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Stereochemistry of the gemini surfactant influences the final fate of cationic liposomes in human tumor cells

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Our previous studies demonstrated that the presence of cationic gemini surfactant (S,S)-2,3-dimethoxy-1,4-bis(N-hexadecyl- N,N-dimethylammonio)butane bromide **1a** in the liposomes formulated with dimyristoyl-*sn*-glycero-phosphatidylcholine, DMPC, increases the cell uptake of the photosensitizer *m*-tetrahydroxyphenylchlorin, *m*-THPC [1, 2], in human colon adenocarcinoma cells [1], murine and human glioblastoma cells [2]. In order to explore the influence of the stereochemistry of the gemini surfactants 1 on the delivery efficiency of *m*-THPC to malignant glioma (GBM) cell lines, liposomes were formulated with DMPC, and either cationic gemini surfactant **1a**, or (S,R)-2,3-dimethoxy-1,4-bis(N-hexadecyl-N,N-dimethylammonio) butane bromide (**1b**).

The stereochemistry of the spacer of the gemini was found to strongly influence the delivery efficiency of *m*-THPC to cells and its intracellular distribution. Unexpectedly, the formulation featuring a higher surface potential and a greater stability toward fusion (DMPC/**1a**), was found to be less efficient in the delivery of the photosensitizer.

Since the liposome internalization pathway depends both on the size and nature of the carrier, we investigated the influence of the stereochemistry of the surfactant on the interaction with cell membranes and the intracellular route of liposomes. In a first approach, a combination of different inhibitors was used to selectively block different pathways (Figure 1a). These effects were evaluated by flow cytometry on human (LN229) and murine (C6) glioblastoma cells. The analysis performed revealed that both DMPC/**1a** and DMPC/**1b** followed the endocytic pathway but that the two formulations were influenced by different endocytic inhibitors (Figure 1b). The results strongly suggest that DMPC/**1a** enter into the cells preferentially through the interaction with rafts and caveolae as demostrated by the filipin-indiced inhibition. On the other hand, DMPC/**1b** seems to enter into the cells preferentially upon interaction with clathrin, as showed by the inhibition exerted by chlorpromazine.

In the second approach, several antibodies specific for organelles involved in endocytic routes were employed. The observations performed by laser scanning confocal microscopy (LSCM) on cells treated with DMPC/**1a** or DMPC/**1b** confirmed data obtained by flow cytometry. In addition, it demonstrated that DMPC/**1a** colocalized preferentially with early endosomes (Rab5+), whereas DMPC/**1b** were found in early and late endosomes (Rab7+) and in lysomomes (Lamp1+).

Finally, in order to study at higher resolution the interaction with plasma membrane a comparative study on either ultrathin sectioned or freeze-fractured samples was carried out by TEM. The observations on both freeze fractured and resin embedded sample (Figure 2), confirmed the intracellular transport of liposomes mediated by cytoplasmic organells, as revealed by LSCM.

These results demonstrated that the different mode of interaction results in a different subcellular fate of the two liposome formulations. After binding to the plasma membrane, DMPC/G**1a** liposomes are internalized trough caveolae-coated vesicles. Then, they are preferentially transferred to early endosomes . On the other hand, clathrin-mediated endocytosis of DMPC/G**1b** leads to the formation of an early endosome, which is acidified and fuses with prelysosomal vesicles containing enzymes to give rise to a late endosome and, finally, a lysosome, an acidic and enzyme-rich environment.

1. C. Bombelli et al., J Med Chem 48 (2005), p. 4882.
2. A. Molinari et al., Int J Cancer 121 (2007), p. 1149.

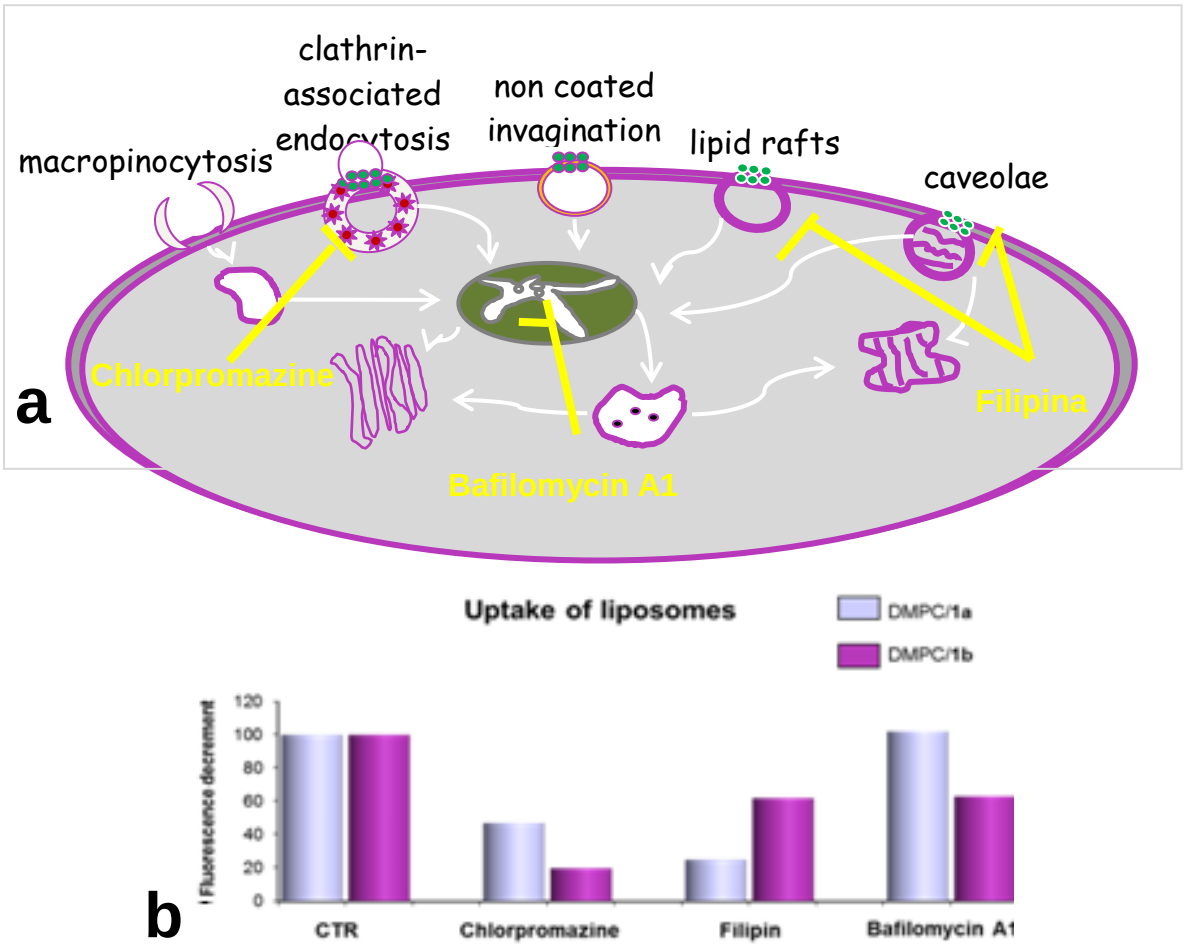

Figure 1. Uptake of DMPC/1a and DMPC/1b liposomes evaluated by flow cytometry in LN229 cells, before and after the treatment with endocytic inhibitors

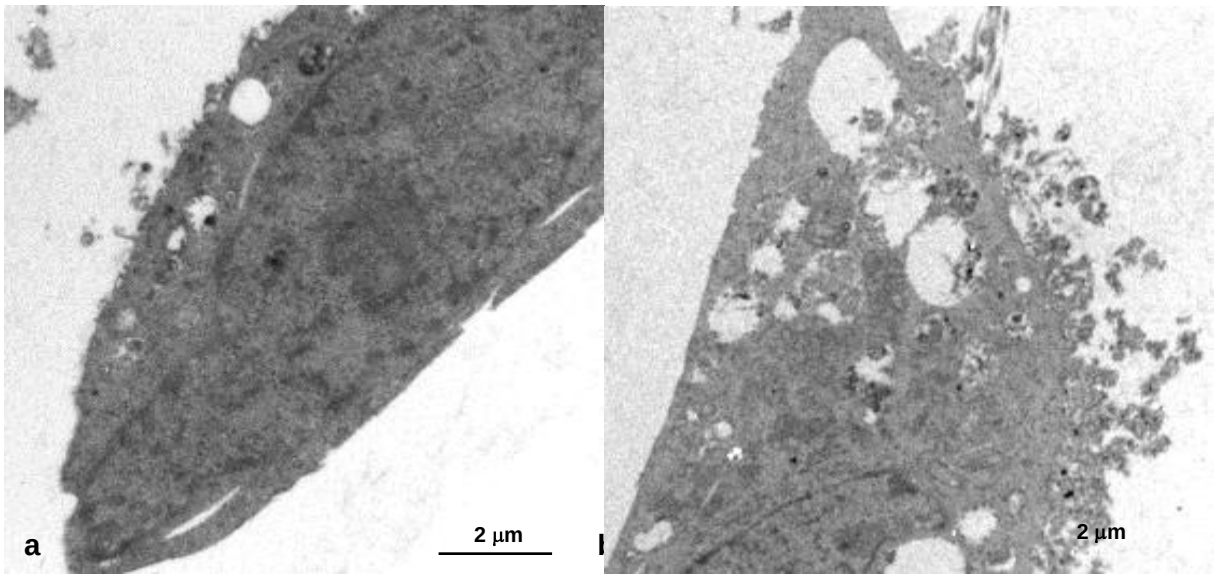

Figure 2. Human glioblastoma LN229 cells interacting with DMPC/a (a) and DMPC/b (b). After 18 h of incubation, DMPC/b liposomes (b) showed to be significantly more numerous on the apical side of LN229 cell, when compared to DMPC/a. In addition, after the interaction with DMPC/b the cytoplasm of glioblastoma cells displayed a number of cytoplasmic organelles (endosomes and lysosomes) (b) higher than cytoplasm of cells treated with DMPC/a formulation.