Encapsulation of staurosporine using a novel intraliposomal stabilization strategy: Therapeutic efficacy in glioma

Rajesh Mukthavaram, Rohit Saklacha, Pengfei Jiang, Ying Chao, Milan Makale, Sandra Pastorino, Valentina Fogal, Wolf Wrasidlo, Santosh Kesari*

Liposome-based delivery systems have been used to enhance drug efficacy and reduce toxicity. The most successful systems have been engineered for long circulation times and stable liposomal encapsulation of the active drug. A long circulation half-life allows the liposomes to accumulate at the tumor either by leakage (EPR effect) from incomplete tumor vessels. An example is the FDA approved PEGylated liposomal delivery of doxorubicin, an anthracycline with antitumor activity. However, success with liposomal anthracyclines has not yet been matched with other much more potent anticancer drug classes.

The objective of the present study was to address this limitation with a highly potent antitumor agent whose clinical use is presently not possible, but which could be made feasible if the compound were to be successfully delivered by liposomes. In order to attain this objective we addressed the hypothesis that it is possible to encapsulate within liposomes a wide variety of drugs (natural products, kinase inhibitors) using a method in which pH gradients are established and manipulated. We selected for detailed study staurosporine which, (1) is a pan protein kinase C inhibitor that cannot be encapsulated within liposomes, and (2) has very potent *in vitro* anti-tumor activity. Staurosporine is not amenable to clinical use because its high affinity binding to plasma proteins (hAGP) prevents effective delivery to tumors. Liposomal encapsulation of staurosporine can in principle prevent interaction with blood constituents prior to tumor delivery.

We successfully encapsulated staurosporine into PEG liposomes using a novel pH gradient method. The precise pH gradient conditions were systematically varied and defined for optimal liposomal uptake of the drug. Our confirmatory tests showed high encapsulation efficiency and a favorable drug release profile. In order to demonstrate effective shielding of the encapsulated drug from blood constituents, we added the liposomal formulation to human serum and measured the concentration of staurosporine in different fractions after separation using size exclusive chromatographic seperation. We also showed the favorable clinical potential of liposomal staurosporine in terms of, (1) anti-tumor potency with different glioblastoma cell lines in culture, (2) radiation sensitization of glioblastoma lines, and (3) anti-glioblastoma effects using an *in vivo* subcutaneous mouse model.