Lung epithelial cell tolerance of trigger conditions for stimuli-responsive inhaled drug delivery systems

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Introduction and Aims

- The rapid removal of therapeutic agents from the airways following inhalation is a significant barrier to achieving prolonged drug delivery via this route.
- Nanoparticles have the potential to increase the residence time of drugs in the airways, acting as reservoirs to facilitate controlled drug release.
- However, the rigid structure of nanoparticles can present a barrier to drug release following administration [1].
- Stimuli-responsive systems, in which the system is modified in response to changes in environment to facilitate drug liberation, offer a solution to this problem.
- The aim of this work was to investigate how human lung epithelial cells would tolerate changes in microenvironment that may be utilised as triggers for controlled drug release.
- The effects of elevated temperatures, acidic and alkali pH and the presence of surfactant were investigated using a human bronchial epithelial cell line, Calu 3, and a simple colorimetric assay to assess cell viability as a function of mitochondrial activity.
- In situ surfactant triggered release from lipid nanoparticles was also studied using confluent Calu 3 cell layers to model the airway epithelial barrier.

Methodology

- The Calu-3 human bronchial epithelial cell line was used between passages 30-45 and cultures were maintained in a humidified 5% CO2/95% atmospheric air incubator at 37°C in cell culture media (CCM) as described previously [2].
- The MTT ([3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [3] was used to assess cell viability following exposure to elevated temperature, altered pH and Pluronic L62D surfactant. Cells were seeded at a density of 1 x 10^4 cells/well in 96-well plates and 24 h later exposed to either the test condition or control solutions for 5, 10, 20 or 30 min. MTT solution (0.5 mg/mL) was then added for 4 h and any formazan crystals generated were solubilised with 100 µL of SDS solution (10% w/v in 50:50 DMF:water).
- Absorbance was measured 16 h later at 570 nm, corrected for background absorbance at 650 nm (SpectraMax 190, Molecular Devices, USA). Absorbance was used to determine cell viability by comparing to negative and positive controls [3]. The assay was performed in triplicate, with three to six repeats for each test condition on each occasion.
- The surfactant triggered release of rhodamine from lipid nanocapsules was quantified using fluorescence spectroscopy (Cary Eclipse, Varian, Australia) following application to confluent cell layers cultured at the air-liquid interface. Cell barrier integrity was monitored during and after sample application [2].

Results and Discussion

- Sigmoidal dose-inhibition relationships were observed between incubation temperatures and percentage cell viability for the various exposure times (Figure 1a). Cell viability diminished at lower temperatures as exposure time was increased from 5 to 20 min, reflected in the decreased temperatures able to reduce cell viability to 50% (IC50) (Table 1).
- A reduction in cell viability was also observed when the pH of the cell media deviated from that of physiological pH (Figure 1b). Increasing the length of exposure time from 5 to 20 min was shown to reduce the pH range at which cells could maintain normal mitochondrial activity, this was reflected in the reduced breadth of the bell shaped curve and the IC50 values. (Table 1)
- Sigmoidal dose-inhibition relationships were observed between Pluronic concentration and cell viability (Figure 1c). Profiles for 10 and 20 min exposure times appear similar, but a more marked reduction in cell viability as a function of concentration was noticed when exposure time was increased to 30 min (Table 1).
- Pluronic surfactant was found to trigger the release of rhodamine from lipid nanocapsules (Figure 2) to a significantly greater level than from control particles (63.9 ± 16.3% vs 22.5 ±15.4% after 1 h, ANOVA, p=0.05). The barrier integrity of the exposed cell layer (monitored via changes in transepithelial electrical resistance) was not compromised (data not shown).

Conclusions

- Cells were less able to tolerate changes in microenvironment as they deviated further from those of normal physiological states and with increased exposure times. However, significant changes in temperature, pH and surfactant can be tolerated, particularly if experienced for less than 10 min.
- This data is useful for the design of stimuli responsive systems which facilitate targeted controlled drug delivery to the airways.
- To illustrate, Pluronic surfactant is capable of triggering release from lipid nanocapsules in situ without untoward effects on cell layer barrier function.

Table 1. Half maximal inhibitory conditions (IC50) for Calu 3 cells subjected to changes in temperature (°C), acidic pH, alkali pH and Pluronic surfactant (mg/mL) as determined by the MTT assay. Mean IC50 ± SD (n=3).

<table>
<thead>
<tr>
<th>Condition</th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>50.9 ± 1.1</td>
<td>49.3 ± 0.8</td>
<td>48.7 ± 1.6</td>
<td>-</td>
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<tr>
<td>Acidic pH</td>
<td>3.0 ± 0.3</td>
<td>4.9 ± 1.3</td>
<td>5.0 ± 1.4</td>
<td>-</td>
</tr>
<tr>
<td>Alkali pH</td>
<td>9.5 ± 0.8</td>
<td>9.1 ± 0.5</td>
<td>8.2 ± 0.3</td>
<td>-</td>
</tr>
<tr>
<td>Pluronic surfactant (mg/mL)</td>
<td>-</td>
<td>24.1 ± 13.7</td>
<td>17.3 ± 6.0</td>
<td>9.1 ± 1.8</td>
</tr>
</tbody>
</table>

Figure 1: Relationship between Calu 3 cell viability (%) and a) exposure temperature (°C), b) pH of applied cell culture medium, and c) applied Pluronic surfactant concentration (mg/mL). Data points represent mean cell viability ± SEM as determined by the MTT assay (n=3 experiments, with three to six replicates per experiment at each test concentration).

Figure 2: Rhodamine release (%) from lipid nanocapsules (LN) and LN treated with Pluronic (LN + Pluronic) at 2.5 mg/mL applied to confluent Calu 3 cell layers. Release was quantified 1 h following sample application as a percentage of the initial LN rhodamine content (n=3 with 3 replicates per experiment).

References


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