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New insights into the activity of Anle138b from in vitro models of Parkinson's disease

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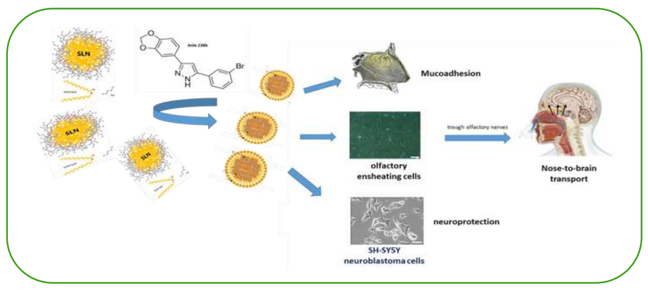
Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder marked by dopaminergic neuron loss and dopamine deficiency. While Levodopa remains the primary treatment, its long-term use leads to motor complications and fails to halt disease progression. Recent research focuses on alpha-synuclein (α-syn) aggregation, a key pathological mechanism. Anle138b, a promising small molecule inhibitor of α-syn and prion protein oligomerization, can cross the Blood-Brain Barrier (BBB), but its poor water solubility limits formulation, reducing bioavailability. Intranasal administration is a promising alternative, bypassing the BBB via the olfactory mucosa for faster brain access and fewer systemic effects.



Aim of research

Anle138b, a promising therapeutic candidate for geriatric use in Parkinson's disease (PD), is hindered by its poor aqueous solubility, which markedly reduces its bioavailability (Antonschmidt et al., 2022). The development of effective water-dispersible delivery systems therefore represents a critical challenge in advancing its clinical application. In this context, solid lipid nanoparticles (SLNs) offer a valuable strategy, as they enable the incorporation of poorly soluble molecules into stable, dispersible formulations suitable for therapeutic use. The present study investigated novel SLN-based formulations containing Anle138b, developed in combination with nutraceutical components, with the aim of improving its delivery profile. Particular attention was given to the in vitro assessment of their stability under different storage conditions, in order to evaluate their potential as reliable candidates for future therapeutic applications in PD.

Materials and methods

Human nasal RPMI 2650 cells, neuronal SH-SY5Y cells and OEC cells were cultured in 96-well plates and exposed to different formulations, namely Anle138b, Anle138b-loaded solid lipid nanoparticles (SLNs), and unloaded SLNs. Triton X-100 was used as a positive control for cytotoxicity.

Cell viability was evaluated by the Sulphorhodamine B (SRB) assay according to Skehan et al. (1990). After treatment, cells were fixed and stained with SRB, unbound dye was removed by repeated washing with acetic acid, and plates were left to air-dry. Protein-bound dye was then solubilized in Tris buffer and absorbance was measured at 492 nm using a Perkin-Elmer UV–Vis photometer. Blank values (background wells) were subtracted from each reading, and cell survival was calculated as the absorbance ratio of treated versus untreated cells. Data are expressed as means ± S.D. from eight replicates per plate, in three independent experiments.

Neuronal differentiation of SH-SY5Y was induced as according to Greco et al. (2021). For neuronal differentiation, SH-SY5Y cells were seeded at a 3 × 10^4 cells/cm^2 and differentiated with 10 µM retinoic acid (RA) and 2% FBS for 6 days, followed by 10 µM RA and 1% FBS for 3 days. For the final 5 days, cultures were maintained in Neurobasal medium supplemented with 50 ng/mL BDNF and 1× B27. Differentiated cells were then exposed for 24h to different Anle 138b, Anle138b SLNs and plain SLNs. Plain SLNs were used as controls, with their volumes adjusted to match the lipid content of the loaded formulations.

Cytoprotection was evaluated by the MTT assay according to Nozhat et al (2022). Cells were seeded in 96-well plates (Becton Dickinson, USA) and allowed to adhere for 24h. The medium was then replaced with fresh medium containing 0.5 mg/mL MTT (Sigma), and cells were incubated for 1h. Viable cells metabolically reduced MTT to insoluble formazan crystals, which were solubilized by adding dimethyl sulfoxide (DMSO). Absorbance was measured at 595 nm using a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, USA), with background correction at 620 nm. Data were normalized to untreated controls (CTR, set at 100%), while 0.1% Triton X-100 served as a positive control.

Results

When SRB test was applied for assessing cytocompatibility in human nasal RPMI 2650 cells, neuronal SH-SY5Y and differentiated neuronal SH-SY5Y cells, then full viability was observed in all tested cell model lines. Both cell types underwent a 24h treatment with various formulations of Anle138b, Anle138b SLNs and plain SLNs and the chosen concentrations for testing Anle138b were 0.1, 1, 10 and 100 µM (**Figure 1**).

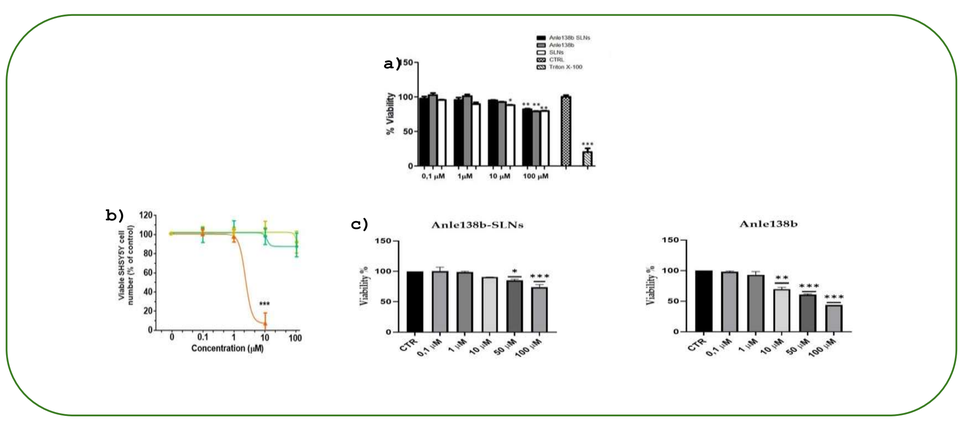


Figure 1:Cell viability in presence of Anle 138b-SLN assessed by SRB in **a)** RPMI cell line; **b)** SH-SY5Y neuronal cells; **c)** Differentiated SH-SY5Y. RPMI 2650 cells, SH-SY5Y neuronal cells and differentiated SH-SY5Y were challenged with free Anle138b, Anle138b SLNs, plain SLNs for 24h at four different concentrations of the drug. Cells were then assayed for vitality using the SRB method. Control (CTRL) cells were untreated cells (100% of vitality), whereas TX-100 (0.1% Triton X-100) denotes positive controls. Data are expressed as the average ± SD of two experiments carried out each in six wells. *** p < 0.001, **p < 0.01. *p < 0.05 for the different treatments vs. untreated.

To evaluate the cytoprotective potential of SLNs, Rotenone was used to induce strong intracellular stress resembling some of the pathophysiological conditions of PD. Differentiated SH-SY5Y cells were incubated with 1 µM Anle138b-SLNs and Anle138b for 24h, and then with 5 µM Rotenone for following 24h. MTT assay results demonstrated that all SLN preparations markedly attenuated rotenone-induced toxicity. In contrast, rotenone alone reduced cell viability by nearly 50% compared to untreated controls and co-treated groups (**Figure 2**).

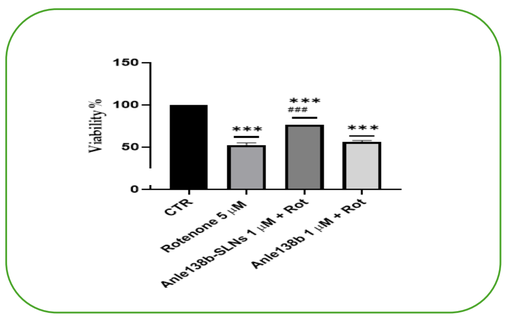


Figure 2. Exposure of Anle138b (free or in the SLNs) to Rotenone. The data are means ± S.D. of two different experiments run in nine replicates and are presented as percent of control. Values were compared by one-way ANOVA following Dunnet test. ***p < 0.0001 in comparison to CTR; ###p< 0.0001 in comparison to Rotenone.

A further advancement in the design of the SLN system consisted in the combination with a multicomponent nutraceutical product (already marketed), intended for support therapy in Parkinson's disease, *i.e.*, DMG-Gold. Again, SH-SY5Y cell viability was maintained close to 100%, irrespectively of the presence of DMG-Gold (**Figure 3**).

DMG-GOLD is a food supplement based on inositol, dimethylglycine and trimethylglycine enhanced by the presence of group B vitamins.

Average quantity of characterizing ingredients	Per Serving daily (6 ml)	*NRV%
Dimethylglycine	396 mg	
Inositol	360 mg	
Betaine	108 mg	
Niacin	16 mg	100
Vitamin B6	1,4 mg	100
Vitamin B2	1,4 mg	100
Vitamin B1	1,1 mg	100
Sweet orange EO	1 mg	
Folate	200 mcg	100
Vitamin B12	2,5 mcg	100

*NRV: nutrient daily reference values for vitamins and minerals for an average adult - Reg. EU 1169/2011

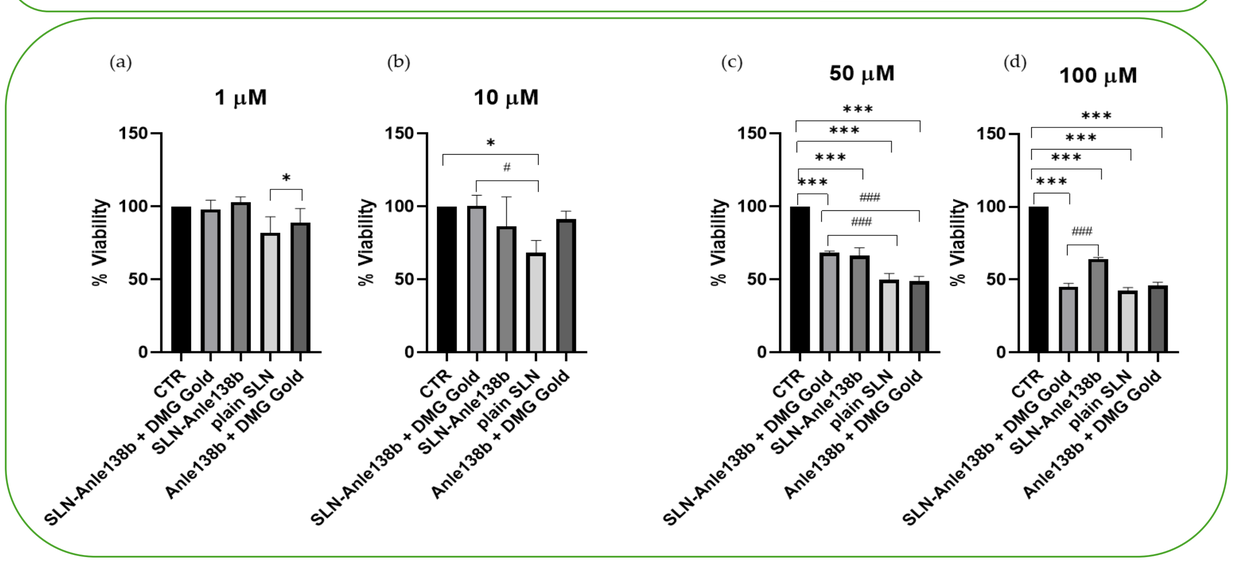


Figure 3. Viability of differentiated SH-SY5Y cells was evaluated by MTT assay after treatment with different concentrations (a) 1, (b) 10, (c) 50 and (d) 100 µM for 24h. The data are means ± SD. of three different experiments and are presented as percent of control. Values were compared by one-way ANOVA following Dunnet test. * p < 0.05, ** p < 0.01, *** p < 0.0001 vs CTR; # p < 0.05, ### p < 0.0001 vs SLN-Anle 138b-DMG Gold.

In parallel with cellular studies, a microfluidic Lab-on-a-Chip (LoC) platform was investigated as a diagnostic tool, designed to operate on a very small scale with minimal numbers of cells and reagents, thereby reducing both costs and time of investigations. The research aimed to develop a LoC system for Parkinson's disease diagnosis using Olfactory Ensheathing Cells (OECs) as the neuronal cell model.

Optimal adhesion was achieved with polymethylmethacrylate/polycarbonate (PMMA/PC) or polymethylmethacrylate/polystyrene (PMMA/PS) substrates coated with Matrigel (4 mg/mL), ensuring OEC viability for up to 24h. Enhanced performance was obtained with devices fabricated by bonding a laser-drilled PMMA substrate to an untreated PMMA layer, which also maintained OEC viability up to 24h **(Figure 4)**.

Figure 4: Representative micrographs of OECs cultured for 24h on LoC devices coated with 4 mg/mL Matrigel. OECs adhered for 24h in LoC channels coated with 4 mg/mL Matrigel, with the solvent layer promoting uniform cell distribution.

Conclusions

This study confirmed the feasibility of using solid lipid nanoparticles (SLNs) to deliver Anle 138b for Parkinson's disease. The formulations proved biocompatible and non-toxic in both nasal and neuronal cell lines, supporting their suitability for therapeutic use. Candidate nutraceutical–SLN combinations tested for intranasal administration further underscored the potential of a multicomponent, non-invasive strategy for PD. Future work will focus on optimizing delivery through microfluidic LoC–models, exploring OEC and their interactions with drug-loaded nanoparticles, to advance innovative formulations toward clinical application.

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