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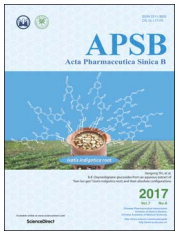

SCO - SHORT COMMUNICATION

A novel nitroreductase-enhanced MRI contrast agent and its potential application in bacterial imaging

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KEY WORDS

Nitroreductase;
MRI contrast agent;
Smart imaging probes;
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Abstract Nitroreductases (NTRs) are known to be able to metabolize nitro-substituted compounds in the presence of reduced nicotinamide adenine dinucleotide (NADH) as an electron donor. NTRs are present in a wide range of bacterial genera and, to a lesser extent, in eukaryotes hypoxic tumour cells and tumorous tissues, which makes it an appropriate biomarker for an imaging target to detect the hypoxic status of cancer cells and potential bacterial infections. To evaluate the specific activation level of NTR, great efforts have been devoted to the development of fluorescent probes to detect NTR activities using fluorogenic methods to probe its behaviour in a cellular context; however, NTR-responsive MRI contrast agents are still by far underexplored. In this study, *para*-nitrobenzyl substituted T_1-weighted magnetic resonance imaging (MRI) contrast agent Gd-DOTA-PNB (probe **1**) has been designed and explored for the possible detection of NTR. Our experimental results show that probe **1** could serve as an MRI-enhanced contrast agent for monitoring NTR activity. The *in vitro* response and mechanism of the NTR catalysed reduction of probe **1** have been investigated through LC–MS and MRI. *Para*-nitrobenzyl substituted probe **1** was catalytically reduced by NTR to the intermediate *para*-aminobenzyl substituted probe which then underwent a rearrangement elimination reaction to Gd-DOTA, generating the enhanced T_1-weighted MR imaging. Further, LC–MS and MRI studies of living *Escherichia coli* have confirmed the NTR activity detection ability of probe **1** at a cellular level. This method may potentially be used for the diagnosis of bacterial infections.

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1. Introduction

Molecular imaging provides a sensitive and specific method for non-invasive, real-time monitoring and visualization of biological processes *in vivo*[1–4]. Magnetic resonance imaging (MRI) has several advantages over other clinical diagnostic techniques for molecular imaging, in virtue of its high spatial resolution, unlimited penetration depth, and lack of harmful radiation[5–7]. Over the past three decades, paramagnetic Gd(III) complexes have been widely developed as contrast agents to dramatically improve detection sensitivity and specificity of MRI by shortening the relaxation times of the surrounding water proton resulting in an enhanced imaging contrast[8–11]. At present, the contrast agents most commonly used in clinical MRI are mainly small molecule gadolinium chelates, such as Magnevist (Gd-DTPA), Dotarem (Gd-DOTA), ProHance (Gd-HP-DO3A), etc. To further improve the enhancing effects and detect physiological changes at the molecular level, the smart MRI contrast agents have been developed and used[12–15]. These probes are capable of monitoring physiological processes by changing signal properties with changes in the physiological environment, such as enzyme[16–20], metal ion concentration[20–23], pH value[24,25], temperature[26], etc. Recently, applications of enzyme-activatable MRI contrast agents have been reported.

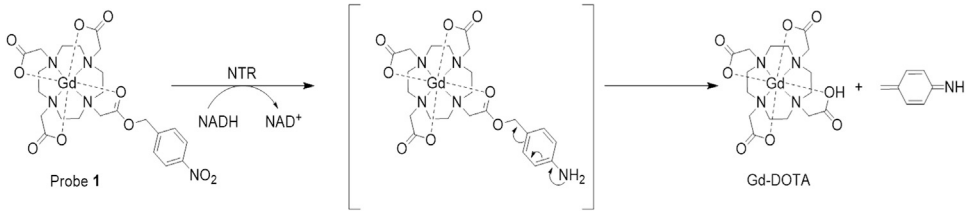
Nitroreductases (NTRs), are a family of flavin-containing enzymes widely exists in bacteria, which can effectively catalyze the reduction of nitroaromatic compounds into hydroxylamines or amines in the presence of reduced nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor *via* an one-electron reduction pathway[27–31]. An increasing number of nitroaromatic compounds have proved to be superior substrates for NTRs opening up the opportunity to develop enzyme-activatable probes, which is, given the role of the NTRs of great significance for environmental and human health[32–34]. Great effort has been devoted to the design of activatable optical probes for sensing NTR activities within hypoxic tumor cells; lately, optical probes for detecting NTR activities in bacterial lysates have been developed as well[33–38]. In comparison, a few NTR-activatable MRI contrast agents have been developed[39,40], and there appears to have been no real time NTR enzymatic activity detection in bacteria using an MRI method.

In this study, we designed and synthesized a novel NTR-enhanced MRI contrast agent: Gd-DOTA-PNB (probe **1**) by conjugating Gd-DOTA with an NTR-sensing moiety, a *p*-nitrobenzyl group. Probe **1** has been characterized by ^{1}H NMR, ^{13}C NMR, MS and evaluated as a new NTR-enhanced MRI contrast agent, which may potentially be used for the diagnosis of bacterial infections. Conceptually, we hypothesized that the *p*-nitrobenzyl moiety (PNB) of probe **1** would be reduced to a primary aromatic amino group by NTR in the presence of NADH, which would then trigger a self-immolative fragmentation through a rearrangement elimination reaction and formation of Gd-DOTA (Scheme 1) resulting a relaxivity enhancement, which could be used for NTR activities detection. The *in vitro* response and mechanism of the NTR catalysed reduction of probe **1** have been investigated through LC–MS and MRI. Further, LC–MS and MRI studies of living *Escherichia coli* (*E. coli*) have confirmed the NTR activity detection ability of probe **1** at a cellular level, which hint to the potential application of probe **1** for the diagnosis of bacterial infections.

2. Materials and methods

2.1. General methods

All chemicals were purchased from J&K (Beijing, China). Commercially available reagents were used without further purification. Unless otherwise noted, all reactions were performed under a nitrogen or argon atmosphere. NTR ($\geq$ 100 units/mg) from *E. coli*, and NADH were purchased from Sigma–Aldrich (Shanghai, China). The lyophilized powder of NTR was dissolved in pure water, and the solution was divided into aliquots suitable for daily experiments. All these enzyme solutions were stored at −20 °C and allowed to thaw before use according to the reported procedure, under the premise of no change of the enzyme activity[33,34]. A stock solution (10 mmol/L) for compounds **1** and **2** were prepared by dissolving an appropriate amount of them in H_2O. The *E. coli* (ATCC 25922) was purchased from American Type Culture Collection (ATCC), USA. MTS (3-(4,5-dimethylthiazol-2-yl)-5(3-carbo-xymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt) was obtained from Promega (Beijing, China). OD values and MTS assays were also measured by TECAN Spark 10 M microplate reader (Männedorf, Switzerland). Thin layer chromatography (TLC) was carried out with Silica Gel 60 F254, and column chromatography with silica gel (200–300 mesh). All ^{1}H NMR spectra were recorded at 600 MHz and ^{13}C NMR spectra were recorded at 150 MHz respectively



Scheme 1 Structure and reaction response mechanism of probe **1** to NTR.

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