“Gene Delivery to Lung Epithelial Cells Using a Cell Penetrating Peptide”

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Research Problem

• More than 70,000 people worldwide suffer from Cystic Fibrosis (a inherited monogenic genetic diseases)
• Lung Cancer is the most common type of cancer in men and the third most common in women
• Seasonal Influenza-associated deaths reached 80,000 Americans in 2017-2018.
• 1 in 1500-3500 people with European ancestry is affected by alpha-1-antrypsin diseases

Could be treated with Gene Therapy
Research limitations

• Approximately 400 clinical trials on gene therapy for lung-related diseases

• Limitations of delivering pDNA
  - Poor internalization by target cells
  - Serum nuclease susceptibility
  - Rapid renal clearance,
  - Phagocyte uptake,
  - Toxic effect due to triggering immune response
Strategies

- Nanopolymeric systems shows potential in delivering pDNA formulations.
- Various polymers have been studied.
- Surfaces modifications: PEGylation, targeting, nuclear localization, cell-penetrating peptides.

Challenges remain:
- Encapsulation efficiency
- Stability of nanoparticles
- Degradation in blood
- Internalization
- Endosomal escape,
- Overall delivery efficiency
- Toxicity
Challenges in aerosol delivery of pDNA

Aim

(1) Develop a novel platform for pDNA delivery to the lungs using a biodegradable polymer PLGA and a cell-penetrating peptide (CPP) as an uptake enhancer

(2) Assess the toxicological effects of these nanoparticles on lung epithelial cells
Particle Size and Zeta Potential

PLGA nanoparticles - Double emulsion technique

pDNA Encapsulation Efficiency (%): 96.72 ± 0.36

Nanoparticles (NPs) characterization (n=3 ± StDev)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Size (nm)</th>
<th>PDI</th>
<th>Zeta Potential (mV)</th>
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</thead>
<tbody>
<tr>
<td><strong>Before coating with CPP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>157.267 ± 1.858</td>
<td>0.146</td>
<td>-3.79 ± 0.65</td>
</tr>
<tr>
<td>NP - DNA</td>
<td>175.300 ± 0.361^C</td>
<td>0.054</td>
<td>-12.57 ± 0.21^C</td>
</tr>
<tr>
<td><strong>After coating with CPP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP CPP</td>
<td>170.033 ± 1.563^B</td>
<td>0.140</td>
<td>12.87 ± 0.65^C</td>
</tr>
<tr>
<td>NP CPP - DNA</td>
<td>167.867 ± 0.493^A</td>
<td>0.021</td>
<td>-0.13 ± 0.05^C</td>
</tr>
</tbody>
</table>

Gomes dos Reis, et al. Delivery of pDNA to lung epithelial cells using PLGA nanoparticles formulated with a cell-penetrating peptide: understanding the intracellular fate. (Under Review)
Can we promote internalization?

**Beas-2B:** non-carcinoma-derived bronchial epithelial cells

Confocal microscope image of internalised NP-DNA-CPP in Beas-2B after 3h incubation (yellow, NP-DNA-CPP; blue, nucleus; brown, cellular membrane).

- Addition of CPP to the nanoparticles were essential to promote cellular uptake within 3h
• A 7.4-fold increase of eGFP expression (p<0.05) was observed after 96h for cells exposed to NP-DNA-CPP.
Challenges in aerosol delivery of pDNA

• ↑ ROS (Reactive Oxygen Species) = ↑ Oxidative Status
• Oxidative Stress in NP-DNA-treated similar to the vehicle (2.5 ± 0.5% of NP-Internalized cells)
• Significant decrease (P<0.01) in treatment with NP-DNA-CPP (83.8 ± 1.2% of NP-CPP internalized cells).
• Decreased IL-8 both 24h and 48h
Toxicity: Apoptosis and Necrosis

- Types of cell death
- Interaction with DNA, lysosomal-escape (release of enzymes)

- More than 80% of live cells
- Slight increase in dead cells (less than 5%)
- Increase in Necrosis at 24h that was reverted at 48h
Summary

Achievements:

- IMPROVED encapsulation efficiency
- STABLE nanoparticles (~170 nm, low aggregation)
- INCREASED internalization within 3h (NP-DNA-CPP)
  - Delivery efficiency (Gene expression after 96h)
- Toxicity (NP-DNA-CPPs did not induce apoptosis, oxidative stress; minor effect on necrosis)
Conclusion

• We developed a safe delivery system to deliver pDNA to the lung

• Further investigation in other lung-related systems

• This study increases the toxicological testing knowledge of nanoparticulate systems, in order to screen and optimize safer therapies before clinical trials.

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