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A corrole nanobiologic elicits tissue-activated MRI contrast enhancement and tumor-targeted toxicity

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ABSTRACT

Water-soluble corroles with inherent fluorescence can form stable self-assemblies with tumor-targeted cell penetration proteins, and have been explored as agents for optical imaging and photosensitization of tumors in pre-clinical studies. However, the limited tissue-depth of excitation wavelengths limits their clinical applicability. To examine their utility in more clinically-relevant imaging and therapeutic modalities, here we have explored the use of corroles as contrast enhancing agents for magnetic resonance imaging (MRI), and evaluated their potential for tumor-selective delivery when encapsulated by a tumor-targeted polypeptide. We have found that a manganese-metallated corrole exhibits significant T1 relaxation shortening and MRI contrast enhancement that is blocked by particle formation in solution but yields considerable MRI contrast after tissue uptake. Cell entry but not low pH enables this. Additionally, the corrole elicited tumor-toxicity through the loss of mitochondrial membrane potential and cytoskeletal breakdown when delivered by the targeted polypeptide. The protein–corrole particle (which we call HerMn) exhibited improved therapeutic efficacy compared to current targeted therapies used in the clinic. Taken together with its tumor-preferential biodistribution, our findings indicate that HerMn can facilitate tumor-targeted toxicity after systemic delivery and tumor-selective MR imaging activatable by internalization.

1. Introduction

Multifunctional therapeutics, or "theranostics", combine several activities such as imaging, diagnostic, or therapeutic functions into a single complexed particle, thus providing a powerful approach to simultaneously detecting, diagnosing, and treating disease [1,2]. Engineering a theranostic commonly entails chemically conjugating different agents to a scaffold, such as a polymer, with each agent imparting a different function (*i.e.* tissue or cell targeting, membrane penetration, drug delivery, imaging). A potential complication with such approaches is the requirement for multiple covalent coupling reactions that increase the complexity and heterogeneity of the complex, and may abrogate the activities of its separate components. Sulfonated corroles can overcome this complication because they can non-covalently assemble with protein-based delivery agents while bearing potential theranostic functions, thus forming a multifunctional particle from only two components.

Sulfonated corroles are macrocyclic molecules with structural similarity to porphyrins and bear several notable characteristics that lend to their use as multifunctional payloads. The amphiphilicity of sulfonated corroles contributes to their water-solubility and tight non-covalent binding to proteins [3–5], thus enabling rapid assembly with carrier proteins and delivery in physiological conditions. The net negative charge of sulfonated corroles prevents their penetration into cells in the absence of a membrane-lytic molecule [3], thus enabling selective cell entry directed by the delivery agent. The incorporation of a metal ligand in the corrole ring can contribute to imaging and therapeutic potential. For example, non-metallated and gallium(III)-metallated corroles are both cytotoxic and intensely fluorescent [3–5], whereas iron(III) and manganese(III)-metallated corroles are non-fluorescent and have exhibited robust antioxidant activity on neuronal tissue [6].

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Whereas fluorescent corroles have been examined as agents for optical imaging of tumors in mice [7] and photosensitization of tumor cells *in vitro* [8,9], the limited tissue-depth of excitation wavelengths limits their clinical applicability. To assess the feasibility of using more clinically-relevant imaging modalities, here we explored the utility of corroles as contrast enhancing agents for magnetic resonance imaging (MRI), thus expanding the theranostics benefits inherent to the corroles.

In the current study, a comparison of *in vitro* T1 relaxation shortening and MRI contrast enhancement activities between sulfonated gallium(III), iron(III), and manganese(III)-metallated corroles singled out the manganese (Mn) compound as a candidate for further evaluation in the context of a tumor-targeted particle. Assembly of the particle, HerMn, resulted from binding of the Mn-corrole (or S2Mn) with the recombinant protein, HerPBK10. The peptide domains comprising HerPBK10 impart functions that can facilitate the targeted delivery (through binding to the human epidermal growth factor receptor or HER) and cell entry of membrane-impermeant molecules [10–13]. Importantly, HER2-3 heterodimers, which are recognized by the heregulin-derived targeting ligand of HerPBK10, are predominantly displayed on HER2+ tumors [14], hence targeting is preferential to HER2+ tumor cells [11,13,15]. In agreement, we have shown elsewhere that HerPBK10 can mediate preferential targeting *in vivo* to tumors expressing relatively higher HER2-3 levels in comparison to tumors expressing low/negligible levels of these receptor subunits [15].

Here we present evidence that the sulfonated Mn(III) corrole can elicit MRI contrast enhancement as well as tumor-selective toxicity when delivered by HerPBK10. The tumor-toxic activity comes somewhat as a surprise given the cytoprotective effects that this corrole has exhibited previously on non-tumor, specifically neuronal, tissue [6]. Hence, in this study, we also probe the mechanism of tumor-cell toxicity elicited by the Mn-corrole, and compare therapeutic efficacy *in vitro* against current targeted and non-targeted therapies used in the clinic. Finally, interrogating the MRI capability of protein-bound *vs.* free corrole yielded findings suggesting that contrast enhancement in tissue is activated by pH-independent mechanisms relying on cell uptake.

2. Materials and methods

2.1. Materials

The recombinant fusion protein, HerPBK10 (comprised of the receptor-binding domain of heregulin-α fused to the adenovirus penton base modified by a carboxy-terminal decalysine tail) [16], was produced and isolated from a bacterial expression system as described previously [13]. S2Mn (chemical structure shown in Fig. 1A) was synthesized, reconstituted in phosphate-buffered saline (PBS), and quantified as described previously [3]. HerMn complexes were assembled non-covalently by combining HerPBK10 and S2Mn as described [10]. Briefly, S2Mn and HerPBK10 were mixed at a corrole:protein ratio of 30:1 (the maximum number of corrole molecules bound per HerPBK10 protein, as determined previously) [3,11] in PBS and agitated gently on ice for 1 h, followed by ultrafiltration (50 K mwco) until the volume was reduced from 12 to <0.5 mL, to remove any free unincorporated corrole and concentrate the particle mixture. Retentates (containing HerMn particles) were recovered and analyzed by absorbance spectroscopy to determine the corrole and protein concentrations in HerMn and resulting corrole:protein ratio in each HerMn preparation, which remained at approx. 30:1 M ratio after filtration (Table S1). The concentration used for cell treatment was based on the corrole concentration in each complex.

2.2. Particle sizing

A Malvern ZEN 3600 Zetasizer Nano was used for Dynamic Light Scattering (DLS) measurement. Samples were pipetted into a low volume quartz cuvette with appropriate concentrations to prevent

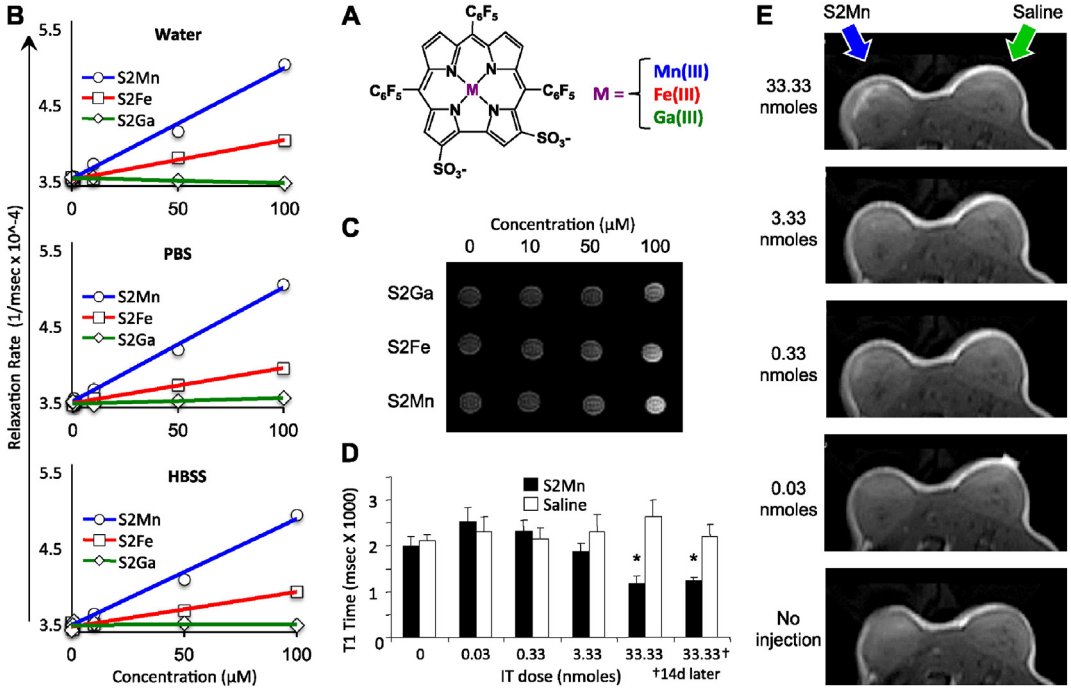

Fig. 1. Effect of Ga-, Fe-, and Mn- corroles on T1 relaxation rate and contrast change. A, Chemical structure of a metallated sulfonated corrole. S2Mn, S2Fe, and S2Ga are specified by replacement of metal ion (M) with Mn(III), Fe(III) or Ga(III), respectively. B–C, T1 relaxation times and contrast image of S2Mn, S2Fe, and S2Ga solutions *in vitro*. Graphs in B show T1 relaxation time measurements of indicated buffer solutions in Eppendorf tubes containing increasing concentrations of each corrole. N = 3 per concentration. Image in C shows the contrast change acquired from PBS containing indicated concentrations of corroles measured in B. D–E, T1 relaxation measurements and MRI of IT-injected tumors. Mice bearing HER2+ bilateral flank MDA-MB-435 tumors received IT injections of S2Mn in right tumors and equivalent volumes of saline in left tumors followed by (D) measurement of T1 change, and (E) MR image acquisition after delivery of indicated doses of S2Mn. In D, contralateral (left) tumors injected with saline are indicated by open bars. *, $p < 0.05$ as determined by t-test comparing S2Mn and saline. N = 8 different recovery intervals.