

The Impact of Permeation Enhancers on Improving the Permeability of Spray-Dried Leuprolide Formulations Using RPMI-2650 Cells

Victoria Hutter³, Khaled Alzahabi¹, Lucas Silva¹, Angelo Consalvo², Paul Shields², Joanne Kelsall³, Ewelina Hoffman³, Victoria Hutter³.

¹Nanopharm Ltd, An Aptar Pharma Company, Cwmbran, UK.

²Enteris BioPharma, Inc., Boonton, USA.

³ImmuONE, Stevenage, UK.

Summary

Leuprolide, a gonadotropin-releasing hormone (GnRH) agonist, is used to manage hormone-related conditions such as prostate cancer, endometriosis, and central precocious puberty [1,2]. Due to poor oral bioavailability resulting from first-pass metabolism in the liver, it is typically administered by injection [3]. While effective, injectable formulations are associated with adverse effects [4]. Intranasal (IN) delivery offers a non-invasive alternative with the potential for quicker systemic absorption [5].

This study evaluated the *in vitro* performance of spray-dried intranasal leuprolide formulations incorporating different permeation enhancers. Three formulations containing β -cyclodextrin, hydroxypropyl methylcellulose (HPMC), or a combination of HPMC and mannitol were compared to unprocessed leuprolide. Permeability and cytotoxicity were assessed using RPMI-2650 nasal epithelial cells. Cytotoxicity was evaluated via metabolic activity and membrane integrity assays, while permeability was measured using air–liquid interface cultures and HPLC.

All formulations, applied in solution, were well tolerated, with no significant cytotoxicity or loss of cell membrane integrity. Spray-dried formulations demonstrated significantly enhanced permeability compared to pure leuprolide ($p < 0.05$). The β -cyclodextrin formulation showed the greatest improvement, increasing apparent permeability 5.1-fold. Formulations with HPMC and HPMC/mannitol increased permeability by 4.5- and 3.7-fold, respectively.

These findings highlight the potential of spray-dried leuprolide formulations with permeation enhancers to provide effective intranasal delivery. Further *in vivo* studies are needed to confirm these results and support development of a needle-free alternative to injectable leuprolide therapies.

Key Message

Spray-dried leuprolide with β -cyclodextrin significantly improved nasal absorption ($p < 0.05$), demonstrating potential as a non-invasive alternative to injections and advancing intranasal drug delivery for hormone-related therapies.

Introduction

Effective management of hormone-related disorders often relies on controlling gonadotropin-releasing hormone (GnRH) activity [1,2]. Leuprolide is an effective therapy but has very poor oral bioavailability due to first-pass metabolism, limiting its administration to injections [3]. While injectable forms achieve therapeutic efficacy, they can cause adverse side effects and reduce convenience [4].

Intranasal (IN) drug delivery presents a promising, less invasive alternative that can bypass liver metabolism and enable faster systemic uptake [5]. However, the nasal mucosal barriers hinder drug absorption, which can be overcome by using permeation enhancers such as β -cyclodextrin to increase solubility and reduce enzymatic breakdown [6].

This research investigated spray-dried leuprolide formulations containing permeation enhancers using an *in vitro* nasal epithelial model. The aim of this study was to improve drug permeability without compromising epithelial barrier function, supporting the development of an effective IN alternative to injectable leuprolide therapies.

Materials and Methods

Formulation Design

Four formulations were assessed: three spray-dried leuprolide compositions incorporating different excipients, and one formulation of pure, unprocessed leuprolide. Composition details are shown in Table 1.

Table 1. Composition of formulations tested

Formulation	Components (% w/w)
1	Leuprolide (75), β -cyclodextrin (25)
2	Leuprolide (75), HPMC 606 (25)
3	Leuprolide (64), HPMC 606 (27), Mannitol (9)
4	Pure leuprolide (100)

Cell Culture

RPMI-2650 cells, (representing human nasal epithelial tissue from the nasal septum of a 52-year-old, male patient with squamous cell carcinoma), were cultured in Eagle's Minimum Essential Medium (EMEM) with 10 %v/v foetal bovine serum, penicillin (100 U/mL), streptomycin (100 μ g/mL), and 2 mM L-glutamine. Cultures between passages 5–25 were used for all experiments.

Cytotoxicity Assessment

Cells were seeded at 50,000 cells/well in 96-well plates and exposed to formulations (0.2–3000 μ g/mL) diluted with complete cell culture medium for 24 h. Cell viability was assessed using the PrestoBlue™ assay, and cell membrane integrity was evaluated using the CytoTox-ONE™ LDH assay, with fluorescence measured using a GloMax microplate reader (Ex/Em: 525/590 nm and 560/590 nm, respectively). Triton-X 100 was used as a positive control for loss of cell membrane integrity.

Permeability Assessment

RPMI-2650 cells were seeded on 1.13 cm² Transwell® inserts and cultured at the air-liquid interface (ALI) for 12 days. Monolayers with transepithelial electrical resistance (TEER) >75 Ω ·cm² were selected. Leuprolide formulations (1000 μ g/mL) were applied to the apical compartment, and basolateral samples were collected every 15 minutes for 90 minutes. Sodium fluorescein (5 μ M) permeability and post-exposure TEER values were used to confirm monolayer integrity. Samples were frozen at –80°C until HPLC analysis. Apparent permeability coefficients (P_{app}) were calculated using:

$$P_{app} = (dQ/dt) / (A * C_0)$$

Where dQ/dt is the rate of drug transport, A is the surface area of the insert, and C_0 is the initial donor concentration. Samples with fluorescein P_{app} >6 $\times 10^{-6}$ cm/s were excluded.

Results

Cytotoxicity

Across all concentrations that were tested, no significant ($p > 0.05$) reduction in viability was observed in any of the formulations. Metabolic activity and membrane integrity remained largely unaffected, confirming the biocompatibility of all formulations tested (Figure 1)

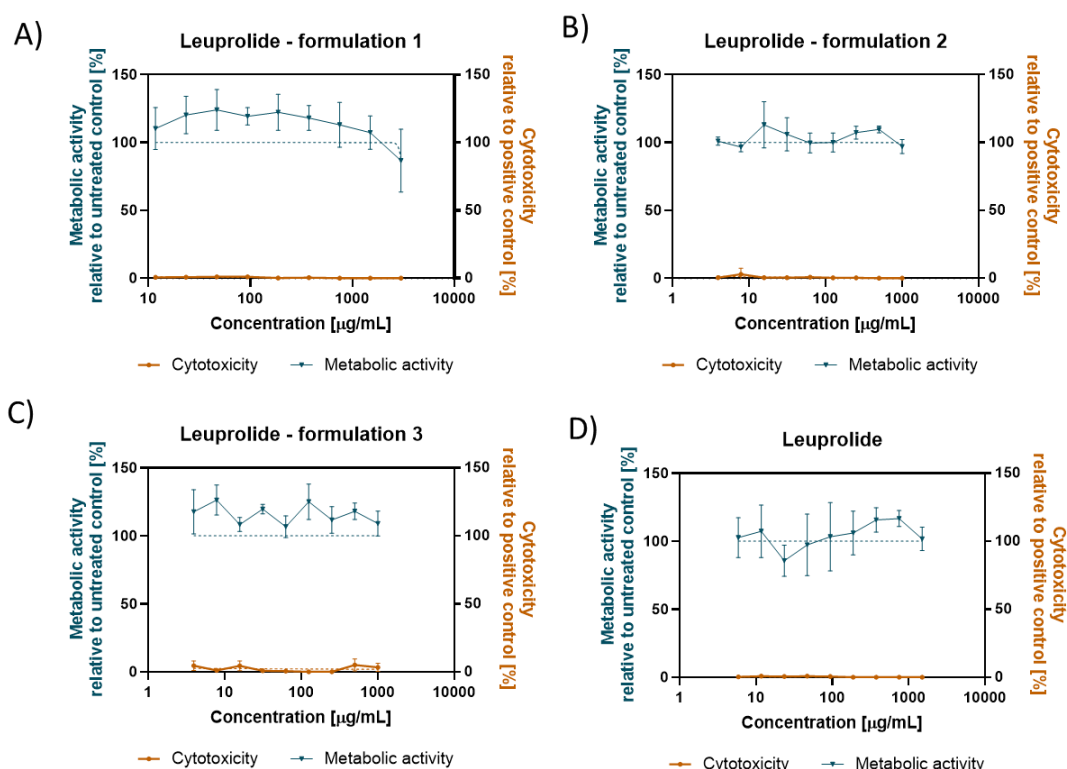


Figure 1: Acute cytotoxicity assessment of three replicates of different leuprolide formulations on RPMI-2560 cells. Dose response curves for epithelial cells (RPMI-2560) exposed for 24 h to three leuprolide formulations (A – C) and leuprolide (D). Metabolic activity (blue) was assessed using the PrestoBlue cytotoxicity assay and cell membrane integrity (gold) was assessed using the CytoTox-ONE™ LDH assay. Data is shown as mean $\pm$ standard deviation, $n=3$.

Permeability Enhancement

TEER values remained stable post-exposure, indicating preservation of epithelial barrier function throughout the assessment. All three spray-dried formulations significantly enhanced the permeability of leuprolide compared to the pure drug ($p<0.05$). Formulation 1 (β -cyclodextrin) yielded the highest P_{app} , with 5.1-fold improvement over unformulated leuprolide. Formulation 2 (HPMC 606) and Formulation 3 (HPMC and mannitol) showed 4.5- and 3.7-fold increases, respectively. These differences, while notable, were not statistically significant ($p<0.05$) between the enhanced formulations.

Table 2. Apparent permeability (P_{app}) values of leuprolide formulations. P_{app} is the mean from $n=4$ cell layers.

Formulation	$P_{app} \times 10^{-6}$ (cm/s)	Standard Deviation
Formulation 1	1.14	± 0.18
Formulation 2	1.00	± 0.13
Formulation 3	0.97	± 0.22
Pure leuprolide	0.22	± 0.07

Discussion and conclusion

Solutions of the spray-dried leuprolide formulations developed in this study, enhanced with various permeation enhancers, demonstrated a marked increase in nasal permeability *in vitro*. Notably, β -cyclodextrin showed enhanced performance compared to other excipients, indicating its strong potential to facilitate drug transport across the nasal mucosa. Importantly, none of the tested formulations

compromised the integrity of the epithelial barrier, suggesting early safety and tolerability of the spray dried formulations in solution for nasal administration use ahead of *in vivo* assessment.

These results highlight the promise of IN delivery as a viable, non-invasive alternative to conventional parenteral routes for leuprolide administration. By improving nasal absorption, these formulations may reduce the risks associated with injections.

This study demonstrates how adopting *in vitro* approaches to screen the efficacy and safety of nasal formulations early in the development phases supports maximising the therapeutic efficacy and success. In conclusion, this study lays a solid foundation for the development of intranasal leuprolide therapies and supports continued research toward clinical translation of this drug delivery approach.

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