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

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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

Physico-chemical characterization

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 plasma pharmacokinetics

Blood pharmacokinetics were assessed on male Wistar rats (250 ± 20 g: N = 3 / group) anesthetized with 50 mg Nembutal/kg b.w., i.p. The rats were tracheotomized, and the left carotid artery was catheterized for blood collection. Gd complexes were injected as a bolus through the femoral vein at a dose of 0.05 mmol/Kg b.w for **(Gd-DTPA)$_6$Lys$_5$** and of 0.075 mmol/Kg b.w. for (**Gd-DTPA)$_4$Lys$_3$**). Gd-DTPA has been used as a control and injected at a dose of 0.1 mmol/Kg b.w. Blood samples (~ 0.2 mL) were collected (with saline replacement) before and at 1, 2.5, 5, 15, 30, 45, 60, 90 and 120 min after injection. The gadolinium content of the blood samples was determined by relaxometry at 37°C and 60 MHz on a Bruker Minispec (Bruker Karlsruhe, Germany). A two-compartment distribution model was used to calculate the **pharmacokinetic parameters** such as the elimination half-life ($T_{e1/2}$), the apparent volume of distribution (VD_β), and the total clearance (Cl_{tot}). The gadolinium concentrations in blood were converted to plasma concentrations by assuming a hematocrit value of 0.53 (blood volume: 58 ml/kg, plasma volume: 31 mL/kg) [3].

Biodistribution

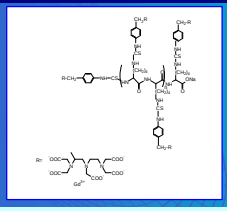
The biodistribution has been determined in rats at the end of the pharmacokinetics experiment. The organs (liver, kidneys, heart, spleen, lungs) were weighted, dried overnight at 60°C, and subsequently were digested (up to 0.4 g each sample) in acidic conditions (3 ml HNO_3, 1 ml H_2O_2) by microwaves (Milestone MSL-1200, Sorisole, Italy). The gadolinium content was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Jobin Yvon JY70+, Longjumeau, France). The results were calculated as percentages of the injected dose / g (%ID/g).

RESULTS

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 dialyzed (cut-off membrane 1000) and then complexed with $GdCl_3$•$6H_2O$. The mass spectrometry confirms the structure (**Figure 1**).

Figure 1. Structures of complexes **(Gd-DTPA)$_4$Lys$_3$** (n=1) and **(Gd-DTPA)$_6$Lys$_5$** (n=2)



RESULTS

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 (**Figure 2**). The **temperature dependence** of the relaxivity at 20 MHz demonstrates that the water exchange is not a limiting facto (**Figure 3**). 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.

Figure 2. The NMRD profiles of (Gd-DTPA)$_4$Lys$_3$ and of (Gd-DTPA)$_6$Lys$_5$ as compared to Gd-DTPA

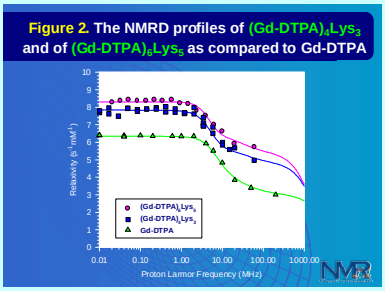
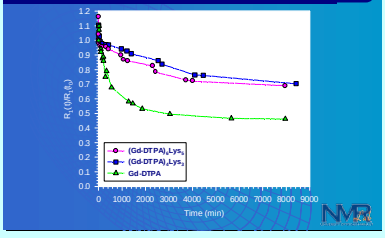


Figure 3. Transmetallation of (Gd-DTPA)$_4$Lys$_3$ and of (Gd-DTPA)$_6$Lys$_5$ as compared to Gd-DTPA

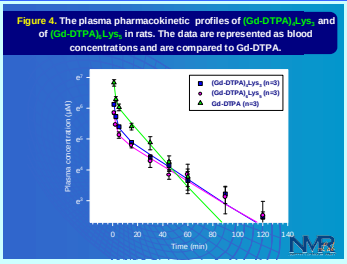


RESULTS

Pharmacokinetic parameters

The pharmacokinetic profile of the two compounds compared to that of Gd-DTPA is presented in **Figure 4**. The pharmacokinetic parameters were calculated by using a bi-exponential fit of the plasma concentration versus time curves. The pharmacokinetic parameters show a **slightly prolonged blood residence time** for **(Gd-DTPA)$_4$Lys$_3$** ($T_{e1/2}$ = 26.4 min; Cl_{tot} = 7.3 ml/kg/min) and for **(Gd-DTPA)$_6$Lys$_5$** ($T_{e1/2}$ = 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.

Figure 4. The plasma pharmacokinetic profiles of (Gd-DTPA)$_4$Lys$_3$ and of (Gd-DTPA)$_6$Lys$_5$ in rats. The data are represented as blood concentrations and are compared to Gd-DTPA.

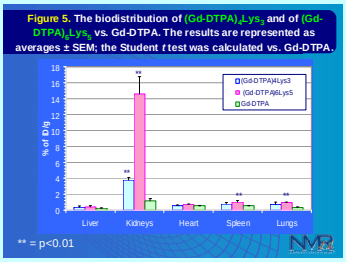


RESULTS

Biodistribution

The biodistribution of the two Gd-DTPA-conjugated polylysine compounds is presented in **Figure 5**. The results show that both compounds are **accumulated in kidneys** in significantly ($p<0.01$) higher concentrations as compared to Gd-DTPA. This characteristic is directly related to the length of the Lys chain, i.e. **(Gd-DTPA)$_6$Lys$_5$** was found in higher concentration (14.6 % of ID/g) as compared to **(Gd-DTPA)$_4$Lys$_3$** (3.8 % of ID/g). In addition, $(Gd\text{-}DTPA)_6Lys_5$ was also found in significantly ($p<0.01$) higher concentrations in spleen and lungs.

Figure 5. The biodistribution of (Gd-DTPA)$_4$Lys$_3$ and of (Gd-DTPA)$_6$Lys$_5$ vs. Gd-DTPA. The results are represented as averages ± SEM; the Student *t* test was calculated vs. Gd-DTPA.



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 did not limit the retention of the two compounds in kidneys, property which is probably not related to the positive charge of Lys. On the other hand, **(Gd-DTPA)$_4$Lys$_3$** is retained in kidneys at a lesser extent than **(Gd-DTPA)$_6$Lys$_5$**, which could be an advantage from the pharmacological point of view.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Bogdanov AA Jr, Lewin M, Weissleder R, *Adv Drug Delivery Rev* (1999) 37, 279-293.

[2] Laurent S., L. Vander Elst, F. Copoix, R.N. Muller, *Invest Radiol* (2001) *36*, 115-122.

[3] Burtea C, Laurent S, Colet J-M, Vander Elst L, Muller RN, *Invest Radiol* (2003) 38, 320-333.