Design of novel cationic liposomes for brain delivery

Francesca Ceccacci [a], Cecilia Bombelli [a], Giovanna Mancini,[b], Beatrice Simonis,[c], Luciano Galantini,[c] Raya Syeda [c], Enrica Donati [b], Stefano Aiello[c], Antonella Cellucci[d], Ivan Fini[d], Domenico Vignone[d], Elena Bracacel[e], Maria Veneziano[e], Odalys Gonzalez Paz[d] ,Annalise Di Marco[d]

[a] CNR-ISB-Sezione Meccanismi di Reazione-UOS di Roma c/o Dipartimento di Chimica, Sapienza Università di Roma, 00185
[b] CNR-ISB, Area della Ricerca di RM1, 00015 Montelibretti (RM)
[c] Dipartimento di Chimica, Sapienza Università di Roma, 00185
[d] IRBM SpA, High Content Biology & Screening Unit, Pomezia, Rome 00071
[e]IRBM SpA, DMPK Department, Pomezia, Rome 00071
e-mail: francesca.ceccacci@uniroma1.it

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With the aging of world population, disorders of central nervous system (CNS) have become a major health problem. Although new neuropharmaceuticals have the potential for treating specific CNS diseases, the treatment is often complicated by the drug inability to cross the blood brain barrier (BBB); in fact, BBB prevents therapeutics from reaching the CNS (>98% of low- molecular-weight drugs and almost 100% of large therapeutics do not cross the BBB). Hence, despite the high prevalence and socio-economic impact of CNS diseases, their treatment is still an unmet need [1].
Being the delivery of the drugs rather than their efficacy the crucial problem in the treatment of CNS diseases, the development of suitable nanocarriers able to cross the BBB and release the drug to the injured districts is a primary objective. In the context of nanocarriers, liposomes represent ideal drug delivery systems because their features can be easily and properly tuned, they are usually characterized by low toxicity and are easy to prepare.
The aim of our work is to develop novel cationic liposomes tailored i) to load high amounts of therapeutics of known efficacy in the treatment of neurodegenerative diseases, ii) to cross the BBB and iii) deliver their cargo to the CNS.
In order to allow liposomes to cross BBB, they need to be functionalized with targeting moieties able to trigger one among the various mechanisms of transcytosis involved in BBB crossing. [2]
Therefore, we designed cationic liposomes, composed of a phosphocoline (DPPC) and a cationic gemini amphiphile (SS or MESO, Figure 1) [3], the presence of a cationic amphiphile should in fact favour the interaction with BBB and the crossing *via absorptive-mediated transcytosis*.
The new formulations were characterized in terms of size, polidispersity, and stability. Then, the formulations showing appropriate size and stability (figure 1) were investigated for their ability to interact with culture cells of brain tissues and of BBB.
The formulations were first tested in a primary porcine brain endothelial monolayer experiment (figure 2) Liposomes were marked with NBD-PE (figure 1) to allow for a comparison of the internalization of the different formulations. Results indicate that the uptake is higher for formulation **2** containing gemini amphiphile SS (figure 2).
Thus, the ability of this formulation to cross the BBB was assessed in a permeability experiment, using an in vitro BBB model.

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Formulation	Compositions
1	DPPC : CHOL : NBD-PE 4.0mM : 1.0mM : 15µM
2	DPPC : CHOL : SS : NBD-PE 2.8mM : 1.0mM : 1.2mM : 15µM
3	DPPC : CHOL : SS : mPEG-DSPE : NBD-PE 2.8mM : 1.0mM : 1.2mM : 0.1mM : 15µM
4	DPPC : CHOL : MESO : NBD-PE 2.8mM : 1.0mM : 1.2mM : 15µM

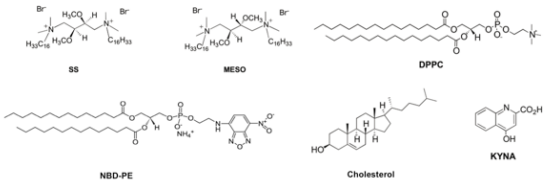

Figure 1. Compositions of liposome formulations in HBSS/HEPES buffer.

For this purpose formulation **2** was loaded with kynurenic acid (KYNA, figure 1), used as a probe, and the permeability of the formulation was compared to that of free KYNA.
The permeability of KYNA encapsulated in the formulations was significantly higher than the permeability of free KYNA, thus suggesting the ability of the liposome to cross the BBB.

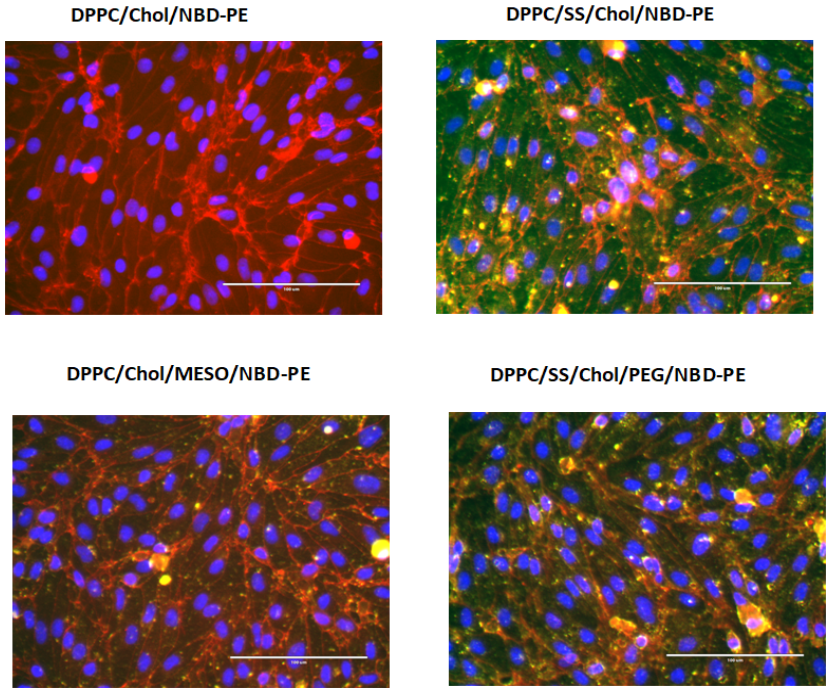

Figure 2. Uptake of liposomes (NBD-green fluorescence) by primary porcine brain endothelial monolayer. Tight junction protein ZO-1 (red fluorescence) staining showed delineated cell-cell contacts. (EVOS FL Cell Imaging System, 40x). Bars correspond to 100 µm.