Different liposomal formulations of selected copper(I) complex bearing amantadine: synthesis, characterization and biological evaluation

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Metal-based complexes represent a highly promising class of agents for cancer diagnosis and treatment, owing to their wide-ranging chemical properties and biological activities. Among the metals investigated, copper has attracted increasing attention due to its pivotal involvement in tumor development and progression, making it a promising target for anticancer therapy. Our previous study focused on the chemical and biological evaluation of a series of novel copper(I) and copper(II) complexes, synthesized via the conjugation of a bis(pyrazol-1-yl) acetate ligand with the biomolecule amantadine. Their antitumor activity was assessed in the human glioblastoma cell lines U87MG and LN18. Among the tested compounds, the copper(I) complex bearing two triphenylphosphine moieties emerged as the most promising, exhibiting pronounced cytotoxic activity. Building on these findings, various zwitterionic and negatively charged liposomal formulations encapsulating the selected copper complex were developed and compared. All liposomes were prepared using the thinfilm hydration method, followed by freeze-thaw and extrusion cycles. Particle size and zeta potential in different dispersions were determined by multi-angle dynamic light scattering (MADLS). The stability of the two most promising liposomal formulations was evaluated in cell culture medium at 37°C and 5% CO_2 for up to 72 hours. Encapsulation efficiency was quantified by ^{1}H NMR spectroscopy and biocompatibility was evaluated in HaCaT human keratinocyte cells. Moreover, mitochondrial metabolic activity was determined using the MTT assay and liposome cellular uptake was analyzed by flow cytometry and confocal laser scanning microscopy (CLSM). Finally, the copper chelator tetrathiomolybdate (TTM) was employed to confirm the metaldependent nature of the observed biological activities.