Cationic liposomes for photodynamic therapy of malignant gliomas: a light and electron microscopy study

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Malignant gliomas represent the most common primary brain tumor: more than 50% of them are Glioblastoma Multiforme (GBM). Photodynamic therapy (PDT) may offer a very good chance of targeted destruction of infiltrating GBM cells, thus increasing the survival time and recurrence-free interval of GBM patients. Among photosensitizing agents, meta-tetrahydroxyphenylchlorin (*m*-THPC) is promising for the PDT of brain tumors. In this study we investigated the transfection activity of dimyristoyl-sn-glycerophosphatidylcholine (DMPC) liposomes, containing the cationic gemini surfactant (S,S)-2,3-dimethoxy-1,4-bis(N-hexadecyl-N,N-dimethyl-ammonio)butane bromide (G1), loaded with *m*-THPC on human (A172, U118, DBTRG, LN229) and murine glioblastoma cell lines (C6).

In order to evaluate the delivery efficiency, the uptake (flow cytometry) and the intracellular distribution (confocal microscopy) of m-THPC, loaded in several formulations of cationic liposomes, were analyzed by making a comparison with those obtained by employing the same chlorin in the pharmaceutical form (Foscan®).

The incubation of all tested glioblastoma cell lines with *m*-THPC/DMPC liposome formulation (in the absence of surfactant G1) slightly increased the value of mean fluorescence channel (MFC) indicating that *m*-THPC chlorin was scarcely transferred into the cells. On the other hand, after 4 h of treatment, liposome formulations containing G1 induced a noticeable increment of MFC value in all glioblastoma cultures. Such an increase appeared to be dependent on the concentration of surfactant in the liposomes.

The liposome-mediated m-THPC delivery was compared with the uptake of the relative pharmaceutical compound, Foscan®. MFC values of samples treated with Foscan® showed to be quite similar to those of samples treated with *m*-THPC/DMPC liposomes but significantly lower than those of liposomic formulations containing the surfactant.

Moreover, the qualitative analysis of the uptake and intracellular distribution of the photosensitizer was carried out by laser scanning confocal microscopy (LSCM), in both human and murine glioblastoma cells. Cultures were treated either with Foscan® or with five liposome formulations. No fluorescent signal was detectable in C6 cell cultures treated with free Foscan® or *m*-THPC/DMPC (Figs. 1 a and 1 b, respectively). The low signal revealed by flow cytometry in these samples was not suitable for the LSCM imaging. When the cultures were incubated with *m*-THPC in G1-containing liposomes, the fluorescence of the photosensitizer was detectable at the membrane level of LN229 and C6 cells (Figs. 1 c and 1 b, respectively). According to the flow cytometric determinations, the intensity of fluorescent signal generally appeared positively related to the G1 concentration. In addition, liposomes were clustered and capped on the plasma membrane of C6 cells.

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The cytotoxicity of chlorin-loaded liposomes was then tested by the cloning efficiency assay performed on both human (U118, LN229) and murine (C6) cell cultures, before and after irradiation with laser light at 652 nm. The cytotoxic effect of liposome formulations was compared with that of Foscan® employed at the same concentrations of *m*-THPC contained in liposome. Different liposome formulations were scarcely cytotoxic in the absence of irradiation.

The irradiation performed on cells treated with chlorin-loaded liposomes reduced drammatically the cell surviving fractions. In particular, cultures treated with m-THPC formulated in G1-free liposomes (i.e. *m*-THPC/DMPC) the irradiation induced the reduction of surviving fraction to 60%. Moreover, in these cultures chlorin-loaded liposomes appeared to be more effective than chlorin formulated in the pharmaceutical form (Foscan®). The presence of surfactant in liposomal formulations dramatically increased the cytotoxic effect of irradiation that destroyed completely the glioblastoma cultures.

In addition, we employed the freeze-fracture method to study the interaction of liposomes with cell membrane of glioblastoma cells. In Figs. 2 a and 2 b several liposomes interacting with the membrane of a glioblastoma cell are shown.

The results obtained by in vitro tests indicated that cationic liposomes: i) transferred m-THPC in glioblastoma cells more efficiently than pharmaceutical formulation; ii) significantly ($p << 0.001$) increased the *m*-THPC cytotoxic effect after laser irradiation; iii) seemed to exert their cytotoxic action in the early phase of interaction with the cells, during adhesion to the plasma membrane.

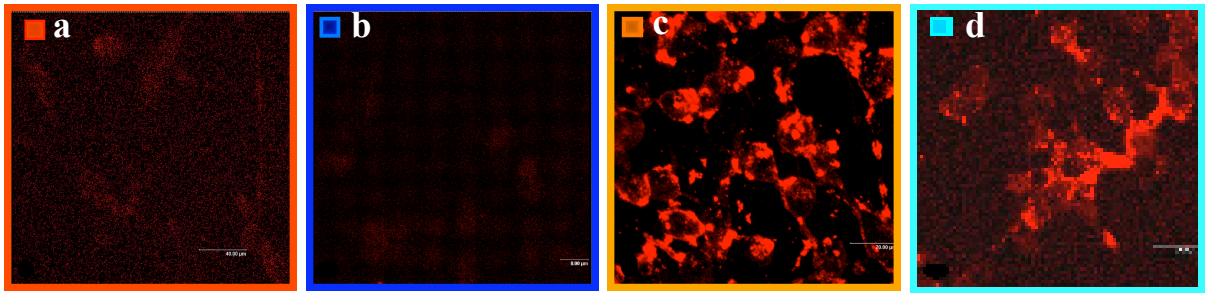

Figure 1. Intracellular distribution of *m*-THPC.

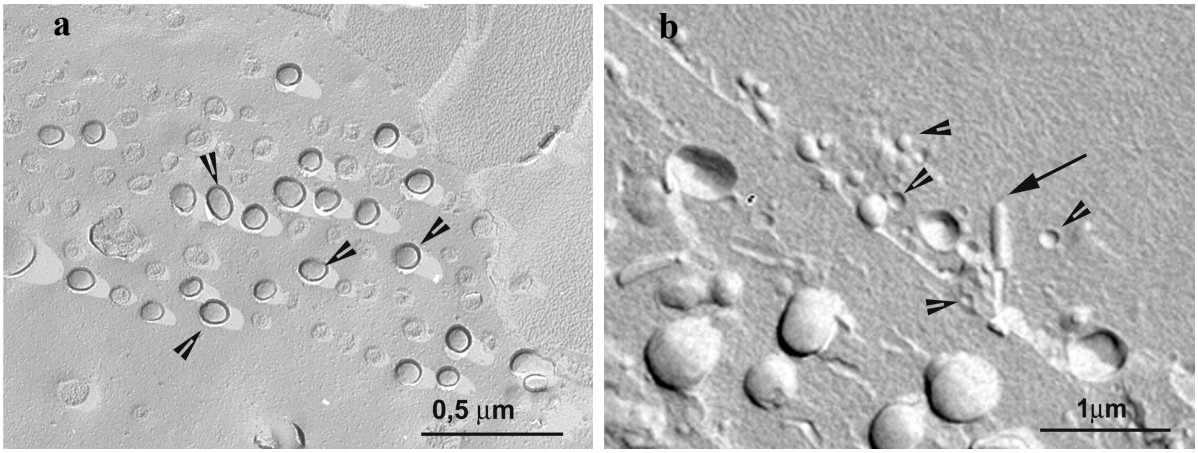

Figure 2. Interaction of liposomes with cell membrane of glioblastoma cells.

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