Synthesis and Characterization of New Low Molecular Weight Lysine-Conjugated Gd-DTPA Contrast Agents suited for MR Angiography

S. Laurent[1], C. Burtea[1], L. Vander Elst[1], R. N. Muller[1]

[1]Dpt of Organic and Biomedical Chemistry - NMR and Molecular Imaging Laboratory, University of Mons-Hainaut, Mons, Hainaut, Belgium

Introduction

The high molecular weight Gd-DTPA-conjugated polylysine compounds were extensively investigated in an attempt to develop optimal polymeric systems for blood pool imaging. However, the longer circulation time of these compounds is currently compromised by the presence of highly charged residues located on their backbone which are able to bind kidney cells as a result of co-operative interactions (1). In order to overcome this major drawback of the polylysine compounds, a new group of low molecular weight contrast agents were synthesized by conjugating the Gd-DTPA moiety directly to the $-NH_2$ group of Lys. The compounds were characterized by relaxometry and by their pharmacokinetic parameters evaluated in Wistar rats.

Material and methods

NMRD profiles were recorded on a fast field cycling relaxometer (Stelar, Italy). Transmetallation by zinc ions was evaluated by the decrease of the water longitudinal relaxation rate of buffered phosphate solutions containing gadolinium complex and $ZnCl_2$.(2) Blood pharmacokinetics were assessed on male Wistar rats (250 ± 20 g). Gd complexes were injected as a bolus through the femoral vein at a dose of 0.05 mmol/Kg b.w for $(Gd\text{-}DTPA)_6Lys_5$ and of 0.075 mmol/Kg b.w. for $(Gd\text{-}DTPA)_4Lys_3$. 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 (elimination half-life, $T_{e1/2}$, total clearance, Cl_{tot}, volume of distribution steady state, VD_{ss}).

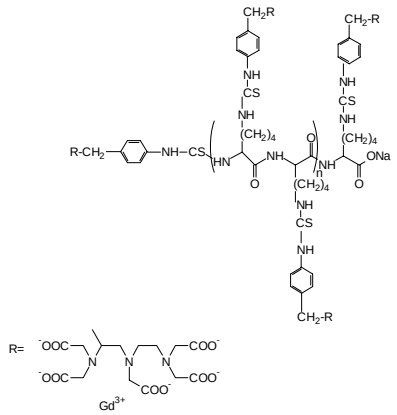
Results and discussion

Synthesis :

The peptides (Lys-Lys-Lys or Lys-Lys-Lys-Lys-Lys) reacted with an excess of p-SCN-Bz-DTPA ligand (Macrocyclics, Dallas, TX, USA) at pH 10 for 24h. The ligand was dialysed (cut-off membrane 1000) and then complexed with $GdCl_3.6H_2O$. The mass spectrometry confirms the structures.

Relaxometric characterization:

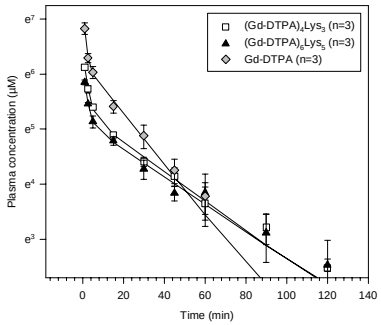
The relaxivity (310 K, 20 MHz) is equal to 5.7 and 6.4 $s^{-1}mM^{-1}$ for $(Gd\text{-}DTPA)_4Lys_3$ and $(Gd\text{-}DTPA)_6Lys_5$ respectively. The temperature dependence of the relaxivity at 20 MHz demonstrates that the water exchange is not a limiting factor. The higher relaxivities at 310 K as compared to Gd-DTPA are related to a longer rotational correlation time subsequent to a higher molecular weight of the molecule. These complexes do not interact with HSA. Transmetallation of the 2 complexes by Zn^{2+} ions shows a higher stability than the commercially used Gd-DTPA derivative.



Structure of complexes $(Gd\text{-}DTPA)_4Lys_3$ (n=1) and $(Gd\text{-}DTPA)_6Lys_5$ (n=2)

Pharmacokinetics

The pharmacokinetic parameters show a slightly prolonged blood residence time for $(Gd\text{-}DTPA)_4Lys_3$ ($T_{e1/2}$ = 26.4 min; Cl_{tot} = 7.3 ml/kg/min) and for $(Gd\text{-}DTPA)_6Lys_5$ ($T_{e1/2}$ = 28.5 min, Cl_{tot} = 6.9 mL/kg/min) as compared to Gd-DTPA ($T_{e1/2}$ = 14.9 min, Cl_{tot} = 8.66 mL/kg/min). The VD_β value (0.3 L/kg for both compounds) is moderately larger than that of Gd-DTPA (0.2 L/kg) and indicates some extravasation towards the interstitial space.



Conclusions

Even though the volume of distribution of the two compounds indicates a slow leakage into the interstitial space, their half-life in blood is slightly prolonged, which makes these compounds suitable as blood pool markers for MRI. The absence of positive molecular charge could limit the retention of the two compounds in kidneys, which is one of the major drawbacks of the high molecular weight polylysine contrast agents.

References

1. Bogdanov AA Jr, Lewin M, Weissleder R, Adv Drug Delivery Rev 1999;37:279-293.
2. S. Laurent, L. Vander Elst, F. Copoix, R.N. Muller, *Invest. Radiol.*, 2001, *36*, 115-122