BNN27 NANOFORMULATIONS FOR NOSE-TO-BRAIN DELIVERY

M. Kannavou [1,2 *], K. Karali [3,4], T. Katsila [5], E. Siapi [5], A. Marazioti [2], P. Klepetsanis [1,2], T. Calogeropoulou [5], I. Charalampopoulos [3,4] and S. G. Antimisiaris [1,2]

[1]Laboratory of Pharmaceutical Technology, Department of Pharmacy, University of Patras, Rio 26510, Greece

[2]Institute of Chemical Engineering, FORTH/ICE-HT, Rio 26504 Patras, Greece

[3]Department of Pharmacology, Medical School, University of Crete, 71003 Heraklion, Greece

[4]Institute of Molecular Biology & Biotechnology (IMBB), Foundation for Research and Technology-Hellas (FORTH), 70013 Heraklion, Greece

[5]Institute of Chemical Biology, National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, 11635 Athens, Greece

* Corresponding author: M. Kannavou, email: mariakannavou@gmail.com

INTRODUCTION

The treatment of neurodegenerative diseases is still an unmet medical need. Small, lipophilic molecules with strong neuroprotective properties, such as BNN27 (DHEA derivative), represent new non-toxic, synthetic compounds for therapeutic applications in brain diseases [1]. Their low solubility and targeted transport to the brain are challenges for optimization of their clinical application.

Intranasal delivery offers an alternative and promising way for brain targeting. In this project, we studied the design of two innovative mucoadhesive nanoformulations for intranasal delivery of BNN27: i) negatively-charged liposomes (LIP) coated with chitosan (CHT) and ii) mucoadhesive nanoemulsions (BNE) with CHT.

METHODS

BNN27-loaded LIP with lipid composition PC or PC/PG 9:1 mol/mol were prepared with thin film hydration method. LIP were coated with CHT by dropwise addition of CHT solution in the liposome dispersion. BNE were prepared by the spontaneous emulsification method using Capmul MCM as the oily phase, Tween 80 as surfactant and Transcutol:Propylene Glycol (1:1) as co-surfactants, according to solubility studies [2]. Size distribution and ζ-potential were measured by dynamic light scattering (DLS) and laser Doppler electrophoresis (LDE), respectively.

For the monolayer studies, hCMEC/D3 cells were seeded on Transwell filters, pre-coated with type I collagen [3] and Rhodamine (Rho)-labeled nanoformulations were used.

For the in vivo study in mice, 25 μL/mouse of each BNN27-formulation was administered into each nostril. After sacrifice, brains were quickly collected and frozen. BNN27 quantification were performed with HPLC-MS [2].

RESULTS

After preformulation studies [2], medium MW CHT-coated PC/PG/BNN27 liposomes at 0.1 w/w CHT/lipid ratio demonstrated highest coating efficiency (table 1) and mucoadhesive properties (Figure 1a). Furthermore, BNEs with 8% w/w or 10% w/w Capmul MCM and 0.3% w/w CHT, displayed optimal physicochemical characteristics, compared to BNEs with Carbopol (CAR) as mucoadhesive agent (Figure 1b). The optimal BNN27 nanoformulations were evaluated for their permeability across a cellular BBB model (hCMEC/D3 cell monolayers) *in vitro* (Figure 2). The translocation of NE-associated Rho and the corresponding permeability values were significantly higher than the corresponding values of LIPs. The *in vivo* fate of the innovative nanoformulations after intranasal administration in mice revealed the superiority of BNE with CHT for more efficient distribution of BNN27 to the brain, compared to liposomal BNN27 (Figure 3).

Table 1: Physicochemical properties of the CHT-coated LIPs. Effect of lipid membrane composition and CHT type and amount.

Lipos	CHT MW-CHT/LIP (*w/w*)	Mean Diameter (nm)	PDI	ζ-Potential (mV)	Coating Efficiency (%)
PC	Non-coated	78.1 ± 1.15	0.281	−3.48 ± 0.49	-
	Low-0.1	123.7 ± 2.17	0.398	3.65 ± 0.27	3.5 ± 0.2
	Med.-0.1	158.8 ± 6.27	0.405	4.57 ± 0.18	4.8 ± 0.6
PC/PG	Non-coated	83.01 ± 1.36	0.263	−12.4 ± 0.689	-
	Low-0.1	822 ± 21.54	0.452	15.2 ± 0.47	79.9 ± 1.5
	Med.-0.1	1147 ± 86.27	0.447	23.1 ± 1.41	85.1 ± 4.5
	Med.-0.3	2563.5 ± 109.6	0.592	24.4 ± 1.273	78.2 ± 0.7
	Med.-0.5	5155 ± 51.24	0.617	29.4 ± 0.689	81.4 ± 1.8

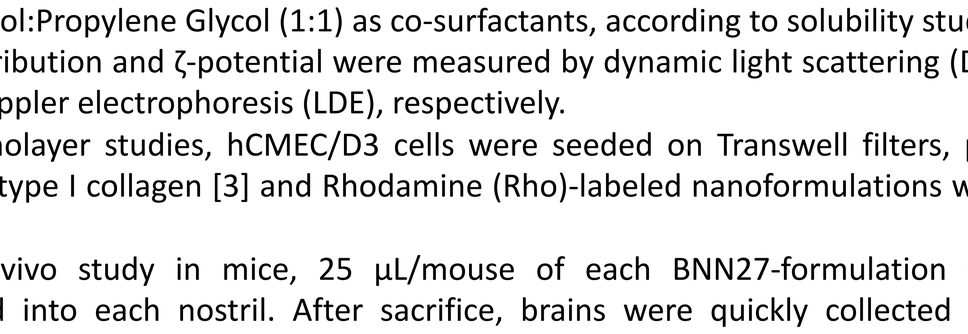

Figure 2: Mucoadhesive properties (expressed as the capability to adsorb mucin (% adsorbed) of: (**a**) CHT-coated LIPs. (**b**) CHT- or CAR-coated NEs. Non-coated formulations were evaluated under identical conditions (in all cases) for comparison.

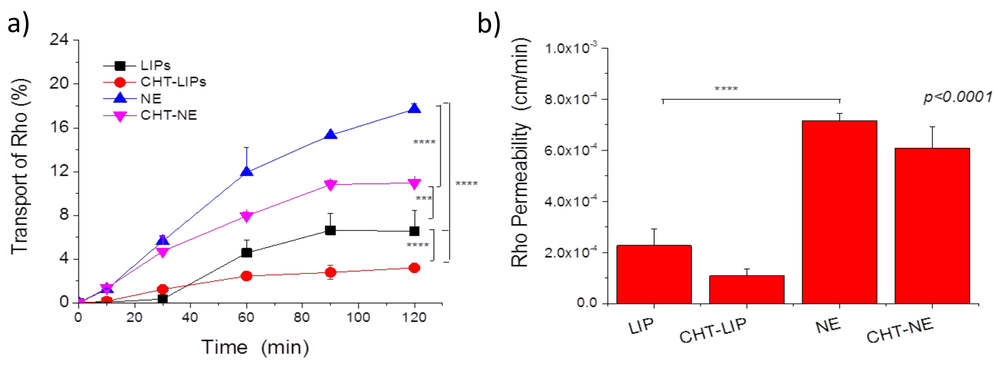

Figure 2: a) Transport of formulation- associated Rho (%) across the monolayer with the time (following incubation with LIP and NE formulations at 0.2 μM Rho concentration). b) Permeability values of formulations (formulation-associated RHO permeability) across the hCMEC/D3 monolayer (calcuculated from the results of Figure 1a).

a)

Samples	Size (nm)	PDI	ζ-potential (mV)
Lip	82.6 ± 1.2	0.240	-14.7 ± 1.2
CHT-Lip	1238 ± 78	0.420	20.6 ± 0.6
BNE	93.44 ± 2.3	0.291	-3.3 ± 0.4
CHT-BNE	150.4 ± 1.8	0.366	7.5 ± 1.8

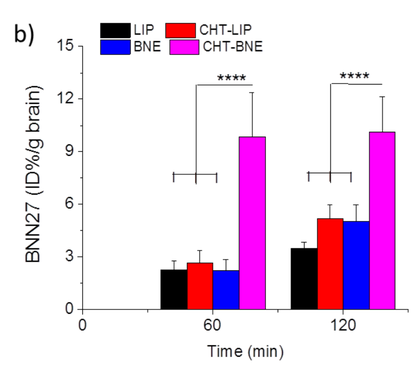

Figure 3: a) Physicochemical properties of the formulations used in the in vivo disposition study. b) Brain disposition of BNN27 1 and 2 h post-intranasal administration of the BNN27 loaded liposomal and NE formulations.

CONCLUSIONS

CHT-BNE formulation developed herein was demonstrated to confer faster and higher nose-to-brain delivery of BNN27 compared to CHT-LIPs. Such NEs could be considered as alternative systems for the brain delivery of lipophilic drugs following intranasal administration. Nevertheless, extended biocompatibility and toxicity studies are required to exclude any potential toxicity issues in relation to the cytotoxicity differences between the LIPs and NEs above-mentioned due to the high surfactant content of NEs.

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