**In Vitro Lens Culture is a Potent Research Tool for Assessing Chemical Toxicity**

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**Introduction**

The crystalline lens is a transparent avascular structure composed primarily of structural proteins and water that is surrounded by a collagen capsule. Activity in the lens continues as the monolayer of epithelial cells that divide, elongate and differentiate to form the regularly arranged lens fibers that are concentrically laid down throughout life. Without a direct blood supply, the lens receives all essential nutrients from the aqueous humor and most of the metabolic, synaptic and transport processes of the lens occur in the epithelial cells. As a result, the lens exists in an environment that is analogous to a single-cell type of tissue culture system. Because of this unique nature, the lens is well-suited for reproducibility in vitro culture studies aimed at elucidating the complex biochemical pathways of the lens and elucidating the metabolic changes associated with cataract formation. Here, we demonstrate how to rapidly identify species differences in lens metabolism. This is illustrated in the comparison of glucose metabolism by rat, dog, and human lenses as monitored by the 24-hr metabolism of 3-Fluorodeoxyglucose by 19F-NMR.

**Experimental Procedures**

Preincubation was conducted because lenses are sensitive to physical injury during dissection which generally becomes visible after 4-6 hr in incubation in control media. Lens free of damage are then transferred to experimental culture media using Teflon coated forceps. After culture, lens are removed from the media, rolled on moist filter paper and immediately weighed. The lenses are then homogenized in ground glass homogenizers.

The avascular lens relies on a number of biochemical functions that are critical to maintaining lens redox, transport and metabolism systems. Drugs can adversely affect these biochemical systems and these changes can lead to cataract formation. The effect(s) of a drug on lens viability can rapidly be assessed through 3-48 hr in vitro lens culture in TC-199 medium with or without the presence of 0.1 ml of the drug in question. A high (0.1 M) concentration is used to rapidly induce the drug effect. Failure of drug effect at this concentration suggests that a metabolite may be the toxicating agent.

After incubation, an initial screening of the following select biochemical parameters should be conducted.

- **Glutathione Peroxidase (GPx)**
- **Glutathione**
- **Glutathione Reductase (GR)**
- **Glutathione levels**
- **β-2-Aminobutyric acid (GABA)**
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- **3-FDGluconic acid**
- **3-FDSorbitol**

**Results**

The results of the uptake and efflux studies suggest that the drug may reduce cellular energy required for the transport systems. ATP levels were only significantly reduced by drug both after 48 hr (A and B) and 24 hr (B) exposure. In contrast, GSH levels are significantly reduced with ouabain and drug both after 48 hr (A and B) and 24 hr (B).

**Conclusions**

- Lens culture studies not only can elucidate the experimental mechanism(s) of cataract formation but also identify the toxicologic mechanism(s) of action of a drug that can alter lens biochemistry and clarity.
- In the above example, while drug-induced cataract developed in a 3-month in vivo time-frame, significant lens effects were detectable within 4 hr in vitro lens culture.
- These studies indicate that in vitro lens culture is a powerful research tool for drug development that can rapidly identify the specific action mechanism(s) of action of a drug to the lens to determine whether the parent compound or its metabolite(s) induces the adverse effect(s).