Formulation Development and Characterisation of Insulin Nanomicelle For Intranasal Administration

Chun Yuen Jerry Wong[1], Tomas Andersen[2], Irene Moroni[2], Michelle Quezada[2], Julie Suman[3], Luis Vitetta[2,4], Jeremy Henson[2], Hui Xin Ong[1,5] & Daniela Traini[1,5].

1. Woolcock Institute of Medical Research, Sydney, Australia, 2. Medlab Clinical, Sydney, Australia, 3. Aptar Pharma, Congers, NY, United States, 4. Faculty of Medicine and Health, University of Sydney, Sydney, Australia, 5. Faculty of Medicine and Health Sciences, Macquarie Medical School, Macquarie University, Sydney, Australia

INTRODUCTION

- Therapeutic proteins are highly unstable and susceptible to mechanical stress, chemical and enzymatic degradation *via* oral administration [1].
- To date, protein therapy has been delivered mostly *via* the subcutaneous route, which can cause unwanted side effects (e.g., skin necrosis, nerve pain, injured capillaries, and topical infection) [2].
- Protein administration *via* a non-invasive intranasal route can circumvent the side effects of injection therapy, improving their acceptability and compliance with treatment.

AIMS & OBJECTIVES

- To evaluate the physicochemical properties, cytotoxicity and effect on cells' monolayer integrity of an intranasal insulin MC-1006 that is a formulation of insulin in nanosized micelles.

METHODS

- **Formulation Preparation**. Insulin nanomicelle MC-1006 was provided by Medlab Clinical Ltd, Sydney, Australia.
- **Physicochemical Characterisation**. To determine the particle size, polydispersity index (PDI) and zeta potential of the formulations, containing blank nanomicelle and insulin nanomicelle (pH 2.5 and pH 4.8), using the dynamic light scattering.
- **Laser Diffraction Analysis.** To evaluate the droplet size of the nasal spray from the 3 formulations.
- ***In Vitro* Cytotoxicity.** To assess the toxicity profile of the nanomicelle formulations on RPMI-2650 cells, a nasal septum cell line (5×10^4 cells/well), by a CellTiter 96® AQueous One Solution Cell Proliferation Assay.
- **TEER Measurement.** To determine the integrity of RPMI-2650 cells' monolayer prior to its exposure to the formulations and 4h after exposure using an EVOM volt ohmmeter.
- **Stability Study.** To understand the effect of storage conditions on the size, PDI and zeta potential of nanomicelle.

RESULTS

- Blank nanomicelle demonstrated comparable physicochemical properties as insulin nanomicelle prepared at pH 2.5 and pH 4.8 conditions **(Table 1)**.

Formulation(s)	Z-average (nm)	PDI	Zeta potential (mV)
Blank nanomicelle	24.0 ± 0.1	0.4 ± 0.0	0.2 ± 0.0
Insulin nanomicelle (pH 2.5)	20.8 ± 0.2	0.3 ± 0.0	3.9 ± 0.5
Insulin nanomicelle (pH 4.8)	17.8 ± 0.0	0.1 ± 0.1	0.8 ± 0.2

Table 1. Z-average, PDI and zeta potential of the prepared nanomicelle (mean ± SD, n = 3).

- Dv_{50} values for the insulin nanomicelle (both at pH 2.5 and 4.8) formulations were significantly smaller than for the blank nanomicelle ($p < 0.05$) **(Table 2)**.

Formulation(s)	Span	Dv_{10} (µm)	Dv_{50} (µm)	Dv_{90} (µm)
Blank nanomicelle	1.7 ± 0.1	35 ± 1	96 ± 1	197 ± 4
Insulin nanomicelle (pH 2.5)	1.6 ± 0.2	25 ± 2	53 ± 3	111 ± 1
Insulin nanomicelle (pH 4.8)	1.6 ± 0.0	16 ± 1	36 ± 1	74 ± 2

Table 2. Aerosolisation property of nanomicelle droplet from VP7 nasal device.

- Insulin nanomicelle at pH 4.8 demonstrated significantly higher cell viability (93.06 ± 1.58%) than insulin nanomicelle at pH 2.5 (86.47 ± 2.35 %) at an equivalent concentration ($p < 0.05$).

CYTOTOXICITY ASSAY WITH RPMI-2650 CELLS

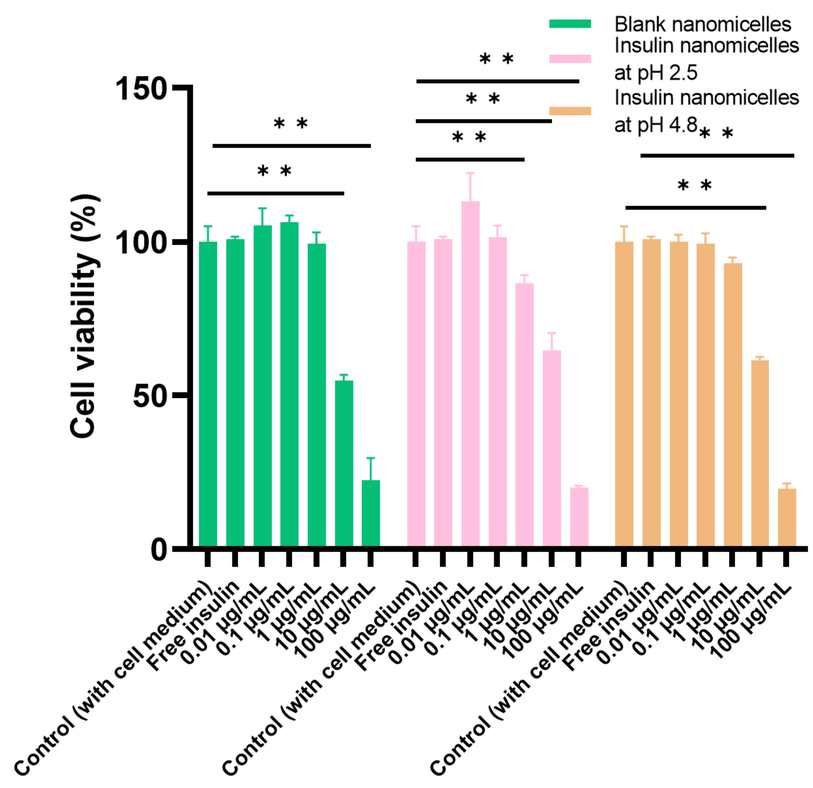

Figure 1. Cytotoxicity of insulin nanomicelle using MTS assay. Nanomicelle formulations up to 1 µg/mL showed comparable cell viability to control and free insulin (100 µg/mL).

- Blank nanomicelle and insulin nanomicelle at pH 4.8 had no adverse effect on the viability and tight junctions of nasal epithelial cells.

TEER VALUES OF RPMI-2650 CELLS

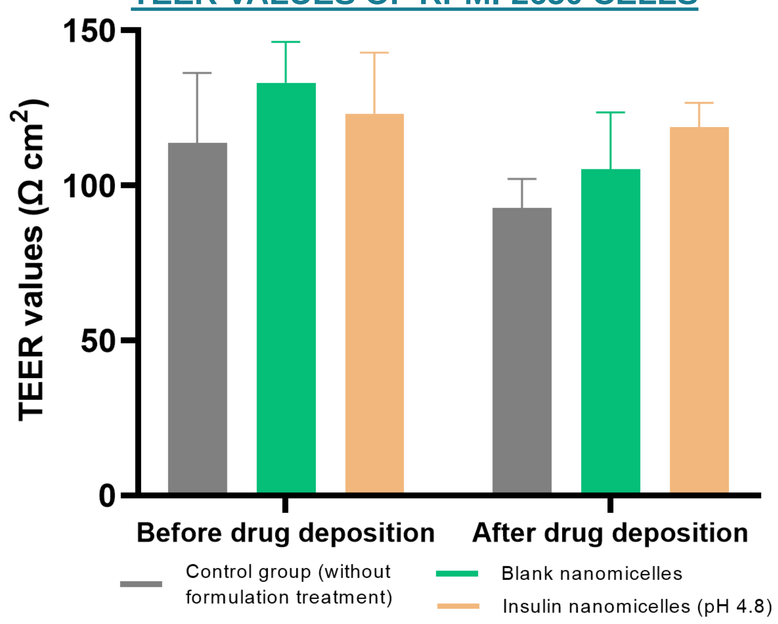

Figure 2. Characterization of cells' monolayer integrity before and after exposure to nanomicelle formulations.

- Insulin nanomicelle at pH 4.8 showed stability when stored at both room temperature and refrigerated conditions (2-5 ^oC).

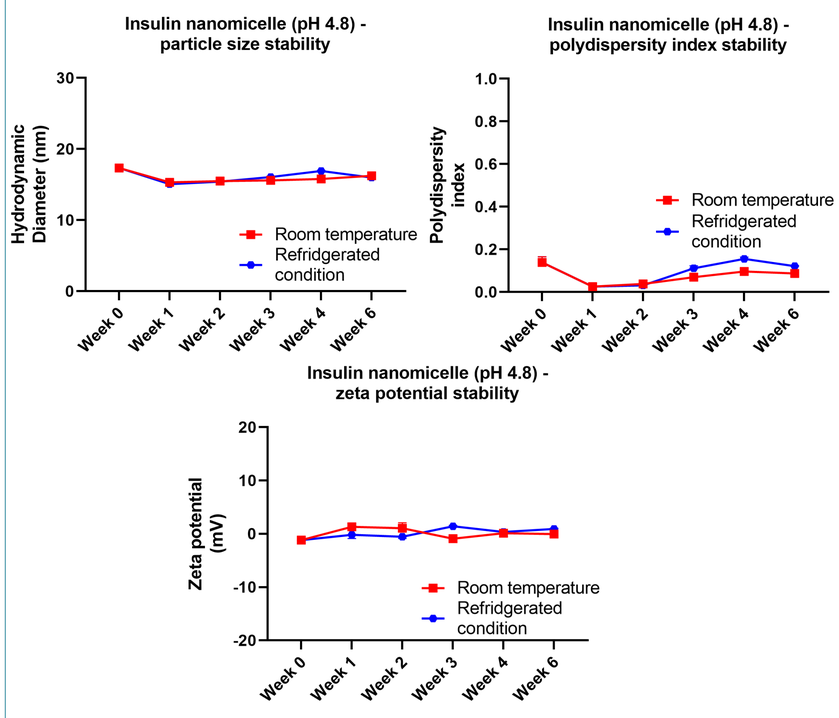

Figure 3. Stability of insulin nanomicelle at room temperature and refrigerated condition.

CONCLUSION

- Insulin nanomicelle at pH 4.8 demonstrated promising physiochemical properties, showed no cytotoxicity at concentrations up to 1 µg/ml and did not disrupt the integrity of the cells' monolayer.
- Further *in vivo* assessment is required to examine the potential of insulin nanomicelle and verify its hypoglycaemic effect on diabetes management.
- Data for the clinical efficacy, safety and tolerability of formulation are warranted.

REFERENCES

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