A novel efficient MRI contrast agent containing two paramagnetic centra

K. Kimpe[1], S. Laurent[2], T. Vogt[1], C. Burtea[2], V. Henrotte[2], L. Vander Elst[2], K. Binnemans[1], R. Muller[2]

[1]Department of chemistry, Katholieke Universiteit Leuven, Leuven, Belgium, [2]Organic Chemistry/ NMR Lab, University of Mons-Hainaut, Mons, Hainaut, Belgium

Introduction

Recently, necrosis avid contrast agents based on Gd^{3+} metalloporphyrins such as Gadophrin-2, have been discovered.[1] The accumulation of this product in tumoral necrosis was tentatively related to an albumin-binding mechanism.[2] If this notion holds true, a similar retention in necrotic tissue should be expected for any agent with a strong albumin binding capacity. In this study, we have prepared and characterized a novel contrast agent by introduction of two metal binding centers in the same ligand (figure 1).

Material and methods

NMRD profiles were recorded on a field cycling relaxometer (Stelar, Italy). The water residence time τ_M of the complex was obtained from the analysis of the temperature dependence of the oxygen-17 transverse relaxation rate (Bruker AMX-300, Germany).[3] Transmetallation by zinc ions was evaluated by the decrease of the water longitudinal relaxation rate of buffered phosphate solutions containing gadolinium complex and $ZnCl_2$.[4] The binding constant and relaxivity value of Gd_2-KA in HSA solution was determined by measuring the proton longitudinal relaxation rate. Blood pharmacokinetics were assessed on male Wistar rats tracheotomized. Gd_2KA was injected as a bolus through the femoral vein at a dose of 0.1 mmol/Kg b.w. Blood samples were collected at different time delays. The gadolinium content of the blood samples was determined by relaxometry. A two-compartment distribution model was used to calculate the pharmacokinetic parameters. The biodistribution has been determined in rats, 2 h after a single i.v. injection of 0.1 mmol Gd/kg. Gd-DTPA has been used as a control. The organs were weighted, dried and subsequently were digested. The gadolinium content was determined by ICP (Jobin-Yvon, France).

Results and discussion

Synthesis: Two DTPA ligands were attached to a bisindole derivative of benzaldehyde bearing three methoxy groups. Hydrazine was used as a 'bridge' molecule, forming two amide bonds, first by reacting with the ethylcarboxylate of the ethyl indole-2 carboxylate group. For the second amide bond, the coupling with DTPA, a coupling agent was used. Under slightly alkaline solutions, the ligand readily formed complexes with gadolinium ions.

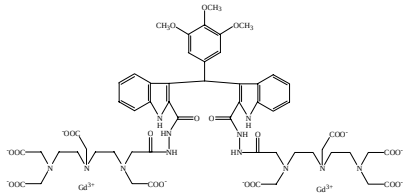

Figure 1: Gd_2-KA

Relaxometric characterization: At 310K, the value of a residence time of the coordinated water molecule τ_M of Gd_2-KA (520 ns) is much larger than that of the parent compound Gd-DTPA (143 ns). The relaxivity of Gd_2-KA is however higher than that of Gd-DTPA and can be related to the reduced motion and a longer rotational correlation time (59 ps for the Gd-DTPA and 183 ps for the Gd_2-KA). Transmetallation of Gd_2-KA by Zn^{2+} ions shows a stability comparable with the commercially used Gd-DTPA complex. The relaxivity of Gd_2-KA is enhanced in HSA solution, indicating a noncovalent interaction. The estimation of the association constant Ka and the value of the relaxivity of the noncovalently bound complex, r_1^c are 1600 M^{-1} and 25.3 s^{-1} mM^{-1} respectively.

Pharmacokinetic characterization and biodistribution: The pharmacokinetic parameters indicate that the elimination of Gd_2-KA is slower than that of Gd-DTPA. The volume of distribution is similar, however the elimination half-lives are much longer with respect to Gd-DTPA, most likely because of albumin binding. The relative low volume of distribution and the longer elimination half-live indicate that the agent is largely confined in the blood vessels for a more prolonged time, so the agent could have some potential as a blood pool contrast agent. Gd_2-KA is eliminated, like Gd-DTPA, mainly by the renal route. The biodistribution of Gd_2-KA is similar to that of Gd-DTPA, with Gd_2-KA showing a slightly higher preference for the liver, which is most likely attributable to its higher lipophilicity.

Conclusions

NMRD data prove that Gd_2-KA has a higher relaxivity than Gd-DTPA and that the relaxivity value increases *in vivo* due to HSA interaction. The interaction with HSA also results in longer elimination half-life and a better confinement in the vascular space as shown by pharmacokinetic evaluation. Biodistribution studies show that the complex is excreted, similarly as Gd-DTPA, by the renal pathway. The agent did not show any necrosis avidity, despite the structural similarity with ECIV-7, [5] and its binding to HSA. This study may therefore discard the proposed mechanism of albumin binding for the necrosis targetability.

References

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