Ionomycin causes opacities in cultured sheep lenses by damaging the outer layers of the cortex

**Background**

Cortical cataracts have been associated with increased levels of calcium. A model has been developed for cataracts by increasing the calcium levels in cultured sheep lenses using the calcium ionophore, ionomycin. This model has been used to test the efficacy of potential therapeutic agents for cataracts, particularly calpain inhibition. The calcium-dependent calpains have been implicated in cataract formation, and are known to cause lens crystallins and cytoskeletal proteins.

**Purpose**

The purpose of these experiments was to determine which parts of the lens were affected by ionomycin and to look for specific evidence of calpain activation during formation.

**Methods**

Lamb lenses were dissected from whole-eye globes and cultured in Eagle's enriched minimum essential medium (EMEM) for 48 hr. Control lenses (n=10) were maintained in EMEM for a further 4 days. Lens opacity was assessed in the test group (n=10) by exposure to JHM lenses. The replication for the calcium in the medium was tested using lenses treated with ionomycin and Urban EMEM (n=5). Remaining lenses were dissected into epithelial, cortical and nuclear fractions. The calcium concentration in each fraction was determined by flame emission spectrometry and the calpains activity by casein zymography. Protocols of lens proteins were assessed by two-dimensional electrophoresis (2-DE) and Western blotting of the cytoskeletal proteins spectrin and vimentin. Spots from 2-DE gels were identified by mass spectrometry and compared with previously mapped lens proteins.

**Results**

The progress of opacification was studied in the 4-day period (Figure 1). In control lenses, a decrease in lens transparency occurred at the 48 hr mark, and this trend continued for 96 hr. The ionomycin-treated lenses showed changes at 48 hr, with a marked decrease in transparency at 96 hr. The difference in protein content was significant and was associated with a decrease in the amount of lens proteins.

**Changes in Lens Proteins**

Detection of vimentin in the soluble lens fractions and cortex was used to confirm the proteolysis on the 2-DE gels (Figure 2). There was evidence of vimentin proteolysis in the epithelium from control lenses. This was prevented by EGTA treatment which is consistent with a role for calpains in this proteolysis. Ionomycin treatment led to a decline in the amount of vimentin and its fragments.

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**Changes in Spectrin**

Spectrin and its breakdown products were determined by Western blotting of the soluble lens fractions (Figure 5). The spectrin bands showed extensive proteolysis of native spectrin (280 kDa) to 150 kDa fragments in all treatments. This proteolysis may be attributed to either calpains or caspase 3. The control and ionomycin-treated lenses also revealed the 145 kDa fragment of spectrin proteolysis, which was not present following treatment with EGTA. In the cortex, the 150 kDa spectrin breakdown product appeared to increase with ionomycin treatment. The spectrin fraction did not possess intact spectrin bands but had both the 150 and 145 kDa breakdown products. Disappearance of intact spectrin in the nucleus was not retarded by EGTA treatment suggesting that the role of this protein was not affected by ionomycin treatment. This observation is also supported by the absence of changes in the 2-DE pattern for the nucleus.

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**Conclusions**

Ionomycin caused opacification of cultured sheep lenses and ionomycin was associated with a rise in lens opacity. The epithelial and outer cortex were most affected by ionomycin treatment with no evidence of change in the lens nucleus. The formation of a liquid fraction between the lens epithelium and outer cortex following ionomycin treatment indicated protein degradation in this region. Spectrin and vimentin proteolysis and changes in cytoskeletal proteins were prominent features of this superficial model. These changes were consistent with calpain activation. However, the limited changes in the lens cortex and the absence of changes in the nuclear region implied that ionomycin had not penetrated to the interior of the lens.

**References**