Non-Invasive Visualization on Drug Delivery of Polymer Drug Conjugates

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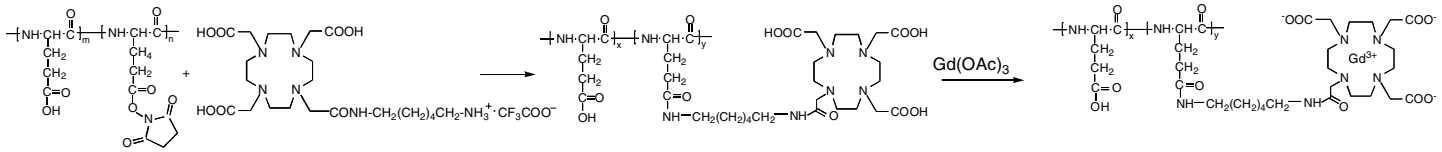
Introduction:

Polymers have become effective carriers in drug delivery system due to advantages such as prolonged retention time and enhanced tumor accumulation. However the in vivo drug delivery mechanism of polymer drug conjugates is not clear based on current biopsy-based method. We intend to explore non-invasive approaches for the evaluation of drug delivery with polymer drug conjugates using contrast-enhanced magnetic resonance imaging (MRI). Here poly (*L*-glutamic acid) (PGA) is used as the polymer carrier and a serial of different molecular weight PGA-1, 6-hexanediamine-(Gd-DOTA) conjugates were prepared. We investigated the size effect of polymer carrier on the in vivo biodistribution of the conjugates by contrast enhanced MRI and quantitative T_1 mapping.

Materials and Methods:

PGA-1,6-hexanediamine-(Gd-DOTA) conjugates were prepared by the reaction of DOTA-1,6-hexanediamine amino group with PLGA N-hydroxysucciminide esters, followed by complexation with $Gd(OAc)_3$ (Scheme 1). Different molecular weight conjugates of a narrow molecular weight distribution were prepared by fractionation with HiLoad 16/60 Superdex 200 prep grade column. The size effect of the conjugates was investigated in female athymic nude mice bearing human breast carcinoma MB-231 xenografts. MR images and T_1 mapping data were acquired alternately on a Siemens Trio 3T scanner. T_1-weighted images were acquired with a 3D FLASH pulse sequence (1.74ms TE, 4.3ms TR, 25 ^{O} RF tip angle, 120mm FOV, and 1.6mm coronal slice thickness). T_1 data were acquired with a gradient echo sequence of multiple flip angles 10, 20, 30, and 45^O, and 300 ms TR. Data acquisition duration for each flip angle was 15 sec for 8 slices, 128x64 imaging matrix, with the imaging resolution 1.0x1.0x2.0 mm^3. T_1 maps were processed by homemade software using the IDL software. Non-linear Levenberg-Marquardt fitting algorithm was used for calculating the T_1 relaxation time. Images were acquired before, continuously in first 20 minutes and at 30, 60, 120, 180, 240, 360 and 1440 minutes post-injection at a dose of 0.03mmol Gd/kg.



Scheme 1. Synthesis of poly(*L*-glutamic acid) Gd(III)-DOTA conjugates

Results:

The conjugates with molecular weight 90, 50 and 20 kDa were chosen to represent high-, intermediate-, and low-molecular weight. The gadolinium content was 54.9%, 41.7% and 42%, respectively, and T1 relaxivity was 8.45, 9.44 and 9.19 mM-1s-1, respectively, for the high, intermediate and low molecular weight conjugates. Figure 1 shows the T_1-weighted 3D MIP images of mice before and at various time points after injection with the conjugates. The size of the polymer conjugates had significant impact on their pharmacokinetics. The blood circulation of the conjugates prolonged with increase of the molecular weights. The low molecular weight conjugate (20 kDa) was cleared from the blood circulation in the first 30 minutes post-injection. The high molecular weight conjugates (90 and 50 kDa) showed much longer blood retention time than the low molecular weight conjugate (20kDa). T_1 relaxation time was calculated by fitting the signal intensity equation (Eq. 1).

$$S(\vec{r}:TR,\alpha) = S_o(\vec{r}) \cdot \frac{1-e^{-TR/T_1(\vec{r})}}{1-\cos\alpha \cdot e^{-TR/T_1(\vec{r})}} \cdot \cos\alpha \quad (1)$$

where $S(\vec{r}:TR,\alpha)$ is the measured signal intensity with varying flip angles (α) and a fixed TR.

T_1 relaxation times are inversely proportional to the changes of conjugate concentration in the tissues. T_1 maps were also constructed in pixel-by-pixel for all time points. Figure 2 shows the color-coded T_1 map for mice injected with the 90kDa conjugates in the first 60 minutes post-injection. The dynamic changes of T_1 relaxation time represented the conjugate's pharmacokinetics. After injection, there was a sharp decrease in T_1 values in the blood pool that corresponds to an increase of conjugate concentration, since the concentration of gadolinium has a linear correlation with $1/\Delta T_1$ (Fig. 2, right plot). The solid tumor tissue had higher T1 values than the surrounding tissue before injection of the conjugates. The T_1 values then gradually decreased over time after the injection, indicating the gradual accumulation of the conjugates. The accumulation of the conjugates in the solid tumor was heterogeneous as shown the T_1 maps.

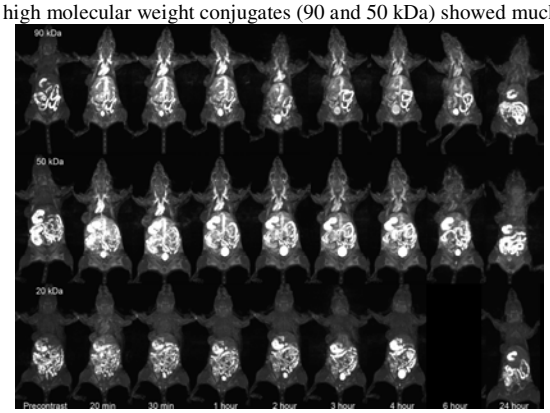


Fig. 1. T_1-weighted 3D MIP MR images of mice injected with the conjugates.

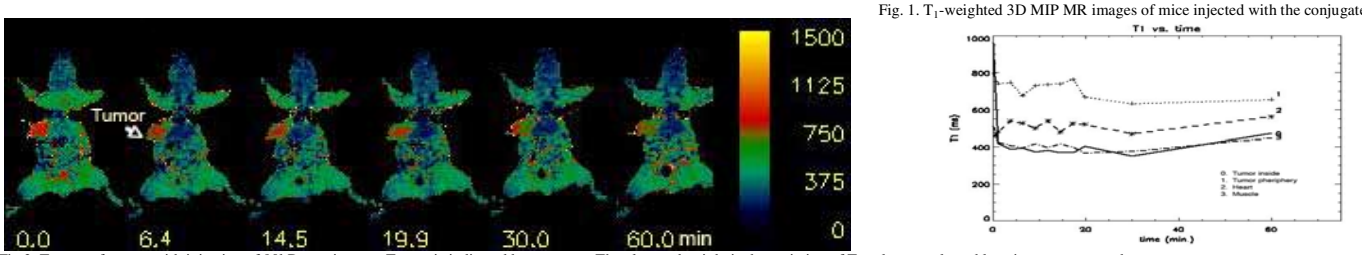


Fig 2. T_1 map of mouse with injection of 90kDa conjugates. Tumor is indicated by an arrow. The plot on the right is the variation of T_1 values at selected locations as annotated.

Conclusion:

The contrast enhanced MRI clearly revealed the sized effect of the conjugates on their pharmacokinetics. The blood circulation of the conjugates prolonged with the increase of the size of the polymers. Quantitative T_1 mapping is effective to visualize the dynamic accumulation of the conjugates in the solid tumor tissue. Contrast enhanced MRI and quantitative T1 mapping are promising for non-invasive monitoring in vivo drug delivery with polymeric drug conjugates.

Reference:

Lu ZR, Wang X, Parker DL, Goodrich KC, Buswell HR, Bioconjug Chem. 2003, 14, 715-9; Li C., Adv Drug Deliv Rev. 2002, 54, 695-713; Gohr-Rosental S, Schmitt-Willich H, Ebert W. and Conrad J, Invest. Radiol. 1993, 28, 789-795