

TARGETED MULTIMODAL LIPOSOMES FOR IMAGING AND RADIONUCLIDE THERAPY

GERBEN A. KONING^{1*}, WILLEM MULDER², GUSTAV J. STRIJKERS²,
KLAAS NICOLAY², SANDER ZIELHUIS³, FRANK NIJSEN³, MARJAN M.
FRETZ⁴, GERT STORM⁴ and GERARD C. KRIJGER¹

¹Department of Radiation, Radioisotopes and Reactors, Delft University of Technology, Mekelweg 15, 2629-JB, Delft, The Netherlands, ²Department of Biomedical NMR, Eindhoven University of Technology, The Netherlands, ³Department of Nuclear Medicine, University Medical Centre Utrecht, The Netherlands, ⁴Department of Pharmaceutics, Utrecht University, The Netherlands

Targeted liposomal carriers have proven successful in the delivery of mainly chemotherapeutic agents [Mastrobattista, E. *et al.* **Adv. Drug Deliv. Rev.** 40 (1999) 103 and Sapra, P. and Allen, T.M. **Prog. Lipid Res.** 42 (2003) 439]. We aim to translate and utilize achievements from that field of drug delivery research towards successful application of similar carriers for both imaging and radionuclide therapy. In that respect we focus on targeted multimodal carriers that combine an imaging (MRI and/or SPECT) and a therapeutic component. The latter can be a radionuclide or a compound that after neutron capture is able to produce radiation. In principle combinations with a chemotherapeutic modality are feasible as well. To achieve this collaboration of various expert groups in imaging and nuclear medicine is required. This contribution gives an update of the progress in the different areas.

Molecular imaging

Within the imaging field there is a great need for agents that allow for non invasive visualization of biological processes at the molecular level. This field of molecular imaging is currently gaining more and more attention. Magnetic resonance imaging (MRI) is an imaging technique which combines anatomical / morphological information with the information that comes from the used contrast agent. In order to apply MRI at the molecular level the relatively low inherent sensitivity has to be improved considerably. Receptor targeted liposomes may be a valuable tool to achieve MRI detectable contrast that allows for imaging and diagnosis of molecular processes. Recently Mulder *et al.* proved the feasibility of in vitro targeting of Gd-bistearylamide (Gd-BSA) containing liposomes to E-selectin overexpressing TNF- α stimulated human endothelial cells [Mulder, W.J. *et al.* **Bioconjug. Chem.** 15 (2004) 799]. The presence of a high amount of Gd atoms in the bilayer of the targeted liposomes contributed to a strong signal increase. The E-selectin targeted liposomes may represent an attractive tool for diagnosis of activated endothelium, which is a hall mark of

* E-mail: g.a.koning@iri.tudelft.nl

various inflammatory processes [Koning, G.A. *et al.* **Endothelium** 9 (2002) 161].

Gd-containing liposomes equipped with an RGD-sequence containing peptide [Schiffelers, R.M. *et al.* **J. Control. Release** 91 (2003) 115] were used for molecular imaging of angiogenesis. A cyclic RGD-sequence is known to have specificity for $\alpha\beta3$ integrins which are overexpressed on angiogenic endothelium. We recently tested these RGD-liposomes equipped with both Gd-BSA and a fluorescent bilayer marker (rhodamine-PE) in a s.c. LS174 human colon cancer xenograft model in nude mice. Only by using this bimodal RGD-liposome we were able to both demonstrate tumor vasculature specific MR imaging and validate this by fluorescence microscopy. Specificity for tumor vasculature was only observed for the RGD-liposomes and not for liposomes with control RAD-peptides or liposomes without peptide.

Neutron capture therapy

We ultimately aim to further increase the multimodality of the above described liposomal systems by including a therapeutic component. To this respect we set out to test the encapsulation of the boron-10 enriched dodecahydrododecaborate ($B_{12}H_{12}$)²⁻ in the aqueous phase of targeted liposomes in order to apply them for neutron capture therapy (NCT). NCT is based on the capture of thermal neutrons by boron-10 or gadolinium-157 atoms. Upon capture of a neutron the boron-10 atom disintegrates and produces α -particles, ⁷Li-nuclei and a γ -photon, whereas the Gd-atom produces auger-electrons and γ -photons. The radiation produced in this process can be lethal to surrounding cells and can be used for therapy of e.g. tumor cells. This approach is attractive as it involves the use of principally non-toxic compounds that can locally, i.e. only in the tumor area, be activated to produce the radiation.

For the targeting we adopted two approaches; first, using the RGD-exposing liposomes for targeting this radiotherapy to the tumor vasculature and second using an EGF-receptor specific antibody for targeting EGFR-overexpressing tumor cells. We tested the in vitro uptake and boron delivery properties of RGD- and EGFR-targeted liposomes in human endothelial cells and ovarian cancer cells, respectively. An increased intracellular localization of boron-10 was observed using the targeted liposomes as compared to non-targeted liposomes. Cells incubated with targeted boron-10 containing liposomes prior to a thermal neutron irradiation showed the highest level of cytotoxicity in cell viability assays [Koning, G.A. *et al.* **Appl. Radiat. Isot.** 61 (2004) 963 and Krijger, G.C. *et al.* **Radiochim. Acta** in press]. We are currently initiating in vivo experiments using these two approaches. We further also aim at combining the NCT-approach with MR imaging for both treatment optimization and evaluation.

Radionuclide therapy

Targeted liposomes are also being used as a carrier for the delivery of therapeutic radionuclides either for systemic or local administration. We are

currently loading liposomes with the lanthanides holmium-166 and lutetium-177. Both radionuclides can be used for therapy as well as imaging using SPECT (Single Photon Emission Computed Tomography). Ho-166 has an additional MR imaging possibility [Zielhuis, S.W. *et al. Curr. Med. Chem.-Anti Cancer Agents* 1 (2005)]. Liposomes containing the chelator DTPA in their bilayer are developed for lanthanide loading. DTPA-liposomes are prepared, targeting ligands can be attached and the whole system can be analyzed prior to loading of the radionuclide. Optimal conditions for liposome loading have been achieved and allow for a one-step liposome labeling procedure that could be performed just prior to use. Preliminary studies on in vivo imaging of a draining lymph node after s.c. administration proved the feasibility of using such liposomes for imaging and future therapy of sentinel nodes. In vitro targeting experiments and studies assessing the anti-proliferative activity of Ho-166 and Lu-177 liposomes targeted to tumor cells are in progress. In conclusion, liposomes provide a highly versatile multimodal carrier system that can combine different imaging modalities together with a therapeutic component in one carrier in which additional cell specificity can be introduced by coupling targeting ligands. Such a multimodal tumor-targeted system ultimately allows for online monitoring of tumor targeting levels, intratumoral localization prior and during radio- and/or chemotherapeutic treatment.