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Arsenic trioxide initiates ER stress responses, perturbs calcium signalling and promotes apoptosis in human lens epithelial cells☆

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Abstract

Identification of novel agents to eradicate the residual lens cell population following cataract surgery provides one mode of preventing PCO formation. The present study investigated the biological mechanism of As_2O_3 cytotoxicity in a human lens cell line and capsular bag system. FHL 124 cell survival was assessed by quantification of total protein content, a cell population measure. Gene changes were detected by Real-time PCR; apoptosis by TUNEL assays. Intracellular calcium was measured by real-time fluorimetric single-cell digital imaging techniques after Fura-2 incorporation. In vitro human capsular bags were generated from donor eyes, which involved sham cataract surgery then use of the *Perfect Capsule* device to form a closed system to deliver As_2O_3 for 2 min. On-going observations were by phase-contrast microscopy. Cellular architecture was examined by fluorescence immunocytochemistry. FHL 124 cells demonstrated a dose-dependent sensitivity to As_2O_3 exposure. A 2 min exposure of As_2O_3 to cells within the capsular bag, using the perfect capsule system, resulted in total cell death when used at 100 mM. As_2O_3 provoked an ER stress response identified through an upregulation of known genes. As_2O_3 depleted the calcium store and consequently lead to reduced calcium signalling. As_2O_3 increased rates of apoptosis. Arsenic trioxide provokes ER stress that leads to down-regulation of calcium signalling resulting in apoptosis. The application of As_2O_3 to cells within the capsular bag for a 2 min window using the *Perfect Capsule* system predicts putative therapeutic benefit in vivo.

Keywords: human; lens; posterior capsule opacification; stress; calcium; apoptosis

1. Introduction

Posterior capsule opacification (PCO) is still a major long-term complication of cataract surgery and likely the most common cause of non-refractive decreased postoperative vision (Apple et al., 1992; Schaumberg et al., 1998). PCO arises from residual lens epithelium cells (LECs) within the capsular bag which is formed at cataract surgery to hold the intraocular lens (IOL), which corrects the hyperopic state caused by the removal of lens content. The remaining lens epithelial cells proliferate and migrate onto the posterior capsule underlying the intraocular lens and into the light path, thus causing a secondary reduction in vision quality (Wormstone, 2002). Recent studies have shown that PCO rates can be diminished by improved IOL design (Hayashi et al., 2001; Nishi et al., 2001). These designs are likely to influence the progression of PCO through physical characteristics. However, many thousands

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of individuals in the UK for example do require secondary surgery involving laser capsulotomy, which places a strain on healthcare resources, time and the quality of a patient's life. Moreover, it should not be assumed that a person who does not undergo corrective laser therapy has optimal vision. Laser therapy is employed when visual loss severely affects the life of an individual. Using laser capsulotomy rates as a barometer for PCO is a useful tool, but a number of patients who do not undergo this secondary surgery are still likely to have diminished visual quality compared with the initial surgical result. Sundelin and Sjostrand (1999) examined this group and found that a significant proportion would benefit from laser capsulotomy. Therefore despite the progress made in the IOL area, the problem is far from resolved. Theoretically, the most efficient way to prevent PCO is to eliminate all the cells within the capsular bag at surgery and a range of cytotoxic agents have been tested in a number of human and animal model systems, such as mitomycin-C (Jordan et al., 2001), 5-fluorouracil (Fernandez et al., 2004), and thapsigargin (Tg; Duncan et al., 1997). None of these treatments have yet to reach the clinic, therefore there is scope to develop further agents to prevent PCO and arsenic trioxide is a promising candidate.

In the present study we investigated the potential benefits of a novel compound, arsenic trioxide (As_2O_3), in the prevention of PCO. Arsenic trioxide (As_2O_3) is a potent anti-tumor agent not only used to treat acute pro-myelocytic leukemia (APL; Miller et al., 2002) but also, more recently, solid tumors (Baumgartner et al., 2004). It has also been reported to have effect on normal cells (Gao et al., 2004). The detailed mechanisms of As_2O_3 cytotoxicity are not completely known, but many preclinical studies have provided insight into the processes involved. The mechanisms include generation of reactive oxygen species, DNA damage, apoptosis induction and inhibition of angiogenesis (see for example Gallagher, 1998; Bode and Dong, 2002 for reviews).

The risk of using a toxic compound is that these general actions will affect the surrounding ocular tissues for example corneal endothelial cells. The Perfect Capsule device recently developed by Maloof and colleagues (Maloof et al., 2003) permits the capsular bag to be sealed off from the environment, permitting cytotoxic agents to be both delivered selectively to the bag but, equally importantly, to be removed during the course of the operation. This method has shown that there was no leakage to surrounding tissues, and the population of epithelial cells remaining in the bags after surgery was reduced (Maloof et al., 2005). Therefore controlled delivery of a toxic drug over a relatively short time frame (e.g. 2 min) appears to be a worthwhile route of investigation (Duncan et al., 2007).

There are at least two reasons for taking As_2O_3 into consideration when choosing effective drugs for PCO. The first is because of the anti-proliferation effect of As_2O_3 proved already (Gallagher, 1998; Bode and Dong, 2002). The other reason is that the biological effects of arsenic (principally the trivalent forms, arsenite and arsenic trioxide) may be mediated by reactions with closely spaced cysteine residues on critical cell proteins (Snow, 1992). Several proteins with a high cysteine content and accessible thiol groups are candidates for interactions with arsenic. If As_2O_3 is to be effective in the clinic to prevent PCO, within the small time window allowed, there should be many accessible SH groups both at the surface and within lens cells. This is indeed the case, as SH groups play a critical role in membrane permeability processes and are important for normal lens cells function (Hightower et al., 1985; Duncan et al., 1988; Hightower et al., 1989).

Although As_2O_3 has been studied extensively in recent years, this work has largely focused on the tumors. However, the functional role of As_2O_3 in the prevention of PCO, which affects a large proportion of cataract patients, has not been investigated. In the present study, we therefore deemed it necessary to test the hypothesis that As_2O_3 can inhibit the development of PCO. Our investigation revealed a cytotoxic action of As_2O_3, which involved ER stress gene activation, suppression of calcium signalling leading to apoptosis. Application of 100 mM As_2O_3 for 2 min in human capsular bags, using the perfect capsule closed drug delivery system, leads to total loss of cell viability and thus appears to be a promising compound to pursue for the treatment of PCO.

2. Materials and methods

2.1. Cell culture

FHL 124 cells were routinely cultured in Eagle's minimum essential medium (EMEM) supplemented with 5% fetal calf serum (FCS; Hyclone, Logan, UT) and seeded on tissue culture dishes (patch assay, western blot, real time PCR), and glass coverslips (calcium imaging).

2.2. Growth assays

FHL 124 cells (Wormstone et al., 2000, 2004; Wang et al., 2005; Duncan et al., 2007) were seeded on tissue culture dishes (Beckton Dickinson labware, New Jersey) and maintained in EMEM supplemented with 5% FCS until confluent regions spanning approximately 5 mm developed. The medium was then replaced with non-supplemented EMEM and cultured for a further 24 h. At this point ($t = 0$ for the experiment) four dishes were isolated and the cells were fixed in 4% formaldehyde for 30 min, and stained with Coomassie blue for 10 min. Excess dye was removed by general wash in PBS and the stained region was measured (see later) to give the initial areas of the patches. Remaining cultures were then placed in experimental conditions over a 4 day period. At the end of each experiment, cells were fixed for 30 min with 4% formaldehyde at room temperature. The cells were then washed in PBS and stained with Coomassie brilliant blue for 10 min. The cells were then washed several times to remove excess dye. For an analysis of total dye uptake (related to total cell protein) the PBS was replaced with 1 ml of 70% ethanol and the dishes agitated for 1 h until all dye was removed. A total of 200 μl of the ethanol/dye mixture from each well was added to a clear plastic 96 well microtitre plate and the absorbance due to the dye was measured at 550 nm using a Wallac