Modelling the Impact of Mucoadhesive Formulations on Intranasal Delivery of RIG-101, a Stem-Loop RNA Therapeutic

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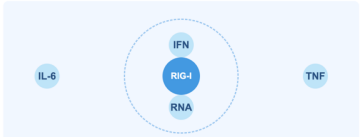
Introduction: RIG-101 Nanoemulsion for Intranasal Delivery

RIG-101: A Potent Immune Activator

RIG-101, a stem-loop RNA and potent RIG-I agonist, activates the innate immune system by stimulating the retinoic acid-inducible gene I (RIG-I) pathway[1]. This activation leads to robust production of interferons and pro-inflammatory cytokines, which are critical components of antiviral defences and immune modulation.

Therapeutic Potential: RIG-101 holds significant potential as a therapeutic candidate for combating viral infections and immune-related diseases due to its ability to induce localized and systemic immune responses[2].



RIG-I pathway activation by RIG-101 leads to production of interferons and cytokines

Challenges of Intranasal Delivery

Intranasal delivery of RIG-101 provides a non-invasive method to engage nasal epithelial and immune cells, triggering rapid, localized immune responses with minimal systemic effects. However, this approach faces several key challenges:

- **Rapid mucociliary clearance** removes therapeutics from the nasal cavity, limiting their residence time
- **Cellular uptake barriers** hinder the delivery of RNA therapeutics to their intracellular targets
- **Endosomal trapping** prevents cytosolic delivery even after cellular internalization[3]

Innovative Formulation Approach

To overcome these issues, RIG-101 is formulated as a nanoemulsion embedded in an Avicel (microcrystalline cellulose) matrix. This dual-component system addresses key limitations:

The Avicel matrix provides mucoadhesive properties that adhere to the nasal mucosa, resisting clearance and prolonging retention in the nasal cavity.

The nanoemulsion enhances cellular uptake by promoting endocytosis or membrane fusion, while facilitating endosomal escape by disrupting endosomal membranes[4].

This synergistic approach ensures sufficient RNA reaches the cytosol to activate the RIG-I pathway, maximizing therapeutic impact while retaining the advantages of intranasal delivery.

Pharmacokinetic Modeling Approach

To evaluate the impact of the mucoadhesive formulation on RIG-101 retention, absorption, and systemic exposure, we employed a physiologically based pharmacokinetic (PBPK) model. This model simulates the deposition and disposition dynamics of the formulation within the nasal cavity and systemic circulation, providing valuable insights into optimizing intranasal delivery systems for RIG-101 and similar RNA therapeutics.

Nanoemulsion Formulation

Preparation Method

- Nanoemulsion phase: 2% w/v RIG-101 in citrate buffer with non-ionic surfactant and fatty acid, homogenized at 2000 rpm for 5 min (Silverson, London, UK).
- Avicel phase: 2% w/v Avicel activated in RNase-free water at 7500 rpm for 10 min.
- Final formulation: Nanoemulsion blended with Avicel using overhead stirring for 5 min.

Final formulation z-average droplet size: 180 nm, optimized for stability and uptake efficiency.

Delivery Characteristics

Simulations based on delivery using a Mucosal Atomization Device (Teleflex MAD 110, North Carolina, USA – 250 µL delivered volume).

Parameter	Size (µm)
10%-undersize droplet	19.9
Median droplet	53.0
90%-undersize droplet	107.0

Data generated using Malvern Spraytec with automatic actuator system (Malvern Panalytical, Worcestershire, UK).

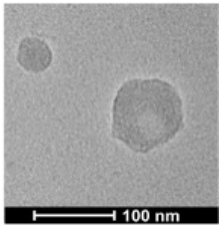


Figure 1: Cryo-TEM image of the RIG-101 nanoemulsion showing internal structure and morphology.

References

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Affiliations and Acknowledgements

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PBPK Model Framework

A physiologically based pharmacokinetic (PBPK) model was developed to simulate the deposition, retention, and absorption dynamics of RIG-101 following intranasal delivery. The model included six interconnected compartments, with drug transfer between compartments governed by first-order kinetics[5,6].

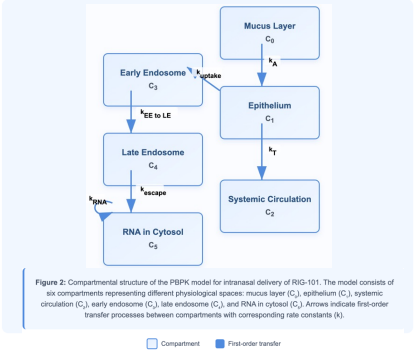

Figure 2: Compartmental structure of the PBPK model for intranasal delivery of RIG-101. The model consists of six compartments representing different physiological spaces: mucus layer (C_0), epithelium (C_1), systemic circulation (C_2), early endosome (C_3), late endosome (C_4), and RNA in cytosol (C_5). Arrows indicate first-order transfer processes between compartments with corresponding rate constants (k).

1 Mucus Layer (C_0)

$$dC_0/dt = -k_a \times C_0$$

where k_a is the clearance rate from the mucus to the epithelium.

2 Epithelium (C_1)

$$dC_1/dt = k_a \times C_0 - k_T \times C_1 - k_{uptake} \times C_1$$

where k_T is the absorption rate from the epithelium into the systemic circulation, and k_{uptake} is the rate of cellular uptake into endosomes.

3 Systemic Circulation (C_2)

$$dC_2/dt = k_T \times C_1 - k_E \times C_2$$

where k_E is the elimination rate from systemic circulation.

4 Early Endosome (C_3)

$$dC_3/dt = k_{uptake} \times C_1 - k_{EE\ to\ LE} \times C_3 - k_{escape} \times C_3$$

where $k_{EE\ to\ LE}$ is the transfer rate from early to late endosomes, and k_{escape} is the escape rate from early endosomes to the cytosol.

5 Late Endosome (C_4)

$$dC_4/dt = k_{EE\ to\ LE} \times C_3$$

where material in late endosomes is considered sequestered.

6 RNA in Cytosol (C_5)

$$dC_5/dt = k_{escape} \times C_3 - k_{RNA} \times C_5$$

where k_{RNA} is the degradation rate of RNA in the cytosol.

Simulation Parameters

Simulations were initialized with a 3.0 mg delivered dose of RIG-101. Numerical integration was performed over 100 minutes to generate concentration profiles for each compartment and calculate systemic AUC.

PBPK Simulation Results

The PBPK simulations revealed significant differences in drug retention, absorption, and intracellular delivery between the RIG-101 nanoemulsion formulations with and without Avicel (Figure 3).

Without Avicel
- Mucus layer (C_0): Rapid clearance within 2-3 hours
- Epithelium (C_1): Transient peak **~0.8 mg** at 2-5h, near zero by 15h
- Early endosome: Peak **0.25 mg** at ~5h
- Late endosome: Plateau at **0.15 mg** by 25h
- Cytosolic RNA: Low peak of **0.05 mg** at ~15h

With Avicel
- Mucus layer (C_0): Extended retention beyond 10h
- Epithelium (C_1): Higher peak **~1.0 mg** at 2-5h, **0.2 mg** at 25h
- Early endosome: Peak **0.32 mg** at ~5h
- Late endosome: Increased to **0.35 mg** by 25h
- Cytosolic RNA: Peak of **0.09 mg** at 15-20h

Compartment	Without Avicel	With Avicel	Increase
Cytosolic RNA (peak)	0.05 mg	0.09 mg	80%
Late Endosome (25h)	0.15 mg	0.35 mg	133%
Epithelium (peak)	0.8 mg	1.0 mg	25%

The Avicel-containing formulation slowed mucociliary clearance, extending residence time in the mucus layer. This facilitated greater epithelial absorption and enhanced endosomal processing, leading to **80% higher cytosolic RNA concentrations** (0.09 vs. 0.05 mg), indicating substantially improved bioavailability.

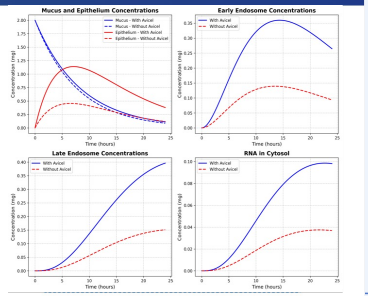

Figure 3: PBPK simulation results comparing formulations with and without Avicel, showing compartmental concentration profiles over time.

Conclusion

In conclusion, the enhanced systemic absorption observed with the mucoadhesive formulation demonstrates its therapeutic potential for RNA therapeutics. By increasing the likelihood of epithelial cell engagement, the formulation facilitates robust activation of the RIG-I pathway, crucial for immunostimulatory effects. Additionally, these findings highlight the utility of PBPK modelling as a cost-effective approach to optimize nasal drug delivery systems, reducing reliance on in vivo experimentation.