, in the current study hydrocortisone had minimal cytotoxicity.

In keeping with our findings, that cell viability decreased when exposed to increasing concentrations of dexamethasone, others found that at 24hrs, Human lens epithelial cells (HLE B-3) displayed a low viability at high concentrations.
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of 2 and 1 mg/ml; however, lower concentrations did not result in any significant decrease in cell viability.

5-Fluorouracil is widely used as an anticancer drug. It has been used in ocular and periorbital surgeries as it is capable of reducing fibrosis and hence has been considered as an important tool for enhanced success rates in ophthalmic surgeries.

In their study on the cytotoxicity of 5-fluorouracil on human corneal epithelial cell (HCEC) and human corneal keratocyte (HCK) cultures, Midena et al. found that it had a dose and time dependent effect on cell viability.

Figure 2. The 48 hour dose response curves revealed that the difference in inhibition rates due to concentrations and across groups were not significant statistically for Hydrocortisone Sodium Succinate and Tobramycin and Pilocarpine Nitrate ($p > 0.05$). The difference in inhibition rates due to Dexamethasone Sodium Phosphate was significant statistically ($p < 0.05$) (2(a)). For 5-Fluorouracil and SDS the inhibition rates were concentration dependent ($p < 0.05$) (2(b) and 2(c)).
effect on both cell lines. However, even at the highest concentration tested, a complete inhibition of cell growth was never observed. This was not entirely in agreement with our results as by the end of 48 hours cell viability was at 29% at a very low concentration of 0.1 µg/ml.

Conclusions

In the present investigation, Tobramycin did not show any cytotoxic effect on the 3T3 cell line. Even at the highest concentration and time point the cell viability was 94% which is in concurrence with other studies23-25.

The next logical step would be to evaluate these drugs on rabbit corneal epithelial cell line (SIRC) followed by human corneal epithelial cells or the three-dimensional model for testing ocular toxicity. The latter would also compensate for the problems in inter-species variation and co-relation, which were the limitations of the current research.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References


