A New Bio-activated Paramagnetic Gadolinium(III) Complex [Gd(DOTA-FPG)] for Tracing Gene Expression

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Abstract

A smart contrast agent, the *β*-galactopyranose-containing gadolinium(III) complex [Gd(DOTA-FPG)](DOTA-FPG = 1-(2-difluoromethyl-4-(1-(4,7,10-triscarboxymethyl-(1,4,7,10-tetraazacyclodecyl))acetamido)phenyl)-*β*-D-galactopyranose) was characterized as being potentially suitable for a bio-activated MRI contrast agent. In this study, the results of the E and M titrations of the [Gd(DOTA-FPG)] were shown an increase in the longitudinal water proton relaxation rate upon addition of HSA. The K_A value of [Gd(DOTA-FPG)] is 9.0×10^2 M^{-1}. The cytotoxicity of [Gd(DOTA-FPG)] was also investigated. The result was shown that the IC_{50} value of [Gd(DOTA-FPG)] to both CT26 and CT26/galactosidase(β-gal) cells was larger than 100 μM, demonstrating that [Gd(DOTA-FPG)] displayed low-cytotoxicity to these cells after β-gal activation. The MR images show a higher intense enhancement in CT26/β-gal tumor with β-gal gene expression but not for the CT26 tumor without β-gal gene expression. Obviously, [Gd(DOTA-FPG)] is a suitable candidate for a bio-activated MRI contrast agent in tracing 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-gal, an often-used gene reporter enzyme in molecular biology, has been explored for smart MR contrasting [1]. In this study, we characterized an enzymatic contrast agent, which could be bound with HSA or β-gal and activated by β-gal *in vitro* and *in vivo*.

Methods

The HSA-binding capability of the contrast agent can be carried out in vitro by the proton relaxation enhancement (PRE) method [2]. This method can be employed to obtain the thermodynamic binding constant (K_A), the number of binding sites (n) and the bound relaxivity of the macromolecular adduct (r_1^b). The cell cytotoxicity of [Gd(DOTA-FPG)] was determined by the ^{3}H-thymidine incorporation assay. Results are expressed as percent inhibition of ^{3}H-thymidine incorporation as compared to untreated cells. To investigate whether sites of β-gal expression could be non-invasively imaged, Balb/c mice bearing established CT26 and CT26/β-gal tumors in their left and right shoulder regions, respectively, were intravenously injected with 0.3 mmol/kg [Gd(DOTA-FPG)].

Results and Discussion

The results of the E and M titrations of the [Gd(DOTA-FPG)] are shown an increase in the longitudinal water proton relaxation rate upon addition of HSA. The binding parameter calculated for the binding site (n) to HSA of the Gd(III) complex are presented in Table 1. The lower K_A value and bound fraction of Gd(III) complex can be used to explain the result of the percentage change in relaxation time (T_1) of Gd(III) chelate in HSA without β-gal decreasing by only 25%. [Gd(DOTA-FPG)] in HSA under sodium phosphate buffer solution at pH = 7.4 in the presence of *β*-galactosidase, the percentage change in longitudinal relaxation time (T_1) significantly decreased by about 60%. Figure 1 shows that the IC_{50} value of Gd(III) complex to both CT26 and CT-26/β-gal cells was larger than 100 μM, demonstrating that Gd(III) complex displayed low-cytotoxicity to these cells after β-gal activation. The MR images of the animal and time-signal intensity change of the tumor were shown in Figure 2. The signal intensity of the CT26/β-gal tumor (3211.9 ± 192.9) is significantly higher than CT26 tumor (1834.8 ± 80.3) ($p < 0.05$) at 10 minutes. This intense enhancement clearly indicates that Gd(III) complex can be activated by CT26/β-gal tumors. To investigate whether the β-gal activity still functionally active in vivo, the tumor sections were also stained with X-gal and examined the sections under a microscope as shown in Figure 3. Histological staining for β-gal activity revealed strong blue color in CT26/β-gal tumors but not in parental CT26 tumors. This result shown that β-gal on the CT26/β-gal tumors was functionally active *in vivo*.

Conclusion

A novel bio-activated MRI contrast agent, [Gd(DOTA-FPG)], was characterized. The ^{3}H-thymidine incorporation assay was displayed low-cytotoxicity of [Gd(DOTA-FPG)]. The MR images show a higher intense enhancement in CT26/*β*-gal tumor with *β*-galactosidase gene expression but not for the CT26 tumor without *β*-galactosidase gene expression. This might result in a new type of contrast agent for tracing gene expression by MRI.

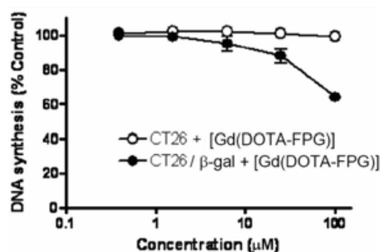

Fig. 1 The cytotoxicity of [Gd(DOTA-FPG)] incubated various concentration of [Gd(DOTA-FPG)] with CT-26 or CT-26/β-gal cells.

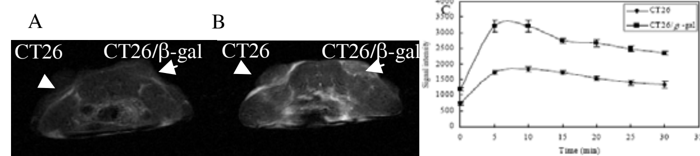

Fig. 2 Representative T1-weighted (TR/TE 100/13 ms) MR images of animal model at 3.0 T MR scanner. (A) precontrast images; (B) at 10 minutes after intravenous injection of 0.3 mmol/kg [Gd(DOTA-FPG)] intense enhancement of CT26/β-gal tumor. (C) time-signal intensity change of the CT26 and CT26/*β*-gal tumors after injection of 0.3 mmol/kg [Gd(DOTA-FPG)].

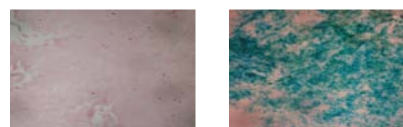

Fig. 3 Histological analysis of functional β-gal in vivo. Sections of CT26 (left) and CT26/ β-gal (right) tumor were stained with β-gal activity by the β-gal Staining Kit and viewed under phase contrast microscope.

Table 1. Parameters of binding studies of [Gd(DOTA-FPG)], $[Gd(cis\text{-}DOTA(BOM)_2)]^-$ and $[Gd(TTDA\text{-}BOM)]^{2-}$.[a]

Complexes	K_A(M^{-1})	n	b	r_1^F ($mM^{-1}s^{-1}$)	r_1^b ($mM^{-1}s^{-1}$)
[Gd(DOTA-FPG)]	$9.0 \pm 0.1 \times 10^2$	1	5.7 ± 0.1	4.4 ± 0.1[b]	25.0 ± 0.1
$[Gd(cis\text{-}DOTA(BOM)_2)]^-$ [c]	$3.2 \pm 0.4 \times 10^2$	2	5.2 ± 0.1	6.8 ± 0.1[b]	35.7 ± 0.9
$[Gd(TTDA\text{-}BOM)]^{2-}$ [d]	$4.6 \pm 0.1 \times 10^2$	1	13.7 ± 0.1	4.8 ± 0.2[b]	65.8 ± 2.7
$[Gd(TTDA\text{-}BA)]^-$ [e]	$1.0 \pm 0.2 \times 10^3$	1	–	6.5 ± 0.3[b]	53.9± 2

[a]The parameter was obtained from PRE (proton relaxation enhancement) measurements (25.0 ± 0.1 °C, 20 MHz, pH = 7.4, 50 mM sodium phosphate buffer solution). [b]These values refer to a solution containing 0.1 mM paramagnetic complex in sodium phosphate buffer solution (pH = 7.4) at 25.0 ± 0.1 °C. [c]Data was obtained from ref.[3]. [d]Ref.[4]. [e]Ref.[5].

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