Evaluation of Phototoxicity of Ocular Medical and Combination Devices

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ABSTRACT

To address ocular device induced phototoxicity, an *in vitro* 3T3 Neutral Red Uptake Phototoxicity test (OECD 432) for chemicals has been modified. Soft contact lenses formulated with three different photo-absorbing compounds (Y, F/C and C) and chemical solutions of these compounds (Y, F and C) were evaluated. Lenses were placed on the bottom of culture wells in direct contact with cells. The plates were incubated for 1 hour followed by exposure to UVA/visible light or remained in the dark. In separate experiments, compounds Y, F and C, used as a part of polymeric lens composition, were tested in the Hanks' Balanced Salt Solution (HBSS), using the standard phototoxicity protocol. An extract of contact lenses in the culture medium was also tested at 100% strength. There was a difference in cytotoxicity response between the three devices with 82%, 22% and 4% cell viability after exposure to light in the presence of Y, F/C and C lenses, respectively. In the absence of light, the cell viability was 92% for all devices. In agreement with results for the intact devices, solutions of compounds Y, F and C were classified as non-phototoxic (Y) and phototoxic (F and C). There was no cytotoxicity from extract of lenses with or without light exposure. Results of a clinical trial, with lenses containing agents Y or C, reported no effect and burning sensations, respectively, while wearing lenses in bright sun. The phototoxicity identified for chemicals in solution does not always translate into phototoxicity of a finished device where this chemical is copolymerized within the device matrix. Therefore, the method described here helps to screen out for the true positive phototoxic devices designed to be placed in contact with eye or skin.

METHOD

Mouse Balb/c 3T3 fibroblasts were cultured in 24-well plates for 24 hours forming a subconfluent monolayer. Twelve replicates of each contact lens were placed on the bottom of wells (containing Assay Medium) in direct contact with cells. A separate 96-well plates were treated with either 8 dilutions of each chemical compound or an extract of the device prepared in the Assay Medium containing 10% serum proteins. A set of separate plates was exposed to a positive control, chlorpromazine. The plates were incubated for 1 hour at 37^0C and 5% CO_2. After 1 hr, half of the wells were exposed to 5 J/cm^2 of UVA/visible light, and the remaining half were placed in the dark. After 50 minutes, the cells containing dilutions were rinsed, and the cells with lenses or extract of lenses were cultured for additional 24 hours (without rinsing). After 24 hr, the cells were examined microscopically, the devices removed, the media decanted, and a 33 µg/mL neutral red (NR) solution was added for 3 hours, followed by NR extraction and spectrophotometric quantitation at 550 nm (Figure 1). Results were presented as the % cell viability for intact lenses and extract, or IC_{50} and the Photo-Irritancy Factor (PIF) and Mean Photo Effect (MPE) for samples of test solutions.

Figure 1: Phototoxicity Test

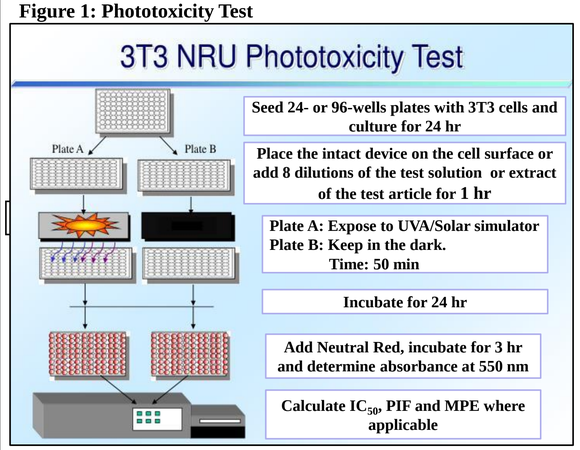

Figure 2: Evaluation of Phototoxicity of Compounds Y, F and C in Solution (96-well plate)

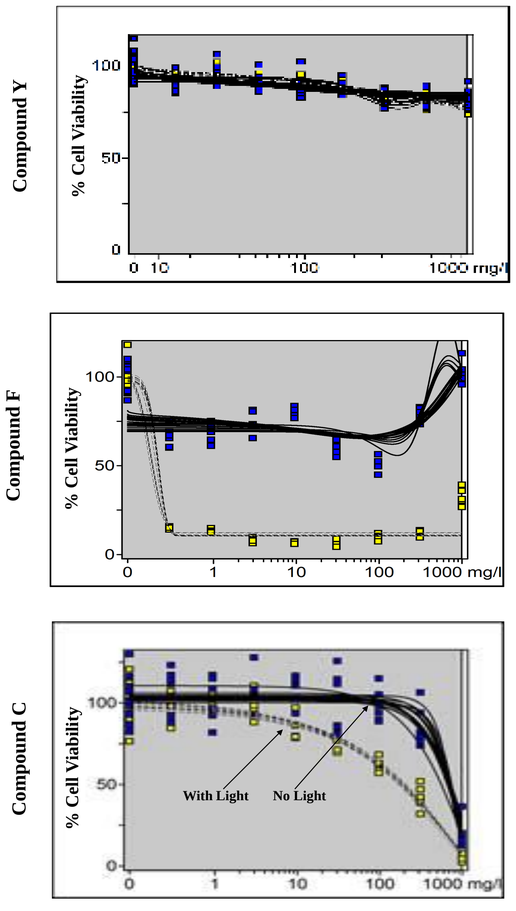

Figure 3: Lens placed on the Cell Surface While Exposed to Light

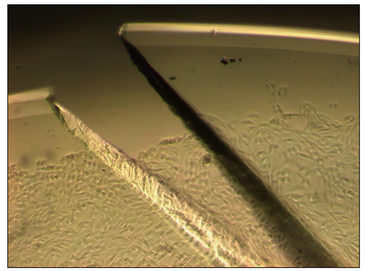

Figure 4: 3T3 Cells after 24 Hour PE and Prior to NR Uptake Test

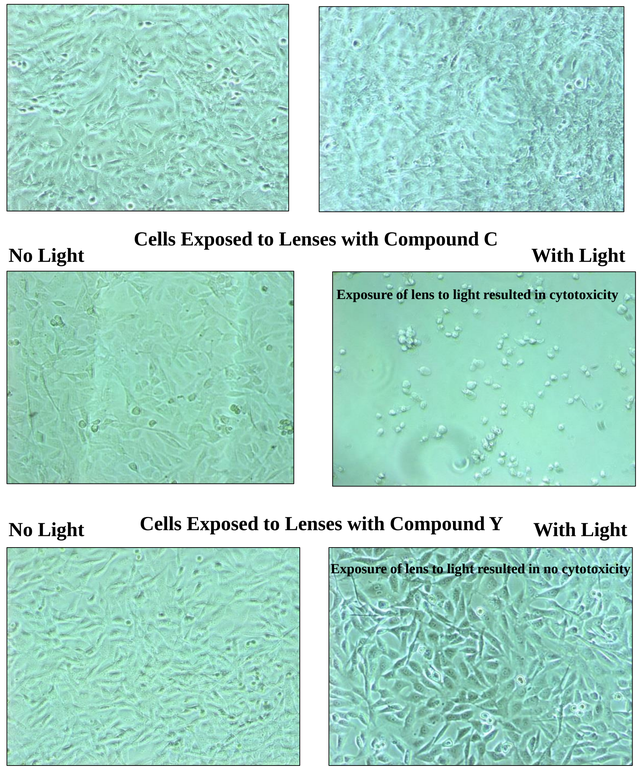

Table 1: Results of Phototoxicity Test of Compounds in Solution (96-well plate)

Compound	IC_{50} (µg/mL)		PIF	MPE	Result (compound in solution)
	With light	With no light			
Y	>1000	>1000	ND	-0.04	Non-phototoxic
F	<0.01	<11	ND	0.25	Phototoxic
C	553	806	2.08	0.17	Phototoxic

Table 2: Results of Phototoxicity Test of Lenses (24-well plate)

Intact Lens Placed on the Surface of Cells or Positive Control	Cell Viability (%) or IC_{50}		PIF	MPE	Result (device)
	With light	With no light			
Lens with Y	82.0	87.6	NA	NA	Non-phototoxic
Lens with F/C	21.6	91.4	NA	NA	Phototoxic
Lens with C	5.4; 1.9 (two separate test)	87.7; 94.9 (two separate tests)	NA	NA	Phototoxic. Caused "burning sensations" on the human eye in bright sun
Positive Control (Solution of Chlorpromazine)	4.02; 3.9 (IC_{50})	25.5; 20.5 (IC_{50})	6.45; 5.24	0.24; 0.29	Phototoxic

CONCLUSIONS

1. A 24-well 3T3 phototoxicity test was established for evaluation of intact medical devices.
2. Testing of contact lenses formulated with three different photo-absorbing agents demonstrated two phototoxic devices (C and F/C) and one non-phototoxic (Y).
3. Extract of contact lenses formulated with compound C was not phototoxic.
4. Contact lenses formulated with compound C caused burning sensation while wearing lenses in bright sun.
5. These results suggest a direct photo-activation of lens copolymerized photo -absorbing compounds (and not lens leachable) by sun light resulting in burning sensation. These clinical events can be predicated based on *in vitro* phototoxicity test.