A New Bioactivated MRI Contrast Agent : Synthesis, Characterization and MR Imaging Studies

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Abstract

A smart contrast agent, the β-glucopyronuronate-containing gadolinium(III) complex GdL_1(L_1 = 1-(2-difluoromethyl-4-(1-(4,7,10-triscarboxymethyl-(1,4,7,10-tetraazacyclodecyl))acetamido)phenyl)-β-D-glucopyronuronate) was synthesized and characterized. Relaxometric studies show that the T_1 change percentage in the value of GdL_1 decreases dramatically (51%) in the presence of β-glucuronidase (β-G) and human serum albumin (HSA). A significant signal change percentage enhanced by MR images was observed for GdL_1 solution in the presence of β-G and HSA. The MR images also shows high intensity enhancement in CT26(+βG) with β-G gene expression but not for the CT26(−βG) without β-G gene expression.

Introduction

MRI offers several advantages over other clinical diagnostic techniques for molecular imaging, including high spatial resolution, non-invasiveness, high anatomical contrast and lack of harmful radiation. However, sensitivity of MRI to depicting small molecule is constrained by the ubiquitous protons in the body, resulting in a high background and lower signal to noise ratio (SNR). Hence, the alternative amplification strategies using smart contrast agents are required to yield a higher sensitivity. Enzyme motif such as β-G, an often-used reporter enzyme in molecular biology, has been explored for smart MR contrast agent. In this study, we characterized an enzymatic contrast agent, which could be bound with HSA or β-G and activated by β-G *in vitro*.

Methods

The spin-lattice relaxation time T_1 measurements were made using a NMR relaxometer operating at 20 MHz and 37.0 ± 0.1 °C (NMR-120 Minispec, Bruker). The percentage changes of T_1 value in these solutions were plotted against the incubation time. CT26 colon carcinoma cells, CT26(+βG) and CT26(−βG) were used in this study. Both CT26(+βG) and CT26(−βG) cells were incubated with 1.0 mM GdL_1, washed by PBS buffer 3 times and scanned by 3.0 T MRI.

Results and Discussion

The purity of ligand and Gd(III) complex were performed by HPLC. In Fig. 1, the kinetics of enzyme-catalyzed hydrolysis of GdL_1 were measured, the T_1 value of GdL_1 decreasing about 17 and 51 % after incubation with different concentration β-G (5μg/ml and 0.1mg/ml), respectively in HSA. In high β-G concentration (0.1mg/ml), the T_1 decreasing of GdL_1 in the presence of β-G and HSA is significantly higher than that of GdL_1 in the presence of low β-G concentration (5.0μg/ml). These results indicated the GdL_1 is a smart contrast agent in the presence of high concentration β-G. The MR images for GdL_1 solution in the presence and absence of β-G in HSA solution were shown in Fig. 2. The MR image results of GdL_1 in the presence of β-G and HSA for 3.0 T MRI indicates that the enhancement (65%) is higher than that of GdL_1 in PBS buffer. In Fig. 3, the *in vitro* MR images of the CT26(+βG) is significantly higher (16%) than that of CT26(−βG). This image clearly indicates that GdL_1 can be activated by CT26(+βG) and worth to notice that β-G on the CT26(+βG) was functionally active *in vitro*.

Conclusion

A new bioactivated MRI contrast agent GdL_1 was synthesized and characterized successfully. T_1 decreasing (51 %) of enzymatic cleavage of GdL_1 in HSA indicates that the HSA and β-G bind to Gd(III) chelate after that the glucopyronuronate residue was removed. The MR images of enzymatic experiment shows the signal change percentage of GdL_1 in the presence of β-G and HSA is higher than GdL_1 in PBS buffer. The *in vitro* MR images show a higher intensity enhancement in CT26(+βG) but not for the CT26(−βG). GdL_1 possesses enzymatic cleavage, as well as higher percentage change in relaxation time and higher signal change percentage of MR images in the presence of β-G and HSA. Therefore, we can conclude that GdL_1 is a potential candidate for a bioactivated MRI contrast agent in tracing gene expression.

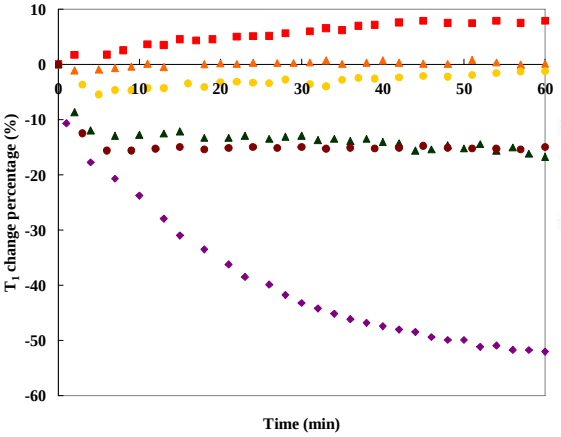

Fig. 1 Change in T_1(%) of enzyme-catalyzed hydrolysis of GdL_1 measured at 20 MHz, 37.0 ± 0.1 °C. (■) 0.5 mM GdL_1 and 0.1mg/ml β-G in 100 mM PBS buffer (pH=7.4 ± 0.1); (▲) 0.5 mM GdL_1 in 100 mM PBS buffer; (●) 0.5 mM GdL_1 and 5.0μg/ml β-G in PBS buffer; (●) 0.5 mM GdL_1 and 0.5 mM HSA in 100mM PBS buffer; (▲) 0.5 mM GdL_1, 0.5 mM HSA

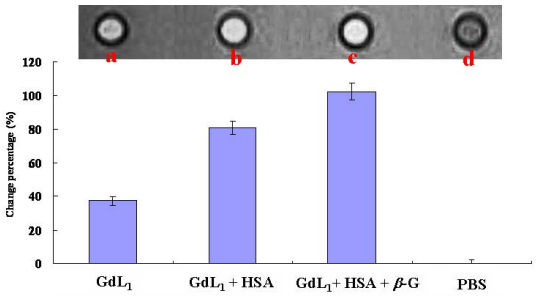

Fig. 2 Representative T1-weighted (TR/TE 100/2.4 ms) MR images of solutions and the signal change percentages in test tubes at 3.0 T MR scanner. (a) 0.5mM GdL_1 in PBS buffer solution; (b) 0.5mM GdL_1 and 0.5mM HSA in PBS buffer solution; (c) 0.5mM GdL_1, 0.1mg/ml β-G and 0.5mM HSA in PBS buffer solution; (d) PBS buffer solution.

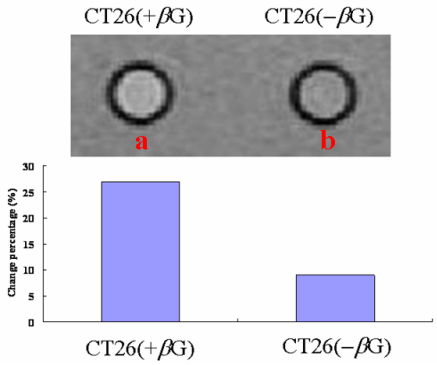

Fig. 3 *In vitro* MR images (TR/TE 150/5.8 ms) of CT26(+βG), CT26(−βG) cells and the signal change percentages in the test tubes at 3.0 T MR scanner. (a) CT26(+βG) cells incubate with GdL_1; (b) CT26(−βG) cells incubate with GdL_1.

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