MR Contrast Agent Coupled to an Antisense Peptide Nucleic Acid - Cell Penetrating Peptide Conjugate

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Introduction Aiming to image the presence of specific mRNAs by MRI, we have developed two intracellular MR contrast agents (CA) composed of a Gd-DOTA complex, a peptide nucleic acid (PNA) sequence and a cell penetrating peptide. One (dsRed CA) was designed to bind to mRNA of dsRed (red fluorescent protein originating from *Discosoma* coral) by its complementary peptide nucleic acid (PNA) sequence, whereas the second one contained a nonsense sequence with no natural counterpart.

Methods The conjugates were synthesized by a continuous solid phase synthesis scheme using Fmoc/Bhoc chemistry for PNA synthesis. Cellular uptake of compounds was confirmed by fluorescence microscopy and spectroscopy in NIH-3T3 mouse fibroblasts (no target sequence) plated in 96well plates as well as by MR analyses of cells in Eppendorf tubes. Internalized fluorescence was measured in a multiplate reader, and microscopic images were made. MRI of cell pellets was conducted at 300 MHz on a vertical Bruker 7T/60 cm MRI Biospec system using T_1- and T_2-weighted spin-echo sequences. Relaxation rates were obtained from axial slices as well as T_1- and T_2-weighted images of sagittal slices.

Results and Discussion Two MR contrast agents, conjugated to PNA and cell penetrating peptide, were synthesized and characterized by ESI-MS. Fluorescence studies showed that both contrast agents could enter efficiently into 3T3 cells in a concentration dependent manner from 0.5 µM to 9.0 µM; and the contrast agent was located in vesicles around the nucleus. The results of *in vitro* MR studies showed a statistically significant increase of the intracellular relaxation rate $R_{1,cell}$ already at a labeling concentration of 0.5 µM (Fig.1, Table 1), thus contrast enhancement was also detectable in the MR images (Fig.2). The increase in $R_{2,cell}$ was less prominent. A preliminary *in vitro* binding assay with an immobilized target oligodeoxynucleotide sequence showed a significant higher specificity of the antisense CA in comparison to its nonsense counterpart.

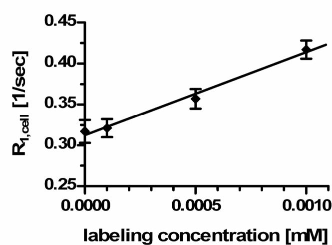

Fig.1. Relaxation rate $R_{1,cell}$ in 3T3 cells after loading with dsRed CA.

Table 1. Intracellular relaxation rates $R_{1,cell}$ and $R_{2,cell}$ in 3T3 cells after loading with dsRed CA for 18 h.

Labeling concentration [µM]	0.5	1.0
$R_{1,cell}$ [% of control]	112 ± 0.5**	132 ± 3.7**
$R_{2,cell}$ [% of control]	104 ± 2.0^{ns}	116 ± 3.5**
n	3, 2	3

Values are means ± SEM; **: p<0.01 statistically different as compared to control (100 ± 0.6 %/100 ± 0.5); ns, not significant, ANOVA with Dunnett´s post test; n: number of experiments, each with two replicates.

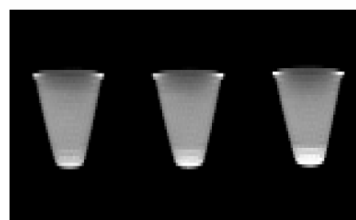

Fig.2. T_1 weighted MR images of 3T3 cells after loading with dsRed CA

Although antisense PNA conjugates have been widely applied to nuclear imaging probes (PET and SPECT), the utilization of such conjugates as contrast agent for MR imaging is very limited. Up to now, there is only a single study on a PNA-linked intracellular MR contrast agent reported (1). Our synthesized contrast agent showed excellent ability for intracellular delivery. These results demonstrate that the presented contrast agent dsRed CA has a potential to be used as a specific targeted contrast agent for MR imaging in cells expressing the dsRed gene. Further cell studies on transgenic cells expressing dsRed will be performed to evaluate the specific accumulation by targeting the mRNA.

References (1) Heckl S et al. *Cancer Research*. 2003; **63**, 4766-4772.