Topical Delivery of Multiple Nucleic Acids in Psoriasis Treatment: Some New Targets

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Background
Psoriasis

- Psoriasis is a chronic inflammatory skin condition that results in hyper proliferation of skin cells (keratinocytes), accumulation of inflammatory proteins and extensive angiogenesis.

- Results in scaling and swelling.

- Mostly observed as patches of thick, red skin with silvery scales.

- Often found on the elbows, knees, other parts of the legs, scalp, lower back, face, palms, and soles of the feet.

- They can also show up in other places; fingernails, toenails, genitals, and inside the mouth.

Treatments available

- T-cell modulating agents
  - Alefacept (Amevive)
  - Efalizumab (Raptiva)

- Tumor Necrosis Factor (TNF) α blockers
  - Adalimumab (Humira)
  - Etanercept (Enbrel)
  - Infliximab (Remicade)

- IL12/IL23 cytokine inhibitors
  - Ustekinumab (Stelara)
  - Briakinumab

- Administered by Infusion, IM or SC

- Topical application of
  - Corticosteroids
  - Vitamin D derivatives such as Calcipotriene (Dovonex) and Calcitriol (Rocaltrol).
  - Topical retinoids such as azorotene (Tazorac, Avage)
  - Calcineurin inhibitors such as tacrolimus (Prograf) and pimecrolimus (Elidel)

- Phototherapy

- Oral-
  - Retinoids
  - Cyclosporine
  - Methotrexate
Objective 1
Development and evaluation of lipid-based delivery system for siRNA and small molecule drug for psoriasis
Background of pyrrolidinium lipid

- Cationic amphiphilic lipid
- Heterocyclic pyrrolidinium head group linked by covalent bond to two oleyl hydrophobic chains
- Fusogenic properties
- Pyrrolidone derivatives incorporated into nanoparticles increase fluidity and enhance permeation
Preparation of CyLiPn

- PLGA
- Capsaicin
- siTNFα
- Cyclic Lipid
- DSPE-PEG-NH₂
- DOPC
Physical characterization of CyLiPn

i) siTNFα-solution, ii) S-CyLiPn, iii) extracted siTNFα from S-CyLiPn, iv) CS-CyLiPn, v) extracted siTNFα from CS-CyLiPn
Uptake of FITC-labeled siRNA

(i) No Treatment
(ii) siFITC-Solution
(iii) LF-siFITC Complexes
(iv) siFITC-CyLiPn
Uptake of FITC-labeled siRNA

(i) No Treatment

(ii) siFITC-Solution

(iii) LF-siFITC Complexes

(iv) siFITC-CyLiPn
Skin permeation studies

- **SC + Epi**
- **Dermis**
- **Recv Comp**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percent Dose Permeated</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Solution</td>
<td>1</td>
</tr>
<tr>
<td>C-CyLiPn</td>
<td>3.5</td>
</tr>
<tr>
<td>Capzasin-HP Cream</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Note: The graph shows that Capzasin-HP Cream has a significantly higher percent dose permeated compared to the other conditions.
Animal Model

1) *In vivo* imiquimod (IMQ)-induced psoriatic like plaque model development in C57BL/6 mice.

- Treatment with IMQ (4mg/g) on the shaven dorsal regions of the mice for 5 consecutive days.
- 2-3 day- wait period for development of psoriatic-like plaques.

2) Secondary treatment with formulations for 5 consecutive days

3) Scoring of the extent of psoriasis area and severity index (PASI) on a scale of 1 to 4 based on extent of erythema formation, skin thickening(plaque formation) and scaling (desquamation).
Histological studies

(i) Normal

(ii) IMQ

(iii) Topgraf®

(iv) S-CyLiPn

(v) C-CyLiPn

(vi) CS-CyLiPn

(vii) CS-Solution
Relative levels of protein expression

- TNFα/β-actin Ratio
- NF-κB/β-actin Ratio
- Ki-67/β-actin ratio
- IL-17/β-actin ratio
- IL-23/β-actin ratio

Protein/β-actin Ratio

- 17 kD
- 345 kD
- 65 kD
- 15 kD
- 19 kD
- 45 kD
mRNA levels of inflammatory biomarkers

**Relative levels of mRNA**

**Tested Protein/β-actin Ratio**

- Normal
- IMQ
- S-CyLiPn
- C-CyLiPn
- CS-CyLiPn
- Topgraf®
- CS-Solution

**MRNA levels of inflammatory biomarkers**

- TNFα/β-actin Ratio
- NF-κB/β-actin Ratio

* indicates significant difference from Normal.
Objective 2

Development and evaluation of lipid-based delivery system for dual siRNA for psoriasis
Preparation of FNALPs

Novel Cyclic lipid

siStat3

siTNF-α

Cationic lipid

DSPE-PEG-2000

Therapeutics encapsulated Lipid nanoparticles (Lip-NPs)
Physicochemical characterization

- Zeta potential was positively charged.
- F-NALPs were stable and showed insignificant change in particle size and zeta potential after 1 month.

<table>
<thead>
<tr>
<th>F-NALP Ave</th>
<th>Particle size (nm)</th>
<th>Polydispersity (PI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>102.0 ± 6.0</td>
<td>0.52 ± 0.01</td>
</tr>
</tbody>
</table>
Physicochemical characterization

- Gel electrophoresis showed siRNA entrapment in F-NALP.
- There was > **80% cell viability** with max concentration of 50 µM of F-NALPs.
Knockdown in HEK 293-GFP cells

No treatment  siGFP-SOLN  siGFP-Lipofectamine  siGFP-F-NALP
Vertical Depth Sections

Epidermis

siFITC-Solution

siFITC-F-NALP

Full Thickness
Tape stripping

**siRNA FITC solution**

**siRNA FITC cy5 liposomes**

Fluorescence intensity (arbitrary units)

<table>
<thead>
<tr>
<th>siRNA-FITC solution</th>
<th>siRNA-FITC lipidosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal skin</td>
<td>tape stripped skin</td>
</tr>
</tbody>
</table>

* denote significant difference.
## PASI Scoring of psoriatic-like plaques

<table>
<thead>
<tr>
<th>Days</th>
<th>CONT (NO IMQ)</th>
<th>CONT (IMQ)</th>
<th>siTNF α-F-NALP</th>
<th>Dual siRNA-F-NALP</th>
<th>siSTAT3-F-NALP</th>
<th>Dual siRNA SOLN</th>
<th>Topgraf®</th>
<th>Blank F-NALP</th>
<th>scr TNF α</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 ± 0.0</td>
<td>4 ± 0.0</td>
<td>4 ± 0.0</td>
<td>4 ± 0.0</td>
<td>4 ± 0.0</td>
<td>4 ± 0.0</td>
<td>4 ± 0.0</td>
<td>4 ± 0.0</td>
<td>4 ± 0.0</td>
</tr>
<tr>
<td>2</td>
<td>0 ± 0.0</td>
<td>4 ± 0.0</td>
<td>3 ± 0.0</td>
<td>2 ± 0.5</td>
<td>3 ± 0.5</td>
<td>4 ± 0.58</td>
<td>2 ± 0.58</td>
<td>4 ± 0.58</td>
<td>4 ± 1.15</td>
</tr>
<tr>
<td>3</td>
<td>0 ± 0.0</td>
<td>4 ± 0.0</td>
<td>3 ± 0.8</td>
<td>2 ± 0.5</td>
<td>2 ± 0.58</td>
<td>3 ± 0.58</td>
<td>1 ± 0.58</td>
<td>3 ± 0.58</td>
<td>4 ± 0.71</td>
</tr>
<tr>
<td>4</td>
<td>0 ± 0.0</td>
<td>4 ± 0.0</td>
<td>2 ± 0.8</td>
<td>1 ± 0.58</td>
<td>2 ± 0.5</td>
<td>3 ± 0.0</td>
<td>1 ± 0.58</td>
<td>3 ± 0.58</td>
<td>4 ± 0.71</td>
</tr>
<tr>
<td>5</td>
<td>0 ± 0.0</td>
<td>4 ± 0.0</td>
<td>2 ± 0.5</td>
<td>0 ± 0.43</td>
<td>1 ± 0.58</td>
<td>3 ± 0.0</td>
<td>0 ± 0.58</td>
<td>3 ± 0.58</td>
<td>3 ± 0.0</td>
</tr>
</tbody>
</table>
IHC analysis

There was significant (p < 0.05) reduction in rete formation, thickening of epidermis and positive brown staining in dual siRNA-F-NALP treated animals for the immune regulating proteins, STAT3, TNFα and IL17.
Inflammatory protein analysis

- NFkB
- Ki67
- IL23
- β-actin

**NFkB expression/β-actin**

**Ki67 expression/β-actin**

**IL23 expression/β-actin**
mRNA levels of inflammatory biomarkers

Relative levels of mRNA

- STAT3
- TNF alpha
- TYK2

mRNA expression/β-actin ratio

- no IMQ
- IMQ
- TNF alpha-F-NALP
- DUAL-F-NALP
- STAT3-F-NALP
- DUAL SOLN
- Topgraf
- BLANK F-NALP
- SCR TNF alpha
Objective 3

Validation of lipid-based drug delivery system for enhanced topical delivery
# Doxorubicin liposomes

<table>
<thead>
<tr>
<th>Particle size (nm)</th>
<th>P.I.</th>
<th>Zeta Potential (mV)</th>
<th>Entrapment Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 - 130</td>
<td>0.10 - 0.20</td>
<td>16.00 - 17.00</td>
<td>99.91±0.10</td>
</tr>
</tbody>
</table>
Time-dependent permeation

Dox solution
Dox cy5 liposome

Fluorescence intensity (arbitrary units)

3 hr
6 hr

*
Effect of tape stripping

Dox solution

Dox cy5 liposome

Fluorescence intensity (arbitrary units)

Dox solution

Dox cy5 liposome

Normal skin

Tape stripped skin

**

*

* * *
<table>
<thead>
<tr>
<th>Depth (μm)</th>
<th>Normal Skin</th>
<th>Hair follicle blocking</th>
<th>Tape stripping only</th>
<th>Tape stripping + Hair follicle blocking</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-60 μm</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td>61-120 μm</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
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<tr>
<td>121-180 μm</td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
</tr>
<tr>
<td>181-240 μm</td>
<td><img src="image13" alt="Image" /></td>
<td><img src="image14" alt="Image" /></td>
<td><img src="image15" alt="Image" /></td>
<td><img src="image16" alt="Image" /></td>
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<tr>
<td>241-300 μm</td>
<td><img src="image17" alt="Image" /></td>
<td><img src="image18" alt="Image" /></td>
<td><img src="image19" alt="Image" /></td>
<td><img src="image20" alt="Image" /></td>
</tr>
<tr>
<td>301-360 μm</td>
<td><img src="image21" alt="Image" /></td>
<td><img src="image22" alt="Image" /></td>
<td><img src="image23" alt="Image" /></td>
<td><img src="image24" alt="Image" /></td>
</tr>
</tbody>
</table>

**Effect of hair follicle blocking**
Effect of Size

Dox DOTAP liposomes

- ~100 nm
- ~600 nm
- ~1100 nm

Fluorescence intensity (arbitrary units)

Size of DOX DOTAP liposomes

***
Effect of Charge

- Positively charged liposomes
  - Dox DOTAP lipo
  - Dox PEG lipo
  - Dox cy5 lipo
  - Dox DOPA lipo
- Negatively charged liposomes
  - Dox DOTAP lipo
  - Dox PEG lipo
  - Dox cy5 lipo
  - Dox DOPA lipo

Fluorescence intensity (arbitrary units)

**Negatively charged lipo**

**Positively charged lipo**

DOTAP liposomes  | cy5 liposomes  | PEG liposomes  | DOPA liposomes
--- | --- | --- | ---

** | * | | **
Effect of CPP

Dox TAT solution  Physical mixture  Dox TAT liposome  Dox TAT liposome w/ hair follicles blocked

Fluorescence intensity (arbitrary units)

- 0  5  10  15  20  25  30  35

**  ***  **
Summary

- The novel pyrrolidium cationic lipid forms stable nanoparticles with entrapment proficiency for therapeutic cargos.

- CyLiPn and F-NALP permeates better into deeper layers of the skin than solution.

- Imiquimod induces psoriatic-like plaques in C57BL6 mice.

- Simultaneous percutaneous delivery of siTNFα/Capsaicin and siTNFα/siSTAT3 inhibits the IL23/TH17 pathway, evident in the pronounced reduction of IL23, STAT3 and other prominent inflammatory biomarkers involved in the immunogenesis of Psoriasis.
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