Quantifying local diffusion in the rat lens by two-photon flash photolysis

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The vertebrate ocular lens is an avascular organ composed of fibre-shaped cells that run from the anterior pole to the posterior pole of the lentoid mass. Often measuring several millimetres or more in diameter, the lens cannot rely on passive diffusion alone in order to maintain homeostasis and transparency. A model of lens micro-circulation has been proposed based upon external electrophysiological, and biochemical data (Donaldson *et al.*, 2001). However, few functional studies to date have focused on transport mechanisms at varying depths within the lens mass. As a transparent tissue composed of highly ordered and regularly-shaped cells, the lens is an ideal system for studying intra- and intercellular transport by optical methods. We have applied two-photon microscopy and image analysis to lenses loaded with a caged fluorescent dye, in order to quantify local diffusion within and between fibre cells at varying depths. We have compared these functional results with our previous structural studies (Jacobs *et al.*, 2001) to elucidate structure-function relationships of fibre cell transport.

Lenses were extracted from adult rats killed by CO_2 asphyxiation in accordance with protocols approved by The University of Auckland Animal Ethics Committee. Lenses were cut in half through the equator and placed in a perfusion chamber containing intracellular medium and 1mM fluorescein bis-(5-carboxymethoxy-2-nitrobenzyl) ether (CMNB-caged fluorescein). The chamber was mounted on the stage of a confocal microscope modified for two-photon excitation. The two-photon laser beam bypassed the scanning system of the microscope and was focused inside a selected fibre cell to uncage the fluorescein by two-photon flash photolysis. Movement of the fluorescein away from this point source, both within and between cells, was imaged in real-time using confocal optics in x-y and line-scan modes. Data were written to hard disk and quantitative analysis of dye movement was performed using custom-written software.

In the lens periphery the spread of the uncaged fluorescein was highly directional, corresponding to radial rows of fibre cells. Deeper in the lens (>300 μm) the cell-cell coupling was approximately isotropic around the cell targeted for photorelease. The directional cell-cell coupling observed at the periphery corresponded to the local expression pattern of gap junctions on opposite broad sides of the hexagonal fibre cells. The isotropic coupling deeper in the lens corresponded to the dispersal of gap junctions in older fibre cells. Quantitative image analysis allowed characterisation of the time courses of differential dye transfer between neighboring fibre cells in different regions of the lens. Cytosolic fluorescein diffusion coefficients were estimated by fitting diffusion equations and by comparison with numerical simulations. Taken together, our functional and structural data are consistent with a lens micro-circulation model in which gap junctions near the lens equator facilitate solute transport to the lens surface where appropriate ion channels and transporters are concentrated.

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