Development of theranostics imaging probe for MRI and EPR imaging

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INTRODUCTION

With transition from conventional "trial and error" medicine to personalized medicine, the new approach using "theranostics" has emerged as a targeted, safe, and efficient pharmacotherapy. The term "theranostic" is defined as a material that combines the modalities of therapy and diagnostic imaging. Thus, theranostics deliver therapeutic drugs and diagnostic imaging agents simultaneously [1].In the present study, we challenged to synthesize theranostics for MRI and electron paramagnetic resonance (EPR) imaging, and applied these newly synthesized drugs to examine and treat a mouse model of brain disease. For therapeutics, the non-steroidal anti-inflammatory drugs ibuprofen and ketoprofen were chosen; for diagnostic imaging agents, paramagnetic nitroxide compounds were chosen. Ibuprofen and ketoprofen alone cannot pass the blood-brain barrier (BBB) due to their carboxylic acid groups; however, the novel probes have an ester group and can cross the BBB. Once reached in the brain, they separate into a drug and an imaging probe. The effectiveness of the novel theranostic compounds was examined in a mouse model of brain disease with both EPR imaging and MRI.

MATERIALS AND METHODS

Theranostics: The target theranostics were prepared by connecting ibuprofen or ketoprofen to 3-hydroxymethylPROXYL by a condensation reaction in the presence of DCC (1.05 equiv.) and DMAP (0.105 equiv.) in CH_2Cl_2. By silica gel column chromatography, ibuprofen-PROXYL or ketoprofen-PROXYL was afforded in yields of 97.5% or 96.7%, respectively. ***Animals*:** Male C57BL/6 mice aged 5 to 7 weeks were used. ***Paramagnetic nitroxides*:** Theranostic imaging probe solutions were injected by tail vein cannulation into the mice under isoflurane anesthesia. ***Magnetic resonance imaging (MRI) measurements*:** MRI of mouse heads was acquired using an MRmini scanner (MR Technology, Tsukuba, Japan) with a 0.5-T permanent magnet. ***EPR imaging measurements*:** All EPR images were obtained with an in-house built 750-MHz EPR imaging system. ***Lipopolysaccharide (LPS):*** LPS from *Escherichia coli* serotype 055:B5 was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Septic mice were prepared by intraperitoneal injection of LPS. **NO measurement:** The NO level in brain tissues was measured by the fluorescence detection method using diaminonaphthalene.

RESULTS AND DISCUSSION

Figure 1 shows the structural formulas of theranostic imaging probes, ibuprofen-PROXYL and ketoprofen-PROXYL. Figures 2A and 2B show EPR images in the sagittal plane of a living mouse head after injection of ibuprofen-PROXYL and ketoprofen-PROXYL, respectively. To confirm the distribution of these probes within the brain, these images are overlaid on MRI images (Fig. 2C and 2D). Co-registered images in Fig. 2E and 2F indicate that these compounds can enter the brain by passing through the BBB. Compared to ketoprofen-PROXYL, ibuprofen-PROXYL was more distributed in both the brain and tongue. The co-registered images in Fig. 2 clearly show that these theranostics act as brain imaging probes. To examine whether these theranostics function as therapeutic drugs, LPS was injected into mice 1 h after administration of ibuprofen-PROXYL. Twenty-four hours after LPS injection, LPS-treated mice were sacrificed, and the NO generated in brain tissues was measured. In Fig. 3 the amount of NO generated in brain tissues of examined mice is shown with or without the therapeutic drug, ibuprofen-PROXYL, indicating that generation of NO in LPS-treated mouse brain was remarkably suppressed by the addition of ibuprofen-PROXYL (Fig. 3, $p<0.001$ by one-way ANOVA). From these results, ibuprofen-PROXYL was also found to act as a therapeutic drug.

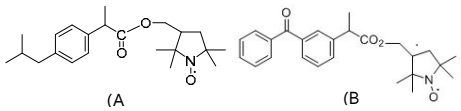

Figure 1: Structure of theranostics. (A) Ibuprofen-PROXYL, (B) Ketoprofen-PROXYL.

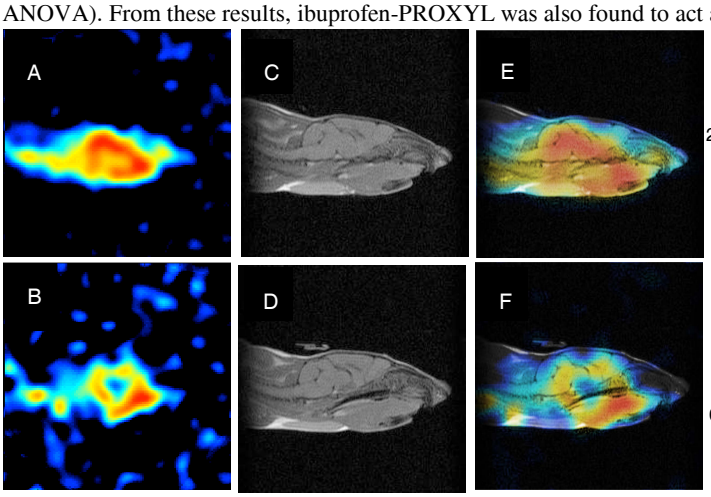

Figure 2 (Left): Distribution of theranostics by EPR imaging and MRI. EPR images of the mouse head for ibuprofen-PROXYL (A) and ketoprofen-PROXYL (B). MRI of mouse brain in sagittal plane (C, D). Co-registration of EPR image on MRI for ibuprofen-PROXYL (E) and ketoprofen-PROXYL (F).

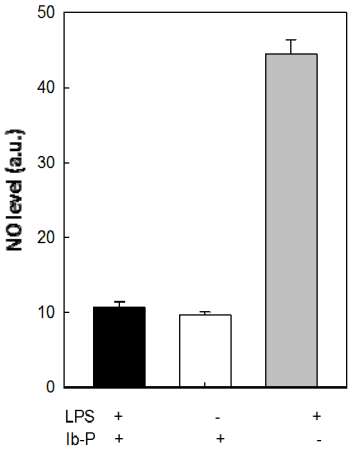

Figure 3 (Right): Generated NO in LPS-treated mouse brain with or without ibuprofen-PROXYL (Ib-P).

CONCLUSION

Two novel theranostic probes that act as both anti-inflammatory drugs that suppress NO generation and as brain imaging probes for EPR imaging and MRI were successfully developed. The present results suggest that both therapeutic action and diagnostic imaging of these theranostic probes can be followed by MRI and EPR imaging.

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Reference: 1) Kelkar S and Reineke T, Bioconjuq. Chem. 2011; 22: 1879-1903.