In vitro Tumor cell targeting with the folate receptor specific MR contrast agent P866

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Purpose: The vitamin folic acid has become a useful ligand for targeted cancer imaging and therapies, because it binds to a tumor associated antigen known as the folate receptor (FR). The expression of high affinity FR is elevated in many tumors of epithelial origin compared to normal tissues. By linking folic acid to tracers, that are detectable with imaging techniques, the folate delivers the tracers specifically to FR positive cancer cells but not to non-neoplastic cells of normal organs, thereby providing cancer specific imaging. The purpose of this in vitro study was to evaluate the specific uptake of the new FR specific MR contrast agent P866 by varying tumor cell lines.

Methods:

Contrast agents: **P866** (Guerbet Group, Paris, France) is a high relaxivity Gd-chelate (contrastophore), which is combined with a folic acid moiety (pharmacophore). P866 has a molecular weight of 9.4 kD. **P1001** (Guerbet Group, Paris, France) represents a non-targeted analogue of the P866, i.e. it is composed of an identical contrastophore but without a folic acid moiety.

Tumor cells and incubation procedure: Samples of $1x10^7$ FR positive human breast cancer cells (MDA MB 468), FR negative human breast cancer cells (MDA MB 435) and histiocytic lymphoma cells (U937) were incubated at 37°C with increasing doses of P866 or P1001 (25, 50 and 100 µM) in 5 ml RPMI media for 24h. In order to investigate the competitive effect of folic acid, cell samples were cultured for 5 days and subsequently incubated with contrast agent in either folate free PRMI media or RPMI media, which contained free folic acid in excess. After the incubation with the contrast agent, cells were washed 3 times with PBS by sedimentation (10 min, 250 g, 20°C). Then, the cells were counted in a Neubauer counting chamber, distributed to Eppendorf test vials and homogenously suspended in 0.5 ml isotonic Ficoll solution. Directly before and after the incubation procedures, the cell viability was determined by the Trypan blue exclusion test.

MRI: MR imaging of the cells in test tubes was performed using a 3T and 1.5T MR scanner (Signa, GE Medical Systems, Milwaukee, WI, USA) and standard circularly polarized quadrature knee coils (Clinical MR Solutions, Brookfield, WI, USA). For measurements of T1-relaxation times, axial spinecho (SE)-sequences were obtained through the cell samples with multiple TR values of 2000, 1000, 500, 250 ms, a TE of 16 (1.5 T) or 15 ms (3T), a FOV of 12 cm^2, a matrix of 256x196, a slice thickness of 5 mm, and one acquisition.

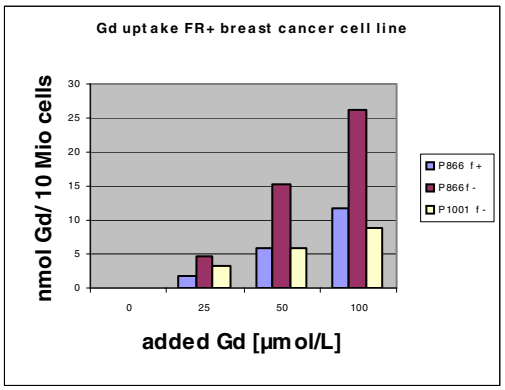
Data analysis: MR images were transferred as DICOM images to a SUN/SPARC workstation (Sun Microsystems, Mountain View, Ca) and processed by a self written IDL program (Interactive Data Language by Research Systems, Boulder, CO, U.S.A.). T1-maps were calculated from the four spin echo images using a nonlinear function least-square curve fitting on a pixel-by-pixel basis. The signal intensity for each pixel as a function of time was expressed as: $SI_{pixel\ xy}(t) = S_{o(pixel\ xy)}[1-\exp(-t/T1_{pixel\ xy})]$. T1 relaxation times of the cells were derived by ROI measurements of the samples on these T1-maps, results were converted to R1-relaxation rates (s^{-1}), and changes in R1 between samples without and samples with contrast media were calculated ($\Delta R1=R1_S-R1_0$). For comparison, gadolinium concentrations within the cell samples were determined by ICP-MS (inductively coupled plasma mass spectrometry).

Results:

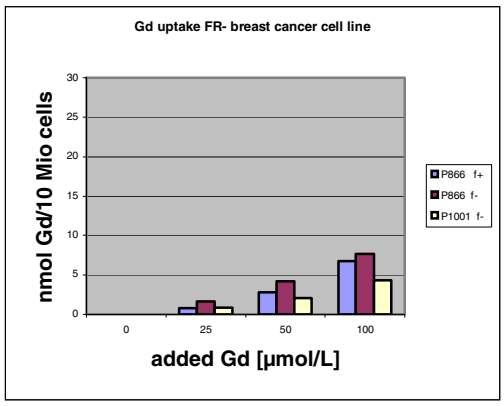
All three cell lines showed an increasing contrast agent uptake after incubation with increasing concentrations of P866 and P1001 (Fig. 1). And for all three cell lines, the cellular uptake of P866 was significantly higher than the cellular uptake of P1001. However the difference between P866 and P1001 uptake was higher for the FR positive MDA MB 468 cells as compared to the FR negative MDA MB 435 cells and the U937 lymphoma cells. This was due to a significantly higher maximal P866 uptake into the FR positive breast cancer cells (26.23 nmol intracellular Gd per 10 Mio cells compared to FR negative breast cancer cells (7.66 nmol intracellular Gd per 10 Mio cells) and the lymphoma cells (2.27 nmol intracellular Gd per 10 Mio cells) after incubation with 100 µM P866. Competition with folic acid in the culture media inhibited the P866 uptake into all three cells lines, this effect was again most prominent for the FR positive MDA MB 468 cells and less pronounced for the FR negative MDA MB 435 cells and U937 lymphoma cells. The positive, but less pronounced competitive effect of folic acid in the FR negative MDA MB 435 tumor cells was most likely due to a minor, but less pronounced expression of the FR compared to the FR positive MDA MB 468 cells.

The P866 uptake resulted in a significant T1-effect for all targeted tumor cells, and this effect was significantly higher at 1.5 T compared to 3T: Maximal changes in R1-relaxation rates ($\Delta R1$) were 0.18 s^{-1} at 1.5 T and 0.1 s^{-1} at 3T for the FR positive MDA MB 468 cells; 0.27 s^{-1} at 1.5 T and 0.07 s^{-1} at 3T for the U937 lymphoma cells and 0.24 s^{-1} at 1.5 T and 0.14 s^{-1} at 3T for the FR negative MDA MB 435 cells.

However, R1 changes were not significantly different between the three cell lines ($p>0.05$). Of note, we cannot exclude a T2-effect, especially in the FR positive cells. Thus, our continuing investigations focus on improvements of our MR technique for the detection of differences in P866 uptake by different tumor cell lines, using a variety of T1-weighted and T2 and T2*-weighted pulse sequences.



a)



b)

Fig. 1. Gd-uptake (measured by ICP-MS) into MDA MB 468 cells, which show a high expression of the FR (a) and MDA MB 435 cells, which show no or only a minor expression of the FR (b) with P866 in folic acid free culture media (f-), P866 in folic acid containing culture media (f+) and P1001 in folic acid free culture media (f-) after incubation of the cells with increasing contrast agent concentrations.

Conclusion: The new FR specific MR contrast agent P866 shows a specific uptake in FR positive breast cancer cells.