Thermosensitive Mn^{2+}-Liposomes for MR-guided Hyperthermia

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Introduction:

Hyperthermia has proven to be an effective treatment concept for locally advanced deep-seated tumors (1,2). MRI could provide non-invasive temperature control of local hyperthermia (3,4). Reaching the therapeutic temperature slightly above 40°C is a crucial factor for the outcome of the hyperthermic tumor treatment (5). It has been shown that paramagnetic liposomes may support MR thermometry (6,7). We demonstrate the feasibility of MR thermometry using new temperature sensitive long circulating liposomes with encapsulated $MnSO_4$.

Long circulating temperature sensitive liposomes (LTSL) composed of 1.2-dipalmitoyl-*sn*-glycero-3-phosphoglyceroglycerol (DPPGOG) (8), DPPC and DSPC showed markedly enhanced circulation time *in vivo* (> 10h) and favourable Mn^{2+}-release at 41-43°C (9).

Material and methods:

LTSL were prepared by the lipid film hydration and extrusion method. Large unilamellar vesicles (LUV) were obtained by extrusion through nanopore filters of 200-nm pore size using a thermobarrel extruder at 60°C. The liposomes were incubated with 300 mM $MnSO_4$. Free Mn^{2+} was removed from the liposome suspension by dialysis (6). The relaxation time T_1 measurements were performed at 0.47 Tesla with a Minispec 120 (Bruker, Germany) by the inversion recovery method. For the first set of measurements the LTSL were heated from 36 to 50°C, for the second set of measurements the sample was cooled down from 50 to 36 °C. The third set of measurements was performed with 10% triton in the buffer to ensure that all Mn^{2+} is set free. The time interval between the measurements was 10 minutes, the temperatures were corrected due to a calibration function.

Results:

Figure 1 shows the changes of the relaxation rate R_1 of the sample in a sigmoid course between 2,1 and 6,9 $(mM \cdot s)^{-1}$ during heating. The cooling of the sample also yields a sigmoid course with R1 of 6,1 $(mM \cdot s)^{-1}$ at lower temperature. The triton measurements show the known temperature dependence of R1 for free Mn^{2+} raising to lower temperatures (see also Fig.1).

This finding clearly indicates a temperature dependent release of the Mn^{2+} from the long circulating liposomes by moderate hyperthermia. Released Mn^{2+} affect the water molecules and shortens the relaxation time T_1, increasing the relaxation rate R_1 respectively.

The sigmoidal course of the cooling scan with a R_1 of 6,2 $(mM \cdot s)^{-1}$ at 37 °C indicates a reencapsulation of Mn^{2+}. For free Mn^{2+} R_1 should rise to nearly 8 $(mM \cdot s)^{-1}$. This reencapsulation is possible because of a complex building of Mn^{2+} with the head group of the lipids, mainly with DPPGOG because of the negative charge of head group of this lipid. Measurements of liposomes with increasing DPPGOG concentration show linearly increasing Mn^{2+} concentration (data not shown) and support our assumption. Future ESR-measurements will show the complex building.

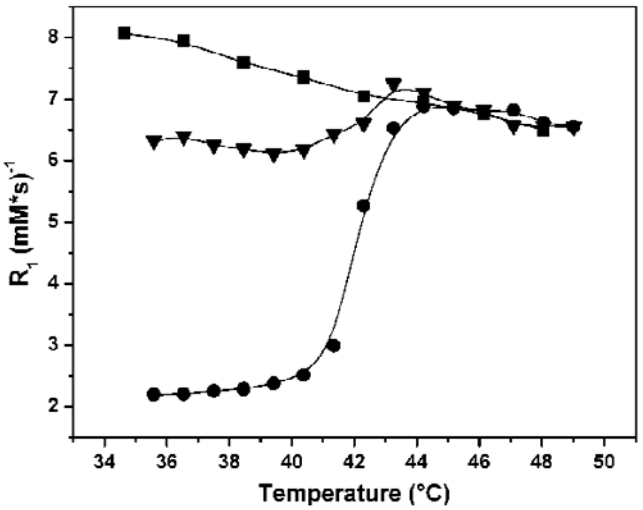

Fig.1:
Change of the relaxation rate R1 of with temperature which indicates the concentration of free Mn^{2+}. The sigmoidal heating scan (●) shows the release of encapsulated Mn^{2+} from the liposomes with a release-temperature of 42-43°C. The cooling scan (▼) shows a partially reencapsulation of Mn^{2+}. Free Mn^{2+} (■) shows an increase of R1 to lower temperature.
(The lines are drawn to guide the eye.)

Conclusion:

In the present study long circulating Mn^{2+}-liposomes were shown to be useful as contrast agent to monitor temperature in hyperthermia therapy. Detection of temperature dependent Mn^{2+}-release demonstrates the technical feasibility of temperature measurement using Mn^{2+}-LTSL. Finally Mn^{2+}/doxorubicin loaded liposomes could be used for in vivo monitoring of liposome concentration distribution and drug release using MRI.

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