Design, synthesis and characterization of new smart MR contrast agents sensitive to pH

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Introduction New gadolinium-based MR contrast agents were designed and synthesized to trace physiological changes of extracellular pH and calcium concentration. DO3A-EP/ DO3A-EPE was appended with -tris-carboxymethyl and -phosphono-ethyl (-EP) / diethyl-phosphono-ethyl (-EPE) moieties on tetraazacyclododecane and complexated with Gd. Phosphonate derivatives are known to have a very high affinity towards calcium (MDP, methylene diphosphonate) and can function as reporters of pH or calcium ions. Ethyl phosphonate was appended covalently on the macrocycle without any amide linkage, which binds reversibly as a function of pH/calcium. Gd-DO3A-EP/-EPE therefore can be used as an extracellular contrast agents reacting to pH/calcium-related changes on cellular activity and neurotransmission. In this paper we demonstrate in-vitro MR relaxation changes induced by changes in pH.

Synthesis 1,4,7 tris(carboxymethyl)-10-(2-phosphono-ethyl)-1,4,7,10-tetraazacyclododecane was synthesized from 1,4,7,10-tetraazacyclododecane (cyclen) by reaction with tert-butyl bromoacetate and diethyl 2-bromoethyl phosphonate, giving an excellent yield of 80%. The corresponding carboxylate derivative DO3A-EP was obtained by cleaving the tert-butyl group by trifluoroacetic acid and anisole at 0°C. DO3A-EPE was synthesized from cyclen by the reaction of ethyl-2-bromoacetate to get the mono-substituted product. It was further appended with tert-butylbromoacetate to get 1,4,7-tris(carboxybutoxyethyl)-10-[N-2(diethyl-phosphono-ethyl)]-1,4,7,10-tetraazacyclododecane. The corresponding carboxylate derivative DO3A-EPE, was obtained by cleaving the tert-butyl groups and treatment of TFA at RT. Yield was 82%.

Methods The gadolinium complexes of DO3A-EP / -EPE were prepared by using GdCl3·6H2O at pH 7-8 on 60-70°C for 18h. For measurements of the relaxivities r1, four different concentrations 0.1, 0.4, 0.7, and 1 mM of the CA were prepared in tubes. For the series with -EP, pH from 4-9 in 0.5 pH steps was adjusted using glacial acetic acid 17 M and NaOH respectively. For the series with -EPE, pH was adjusted from stock solution at pH 8 adding solid p-toluenesulphonic acid and LiOH without changing the concentration of the stock solution separately.

Up to 52 tubes were measured simultaneously using a spin echo sequence with TE 13 ms / TR 0.05 - 8 s or TE 13 - 1248 ms / TR 14 s (21°C, 200 / 300 MHz). Fitting of T1,2 values was done voxelwise on selected ROIs using MATLAB. Relaxivity r1,2 was calculated from the slope of R1,2(c) versus the CA concentration by an error-weighted linear regression.

Results and Discussion In T1-weighted images (Fig. 2 top), different MR image intensities at different pH levels were observed due to changes in the relaxation rates. R1 values from samples with four different pH values are plotted below. With increasing pH, the relaxivity, i.e. the slope of the relaxation rates R1 over concentration, decreased. An overview of all results from tubes with different pH values is given in Fig. 2 bottom. The inflexion point occurs at a pH of approximately 6.5. r1 relaxivity of Gd-DO3A-EP increased by 70% from 2.3 to 3.9 s⁻¹mM⁻¹ (pH 7.5 to pH 5.5); r2 relaxivity increased by 57% from 2.8 to 4.4 s⁻¹mM⁻¹ (pH 7.5 to pH 5.5). For Gd-DO3A-EPE, r1 increased by 50% with (5.3 / 6.2 / 7.9 ± 0.1) s⁻¹mM⁻¹ at pH 8 / 6 / 4, respectively.

Gd-DO3A-EP and Gd-DO3A-EPE show similar relative changes of the relaxivity in the pH range from 8 to 4. In -EPE it is hypothesized that the relaxivity decrease at pH 8 to 4 is due to the formation of ternary adducts with anionic species like carbonate (CO3²⁻) and hydroxide anions (OH⁻) which displace the inner sphere water molecule(s) as described by Aime [1].

In conclusion, these data demonstrate the potential of the newly synthesized ligands as novel smart contrast agents responsive to pH. Since Gd-DO3A-EPE is an uncharged molecule, it may be preferable to Gd-DO3A-EP in some applications, for example when crossing of lipid membranes is desired in vivo.


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References

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