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The development of an innervated epithelial barrier model using a human corneal cell line and ND7/23 sensory neurons

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Abstract

The corneal epithelium is a highly innervated tissue and hence in vitro models that mimic the effects of chemicals or radiation (e.g. ultra violet) on this important barrier should include consideration of the potential role of innervation. A sensory neural cell line, ND7/23, was incorporated into a 2D and 3D model of a corneal epithelium, using a human corneal cell line, and effects on barrier integrity were neither adverse nor stimulatory. In the 3D model the nerve cell bodies were separated from the corneal epithelium, via a porous polycarbonate insert membrane. The ND7/23 cells were induced to form neurites and cease division when cultured in the keratinocyte medium employed for the corneal cells. In the absence of calcium, the epithelial barrier function was lost, shown by enhanced fluorescein leakage and relocation of ZO-1 and E-cadherin from the cell membrane. At 60 μM calcium, and above, the corneal cells formed tight junctions, with peripheral membrane location of ZO-1 and E-cadherin. The presence of the ND7/23 cells did not compromise or enhance the time taken to form these junctions, when monitored at 24-h intervals over 72 h. Both male- and female-derived human corneal cell lines showed a similar tight junction functional response to different medium calcium concentrations in the presence or absence of the ND7/23 cells. Once differentiated in keratinocyte medium, patch-clamped ND7/23 cells were capable of producing a whole-cell current when exposed to low pH (5.4), indicative of the presence of active pH-gated ion channels.

Keywords: ND7/23 sensory neurons; Human corneal cell line; Calcium-regulated adhesion molecules; Acid-sensing ion channels; Patch-clamp

Introduction

The need to identify ocular irritant chemicals via in vitro techniques has been highlighted by the EU Chemicals policy (EU Strategy for Future Chemicals Policy: EU Commission White Paper, 2000). In addition there is an EU requirement to use in vitro validated methods where available to provide toxicity data (European Union Directive 76/768/EEC). A number of international validation trials have concluded that additional refinement of in vitro methods for predicting human eye irritation are required (Balls et al., 1995;

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Brantom et al., 1997). In response to the EU Chemicals Policy document, Worth and Balls (2002) concluded that "in the short-term, the in vitro tests currently being used in-house must be demonstrated, to be valid for the purposes to which they are being used, or new in vitro tests will need to be developed and validated".

Araki-Sasaki et al. (2000), demonstrated using a transfected human corneal cell line, that substance P affected E-cadherin expression, raising the possibility of corneal epithelium barrier function being modulated in vitro by neuroactive molecules. In vivo, such modulation of differentiated functions occurs with substance P from the trigeminal nerve, linked to neurotropic keratitis (Foster, 1987). The sensory-derived ND7/23 cell line can be stimulated to differentiate and form neurite-like projections in the presence of nerve growth factor and is capable of producing neuroactive molecules, including substance P (Suburo et al., 1992; Wood et al., 1990). A female human transfected corneal cell line (Kahn et al., 1993; Kruszewski et al., 1997; Ward et al., 1997a) has been shown to form tight junctions in a 3D model and responds to surfactant based-formulations in a reproducible way (Clothier et al., 2000).

Garle and Fry (2003) noted that few workers developing alternatives to the Draize eye test (Draize et al., 1944) considered the problem of effects resulting from sensory innervation. Suuronen et al. (2004) reported the generation of an innervated model using dorsal root ganglia from the chick.

We describe the development of a 3D model employing an available neural cell line and human transfected corneal epithelial cell lines. Barrier function across the corneal epithelium in the presence or absence of ND7/23 cells was assessed via retention of the calcium-regulated control of the adhesion junction molecules ZO-1 and E-cadherin (Kemler, 1993; Kimura et al., 1996; Stuart and Nigam, 1995), fluorescein leakage (Clothier and Sansom, 1996; Tchao, 1988), and culture activity via the resazurin assay (Clothier and Sansom, 1996; O'Brien et al., 2000). This was undertaken as a prelude to the evaluation of substance P and other neuroactive molecules in the development and restoration following damage of the corneal barrier function in vitro.

It was necessary to evaluate the type of insert membrane for its capacity to support adhesion of the cells because previous work indicated that responses to surfactants in Madin-Darby canine kidney (MDCK) cells were modulated by the type of insert membrane upon which the cells were grown (Ward et al., 1996, 1997b). The female transfected corneal cells (Kahn et al., 1993; Kruszewski et al., 1997; Ward et al., 1997a), are routinely grown on Cellagen^{TM} inserts, and do not attach to Anopore membranes. To achieve physical separation of the neural and corneal cell bodies, as occurs in vivo, in the 3D models the two cell types were grown on either side of a 0.4-μm pore polycarbonate membrane. The 3D co-cultures were performed in the keratinocyte medium as previously used for the corneal cells (Clothier et al., 2000; Ward et al., 1997a). Calcium-free keratinocyte medium is commercially available, enabling the assessment of calcium regulation of functions in vitro under different co-culture conditions.

To demonstrate the presence of living ND7/23 cells in the cultures, non-cytotoxic dye/s for the neural cells were investigated, so that ND7/23 cells and if possible neurites could be visualised and repeatedly monitored in the 3D model, both prior to and following adverse insults. Functionally, the activation of currents was investigated during exposure to low pH, indicative of acid-sensing ion channels (ASICs) as found in dorsal root ganglia (DRG) neurons (Akopian et al., 2000). Similar currents were investigated in ND7/23 cells differentiated with NGF. As vanilloid receptors (TRPV1) present on native DRG neurons also respond to low pH, capsaicin (a specific TRPV1 agonist) was also applied to determine the nature of any pH 5.4 activated current.

Materials and methods

Cell culture

All cells were cultured in the appropriate culture media and vessels and grown in a saturated humidified 5% CO_2/95% air atmosphere at 37 °C.

Cell origins and medium composition

Two SV40-transfected human corneal epithelial cell lines were employed. One, from a male, was a kind gift from Araki-Sasaki (Araki-Sasaki et al., 1995) and was originally grown in RPMI-1640 containing serum (Sigma Ltd., Poole, UK). These cells were educated to grow in the same medium as the other corneal cell line derived from a female, a kind gift from the Gillette Company Ltd. The medium combined Keratinoycte Basal Medium (KBM; Cambrex Bio Science Ltd., Workingham, UK) with the associated bullet kit (Cambrex Bio Science Ltd.) of growth factors (hydrocortisone, bovine pituitary extract, epidermal growth factor, and penicillin/streptomycin) to generate the Keratinocyte Growth Medium (KGM; Kahn et al., 1993; Ward et al., 1997a). To control the basal level of calcium accurately, calcium was added as calcium chloride (Cambrex Bio Science Ltd.).

ND7/23 cells were obtained from ECACC (9090903; Salisbury, UK).