Rat glioma C6 cells labeled with a fluorinated Gd-GlyMe-DOTA-complex

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Introduction

Fluorine is of interest in medical chemistry and diagnostics, because of its very low natural abundance in living organisms, the similary bond length and van der Waals radius in comparison to hydrogen. Furthermore the high MR sensitivity makes fluorine interesting for ^{19}F-MRI. Fluorinated Gd-contrast agents allow to record ^{19}F-images without any background signals and classical ^{1}H-MR images at the same time.

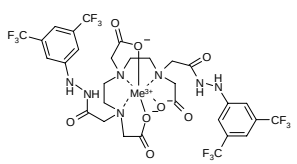
CF_3-groups within a molecule increase its lipophilicity, therefore, 3,5-Bis(trifluoromethyl)benzyl derivatives can pass the cell membrane without any active transport. 3,5-Bis(trifluoromethyl)benzoic acid hydrazide, 3,5-Bis(trifluoromethyl)benzyl amide, 3,5-Bis(trifluoromethyl)benzamidine, 1-[3,5-Bis(trifluoromethyl)phenyl]thiourea, 3,5-Bis(trifluoromethyl)phenylalanine and 3,5-Bis(trifluoromethyl)phenylhydrazine were selected as model compounds to compare Gd-DTPA- and Gd-GlyMe-DOTA-complexes with respect to their T_1-times. Furthermore the lipophilic properties of these Gd-complexes and also of different M^{3+} ions complexes of 3,5-Bis(trifluoromethyl)phenylhydrazine- DTPA, -GlyMeDOTA and -GlyMeAsp-DOTA were determined.

Methods

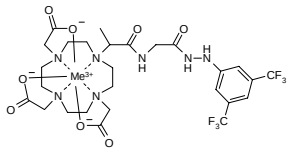
The Gd-DTPA-ligands were synthesised by reaction of DTPA-bis-anhydride at 60°C in dry DMF with the appropriate 3,5-Bis (trifluoromethyl)phenyl derivatives. 1-[3,5-Bis(trifluoromethyl)phenyl]thiourea reacted by loss of the $H_2NC(S)$-group to the 3,5-Bis(trifluoromethyl)aniline-DTPA derivative. The Me^{3+}-complexes are obtained upon the addition of the appropriated lanthanoid salt (La^{3+}, Eu^{3+}, Gd^{3+}, Nd^{3+}, Er^{3+}, Dy^{3+} and Sm^{3+}) and Y^{3+} salt in an aqueous/ethanol solution of the ligand and subsequent neutralisation with 0.1M NaOH.

Tris-t-butyl-GlyMe-DOTA was used for the synthesis of GlyMe-DOTA and GlyMeAsp-DOTA ligands. After activation with TBTU and DIPEA, it reacted with the fluorinated benzyl derivatives at room temperature for 24 h. The t-butyl esters were cleaved of with trifluoroacetic acid. The ligands were complexed in the same way as the DTPA derivatives.

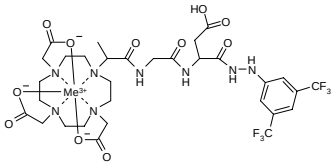
All complexes were purified by HPLC and the chemical structures were characterised by ESI-MS, 1D- and 2D-MR experiments. The ^{1}H- and ^{19}F-T_1-measurements of the Gd-complexes were performed at 8,4T and 293K sample temperature in 1mmol/L aqueous, in the case of DTPA-complexes in aqueous/DMSO solutions.



Bis(3,5-bis(trifluoromethyl)phenylhydrazine)-DTPA-complex



3,5-Bis(trifluoromethyl)phenylhydrazine-GlyMe-DOTA-complex



3,5-Bis(trifluoromethyl)phenylhydrazine-GlyMeAsp-DOTA-complex

The lipophilicity is determinated from methanol-water partition coefficient (log k'w) of each complex by reversed phase chromatography at 40°C column temperature. The methanol content is adapting in different runs with respect to the hydrophobic interaction of the analytes with the RP packing. Plotting the logarithm of retention against the methanol content and an extrapolation to 0% MeOH gives the log k'- value.

3,5-Bis(trifluoromethyl)phenylhydrazine was selected for cell culture experiment. Rat glioma C6 cells were incubated for 30 minutes with 0 mM, 1.3 mM, 2.5 mM, 3.8 mM and 5.1 mM of the Gd-complex for MRI experiments and 0 µM, 1 µM, 2.5µM, 6.4 µM and 63.4 µM for ICP-MS measurements.

Results:

All fluorinated Gd-complexes show shorter ^{1}H-T_1- times compared to Gd-DTPA-BMA (T_1=0.244 mmol·s) with one exception.[1] All relaxations times are within the range of 0.17 mmol·s and smaller except for Bis-(3,5-bis(trifluoromethyl)aniline)-Gd-DTPA (table 1). The very long ^{1}H-T_1- time of this complex is due to the increased steric hindrance for the water exchanges because of a smaller distance of the aromatic system to the Gd-center. ^{19}F-NMR spectra of the complexes exhibit a sharp signal around $\delta \approx -63$ ppm. All ^{19}F-T_1-times including Bis-(3,5-bis(trifluoromethyl)aniline)-Gd-DTPA are very similar at within 0.08-0.09 mmol·s (table 1).

Reversed phase chromatography showed that the lipophilicity of the fluorinated Gd-DTPA-complexes is higher (log k'w > 3.8) than the Gd-GlyMe-DOTA-complexes (log k'w < 3.6).

Substance	^{1}H-T_1 [mmol·s] Gd-DTPA	^{1}H-T_1 [mmol·s] Gd-GlyMe-DOTA	^{19}F-T_1 [mmol·s] Gd-DTPA	^{19}F-T_1 [mmol·s] Gd-GlyMe-DOTA	log k'w Gd-DTPA	log k'w Gd-GlyMe-DOTA
3,5-Bis(trifluoromethyl)benzoic acid hydrazide	0.139	0.161	0.085	0.091	3.869	2.845
3,5-Bis(trifluoromethyl)benzylamide	0.150	0.140	0.087	0.082	5.621	2.864
3,5-Bis(trifluoromethyl)benzamidine	0.102	0.125	0.088	0.083	5.378	2.798
3,5-Bis(trifluoromethyl)aniline	1.598	0.134	0.079	0.081	5.440 / 6.167	3.598
3,5-Bis(trifluoromethyl)phenylalanine	0.147	0.132	0.091	0.084	2.817	2.519
3,5-Bis(trifluoromethyl)phenylhydrazine	0.127	0.106	0.084	0.079	5.633	3.055

Table 1: ^{1}H-, ^{19}F-T_1-times and log k'w-values of the six Gd-complexes.

Exchanging the metal ion (M^{3+}) has no effect on the lipophilicity. Among the three different ligands of 3,5-Bis(trifluoromethyl)phenylhydrazine, the DTPA-complex has the highest lipophilicity (log k'w = 5.63). The GlyMe-DOTA-complexes (log k'w = 3.06) are more lipophile then the GlyMeAsp-DOTA-complexes (log k'w = 2.53).

MRI experiments and ICP-MS measurements proof the possibility to label C6 cells with 3,5-Bis(trifluoromethyl)phenylhydrazine-Gd-GlyMeDOTA. Furthermore, the ^{19}F-MRI signal of the Gd-complex in labelled C6 cells is sensitive enough to be detected on a Biospec 4.7 T MR scanner.

Discussion

All synthesised lipophilic fluorinated Gd-complexes show very short ^{19}F-T_1- times. The lipophilicity of the M^{3+}-complexes has been varied by changing the functional amino group (i.e. $-NH_2$-; NH_2-NH- or NH_2-C(O)-) or by adding another spacer molecule (Asp). It was shown that ^{19}F-MRI can be an alternative to the conventional ^{1}H-MRI. Because of the high signal intensity (4 trifluoromethylgroups in DTPA-complexes) the contrast agent concentration can be kept low. ICP-MS measurements can be used additionally to confirm cell labelling.

[1] Fossheim, S. (et al.) JMRI 1997, 7: 251