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Abstract zur Diplomarbeit

Fachgebiet: Kontaktlinse
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Thema: **Comparison of Agar Overlay Test and UTT Assay Methods for Assessment of Cytotxicity of Contact Lens Care Products**
Toxizitätseinschätzung von Kontaktlinsenpflegemitteln an Zellkulturen - Vergleich von Agar Overlay Test und MTT Assay

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Purpose

The purpose of this work was to compare two methods of cytotoxicity for the assessment of contact lens care products. One aim was to determine the advantages and disadvantages of each method as well as things in common and differences. In addition, a statement should be made about how practicable and how suitable these two methods are. For this purpose six brands of surfactant cleaners and ten brands of rewetting drops were tested with the Agar Overlay Test and the MTT Assay methods.

Methods

To carry out the Agar Overlay Test it is necessary to have a monolayer of L929 mouse fibroblasts grown in a Petri dish and dyed with neutral red. Then the cells are exposed to a sterile non-toxic filter paper soaked in the test sample for 24 hours. Finally, the degree of cytotoxicity is determined by both decolourisation and the degree of lysis of the cells. Each sample was tested three times; the tests were carried out in a masked way.
To carry out the MTT Assay 96-well micro titre plates are seeded with L929 mouse fibroblast cells and after overnight growth exposed to four concentrations of each test sample. On the following day, MTT solution is added: this is metabolised by viable cells to produce a blue insoluble dye (formazan). After growth of cells under test conditions for a specific time, the cell culture medium is removed, and the blue dye solubilised in DMSO. The degree of cytotoxicity of a test sample is determined by the measuring and comparing the absorption at 570 nm and 690 nm of test and control (untreated) wells of the plates.
Six brands of surfactant cleaners and ten brands of rewetting drops were tested with the Agar Overlay Test at 100% concentration. Then, using the MTT Assay, the concentration at which the cell activity has decreased to 50% (IC50), was determined. Finally, all test samples for which IC50 concentrations had been obtained by MTT Assay, were retested by Agar Overlay Test at these IC50 concentrations as a final check. All experiments were carried out together with both a positive and a negative control.

Results

All test samples at 100% concentration which are toxic with the Agar Overlay Test were also toxic with the MTT Assay. With one exception, for all test samples which are non-toxic at 100% concentration with the Agar Overlay Test no IC50 values could be determined with the MTT Assay. All test samples tested at IC50 concentration appeared to be non-toxic with the Agar Overlay Test.

Discussion

Since the same cell line was used and the order of cytotoxicity does not vary significantly with both tests, it is legitimate to compare these tests. The MTT Assay is more objective, more sensitive and allows a more quantitative comparison of the cytotoxicity of different test samples. Using the MTT Assay, cells are directly exposed to the test sample, whereas the Agar might act as a protection for the cells. It is not clear if solutions with particles in it, such as OptiFree Daily Cleaner, get through the agar and to the cells. This might lead to an underestimation of the level of cytotoxicity in such samples. The fact that all test samples tested at IC50 concentration appeared to be non-toxic with the Agar Overlay Test means that samples or concentrations that seem to be completely safe with the Agar Overlay Test may in fact still have cytotoxic properties. This supports the assumption that the MTT will detect cytotoxicity in lower concentrations of solutions than the Agar Overlay Test. The Agar Overlay Test that has only four possible categories of cytotoxicity into which test samples are placed. The MTT Assay, however, is entirely quantitative, and results in IC50 concentrations are stated in ppm on a continuous scale. Therefore the MTT Assay is more able to differentiate similarly toxic samples.

Conclusion

The MTT Assay is preferable to the Agar Overlay Test for the assessment of cytotoxicity of contact lens care products, though there is scope for further modification to both methods to make them better suited to assessment of such products and to the in vivo – conditions.