

COMPLEX FORMATION OF MN(II) AND DPPGOG IN LONG-CIRCULATING THERMOSENSITIVE LIPOSOMES FOR NON-INVASIVE THERMOMETRY?

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Introduction

Thermosensitive liposomes (TSL) with encapsulated MR-active manganese(II) ions could be used for non-invasive thermometry and for active loading of liposomes with doxorubicin (DOX), a potent chemotherapeutic agent. TSL may then be used for direct drug targeting in combination with hyperthermia, temperature measurements and online DOX determination at the tumour site with MRI simultaneous to the treatment of solid cancers. During characterization of TSL by NMR an unexpected hysteresis of the T₁ relaxation time during a heating and cooling cycle was observed. This finding will be described here. We postulate a complex formation between manganese(II) ions and the negatively charged head group of the lipid DPPGOG as potential reason for this observation.

Methods

DPPE/DSPC/DPPGOG 5:2:3 (m/m) TSL were prepared by the lipid film hydration and extrusion method. The size and ζ -potential was determined by Photon Correlation Spectroscopy and T_m was determined by Differential Scanning Calorimetry. The TSL were either loaded with NaCl, MnSO₄ or Gd-DTPA. All T₁ relaxation time measurements were performed on a 0.47T-NMR-Analyzer (Minispec, Bruker, Germany). The loading of TSL with DOX was performed with a pH gradient method.

Results

The manganese ions were released at the phase transition temperature of the TSL (42°C, measured with DSC). The T₁ relaxation time of TSL showed a sigmoidal decrease during heating and a characteristic hysteresis curve during cooling. The sigmoidal T₁ –increase during cooling indicated a reencapsulation of manganese. This phenomenon was not observed when the MRI-contrast agent gadodiamide was encapsulated into the TSL. The TSL with encapsulated manganese(II) tended to form sediments while TSL with encapsulated sodium chloride did not. Loading experiments of TSL with doxorubicin yielded to the formation of aggregates when separating nonencapsulated doxorubicin from encapsulated on Sephadex G50 columns. Measurements of TSL with increasing DPPGOG concentration showed linearly increasing manganese concentration, supporting our assumption. In a pilot experiment, the titration of the ζ -potential of empty TSL with increasing manganese(II) concentrations showed also a complex formation.

Conclusion/Outlook

We found evidence for a complex formation of manganese(II) and the anionic lipid DPPGOG in our long-circulating TSL. To demonstrate the complex formation, additional EPR-measurements are planned. In aqueous solutions manganese(II) prefer the formation of the octahedral $[\text{Mn}(\text{II})(\text{H}_2\text{O})_6]^{2+}$ complex, that could be easily followed using EPR.