Synthesis and Characterization of the tumor targeting contrast agent $[Gd(TTDA)]^{2-}$ derivative for MRI

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Abstract

Bombesin (BN) is a 14 amino acid peptide which target to prostate, breast and colon cancer, was synthesized by peptide solid phase synthesizer (PS-3). The purpose of this study is to design and synthesize a new complex GdL1 for MRI and characterize its Gd(III) complexes. More complete thermodynamic and kinetic evaluation including protonation constant, thermodynamic stability constant, conditional stability constant, selectivity constant, modified selectivity constant and dissociation rate constant are need to ascertain *in vivo* stability. The exchange rate between inner sphere water and bulk water will also studied using temperature dependence of relaxivity by ^{17}O NMR technique. In addition, we develop and synthesize a novel NIRF probe for optical imaging. The Bombesin peptide was used to conjugate with GdL1 and fluorescence dye for MRI and optical imaging for targeting to prostate, breast and colon cancer.

Introduction

Magnetic resonance imaging (MRI) has become the leading tool for imaging fine details of anatomy as well as physiology. Optical imaging is providing a sensitive and specific method for the detection and localization of the biochemical appearance *in vivo*. Bombesin (BN) is a tetradecapeptide, which was first discovered in the skin of the European frog Bombina bombina [1]. The bombesin receptor subtype 2-gastrin-releasing peptide receptors (GRPR) has been shown to be overexpressed on various human tumors thus has been a successful target for the detection and treatment of these cancers [2]. A ligand (L1) (L1 = nitrophenol containing TTDA derivative) was synthesized and characterized. The ligand protonation constant (ΣpKa), thermodynamic stability constant and water exchange rate (k_{ex}^{298}) for GdL1 was significantly higher than that of $[Gd(DTPA)]^{2-}$.These studies demonstrate that GdL1-BN can be used for MR imaging of GRPR-expressing prostate cancer and may be useful as a diagnostic agent or for monitoring therapeutic regimens.

Methods

A modified BN peptide substrate was synthesized by peptide solid phase synthesizer, then conjugated with L1, purified by preparation HPLC and characterized by ESI-MS. *In vitro* studies, PC-3 (which have a relative high GRPR overexpression level) and KB (normal GRPR expression) cell lines were incubated with GdL1-BN washed by PBS buffer and scanned by 3.0 T MRI. The nude male mice were injected with PC-3 and KB cells into the left and right lateral thigh and produced a high yield of tumor into the lateral thigh of nude mice after one to two weeks. MR imaging studies were performed with a 3.0 T MR imager and a high-resolution animal coil.

Results and Discussion

L1-BN were synthesized and characterized by HPLC and ESI-MS. The *in vitro* specific targeting study images using fluorescence microscope. The PC-3 cells incubated with EuL1-BN and the imaging shown specific targeting ability. In the other hand, *in vitro* fluorescent imaging can exhibit detailed microscopic information at the subcellular level(Fig 1.). The MTT assays using the PC-3 cell line was performed to analyze the potential cytotoxicity of GdL1-BN. However, GdL1-BN was shown noncytotoxicity at lower doses. The internalization of GdL1-BN into positive cell line was confirmed by *in vitro* MR imaging study (Fig 2.). With the GdL1-BN conjugates, the detection of the PC-3 cell line occurred with a noticeable MR contrast (T_1-weighted MR images). The enhancement of signal intensity was increased 59% for the PC-3 cell line. The signal intensity of positive cells in the present of GdL1-BN is significantly higher than that of positive cells only. No signal intensity change was observed for the negative cells in the presence and absence of GdL1-BN. The internalization of GdL1-BN into PC-3 cell tumor was also confirmed by *in vivo* study. Figure 3 shows the T_1-weighted fast gradient echo images of a tumor-bearing mice before (Fig. 3A) and 15min after (Fig. 3B) intravenous administration of GdL1-BN. The signal intensity of PC-3 cell tumor on the left was higher than that of KB cell tumor. In other words, the GdL1-BN was readily internalized into GRPR overexpressing tumor cells.

Conclusion

We have successfully prepared and characterized biocompatible GdL1-BN. The results of GdL1-BN presented suggest that it has high affinity for GRPR, weakly cell cytotoxicity, specific tumor localization and exhibits well imaging characteristics. The results in this study demonstrate that the bimodal Bombesin peptide analogs can be used to detect GRPR positive cancer with MRI and fluorescence microscope at the cellular and anatomical level.

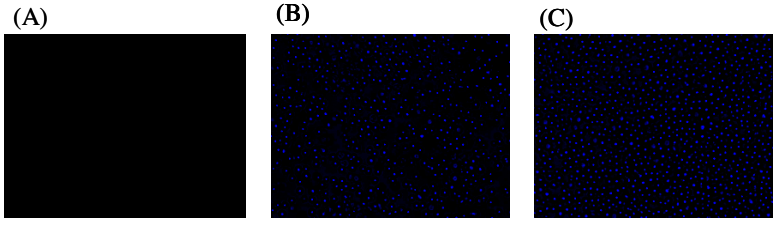

Figure 1. The *in vitro* specific targeting study images using fluorescence microscope. (A) cells without incubated with EuL1-BN.(B) cells incubated with EuL1-BN at 4 °C. (C) cells incubated with EuL1-BN at 37 °C.

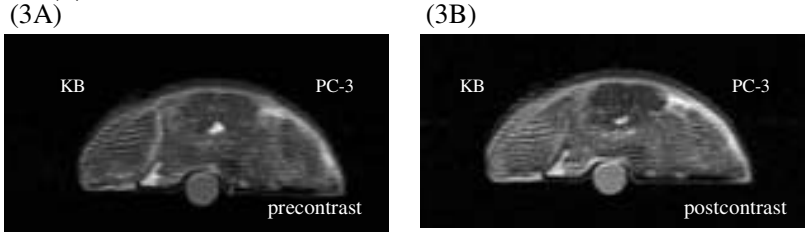

Fig 3. T_1-weighted in vivo images of pre- and post-injection of GdL1-BN (0.15 mmol/kg)

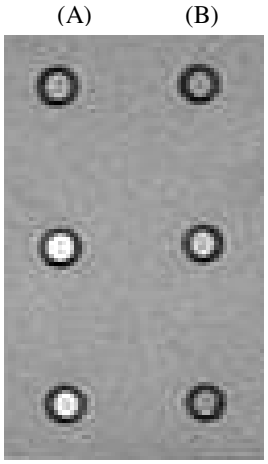

Fig 2. T_1-weighted MR images of positive and negative cells after the treatment with or without GdL1-BN. MRI was performed with a clinical 3.0 T MR scanner, and knee coil; All samples were scanned by a fast gradient echo pulse sequence (TR/TE = 150/2.4), (A) PC-3 cells; (B) KB cells. The upper row was the cells treatment without GdL1-BN. The second row was cells treatment with GdL1-BN (2 mM). The lower low shows the cells treatment with GdL1-BN (1mM).

References

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