

A Boron/Gd/LDL adduct for Imaging-guided Neutron Capture Therapy

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Introduction. Boron neutron capture therapy (BNCT) is a binary therapy based on the selective uptake of sufficient amounts of the stable ^{10}B isotope by tumor cells, followed by irradiation with low energy thermal neutrons. The success of BNCT depends upon the selective delivery of ^{10}B atoms to tumor cell. In fact, in order to be effective, BNCT requires 20-30 μg of ^{10}B per g of tumor and a low concentration ($<5\mu\text{g}$ of ^{10}B per g of healthy tissue). For these reasons, "in vivo" visualization of ^{10}B distribution is important. Thanks to its superb spatial resolution MRI appears to be the most appropriate technique to tackle this task. In this work a new compound containing a carborane unit and a Gd-containing complex (Gd-DCL, Figure 1) has been synthesized. The compound contains a palmityl chain that promote the binding to LDL (Low Density Lipoproteins). The supramolecular B/Gd/LDL adducts accumulate at tumor cells that overexpress transporters for these lipoproteins. Several examples of successful delivery of drugs and imaging agents through targeting of LDL receptors have already been reported. Furthermore, the combination of the boron and gadolinium compounds may be beneficial for enhancing the radiation dose to the tumor as ^{157}Gd (15% natural abundance) owns a very good cross-section for neutron capture..

Methods. LDL adducts have been prepared by incubating LDL and Gd-DCL at different molar ratio for two hours at 37° . Gd-DCL form highly stable micelles that prevent the binding with LDL. Micelles were disrupted by adding an excess of β -cyclodextrin before the incubation with LDL. The unbound complex was eliminated by dialysis.

The cellular uptake of the Gd/B labeled LDL was first tested in vitro on HepG2 (human hepatoblastoma cancer cell line) and B16 (murine melanoma) tumor cells line. In vivo, Gd/B LDLs were administered to mice subcutaneously inoculated with B16 cells line. Magnetic Resonance Imaging (MRI) was performed at 7T before, 6 and 24 hr post-contrast injection. The internalized Gd and B were determined by ICP-MS analysis.

Results. Each LDL particle can load up to 180 imaging probes (Gd-DCL) that correspond to 180 Gd and 1800 B atoms respectively. The millimolar relaxivity of Gd-DCL bound to the LDL surface was of $15\text{ mM}^{-1}\text{ s}^{-1}$ at 20MHz and 25°C . The adduct size, determined by Dynamic Light Scattering measurements, does not change with respect the native protein (22 nm). The cellular labelling experiments proved that, after 16 hours of incubation in the presence of 10-40 $\mu\text{g}/\text{ml}$ of Gd/B containing LDL the amount of internalised Gd is sufficient to generate hyper intense signals in the corresponding MR images (Figure 2). The Signal Intensity (SI) measured on cells is directly proportional to the amount of Boron internalized and the B/Gd molar ratio found into tumor cells by ICP-MS measurements remains ca. 10 thus indicating the total absence of Gd-DCL degradation or Gd release upon incubation. The amount of Boron internalized by HepG2 and B16 cells "in vitro" was of 30 and 36 $\mu\text{g}/\text{g}$ of cell.

B16 tumor bearing mice showed a good tumor signal intensity enhancement (Figure3, 30-40%), 6 and 24 hours after the injection of of Gd-DCL (0.02mmol/Kg in Gd). The tumor SI reports about the Boron concentration.

Conclusions.

LDLs act as efficient carriers for the delivery of a new imaging probe containing Gd and Boron. It follows that imaging-guided BNCT appears possible as, from the signal enhancement generated by the paramagnetic Gd(III) complexes, we access to the key information that the ^{10}B concentration threshold has been reached.

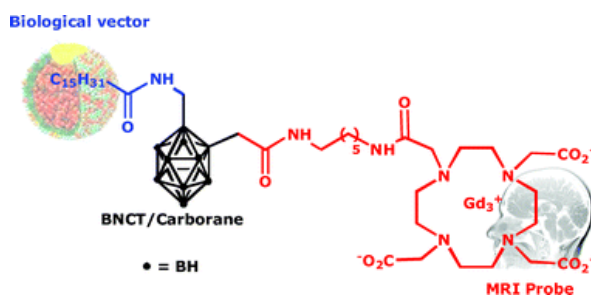


Figure 1

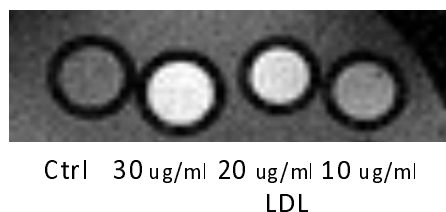


Figure 2

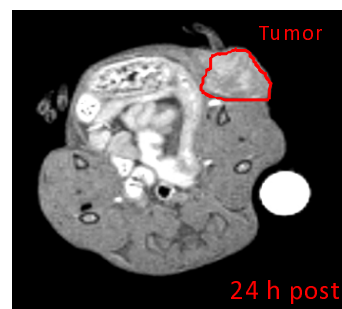


Figure 3