Mn Porphyrins as Novel Molecular Magnetic Resonance Imaging Contrast Agents

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Abstract

Background and Purpose: In this study, we investigated the potential of a new class of therapeutic Mn porphyrins as molecular MRI probes for prostate cancer imaging. Two compounds of different bioavailability were investigated: Mn(III) *meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin (MnTE-2-PyP⁵⁺) and Mn(III) *meso*-tetrakis(*N*-*n*-hexylpyridinium-2-yl)porphyrin (MnTnHex-2-PyP⁵⁺). These compounds have previously been shown to have adjunctive antineoplastic activity through their actions as powerful superoxide dismutase mimics, peroxynitrite scavengers, and modulators of cellular redox-based signaling pathways. Strong paramagnetic MRI contrast properties and affinity for cancer cells suggest their potential application as novel diagnostic imaging agents.

Materials and Methods: MRI experiments were performed at 7.0T on a Bruker Biospec horizontal bore scanner. All *in-vivo* experiments were performed on 12 C57 black mice implanted with RM-9 prostate cancer cells on the hind limb. Two mg/kg of MnTnHex-2-PyP⁵⁺ (n=6) and 8 mg/kg MnTE-2-PyP⁵⁺ (n=6) were administered intraperitoneally 90 minutes before imaging. All the images were collected using a volume coil and processed using Paravision 4.0.

Results: Phantom studies reveal remarkably high T1 relaxivity changes for both metalloporphyrins, which are twofold to threefold higher than commercially available gadolinium chelates. Observable detection limits using conventional T1-weighted MRI are in the low micromolar range for both compounds. *In vivo*, MR relaxation changes in prostate tumor xenografts were readily observed after a single injection of either MnTE-2-PyP⁵⁺ or MnTnHex-2-PyP⁵⁺, with tumor contrast to background ratio greatest after MnTE-2-PyP⁵⁺ administration.

Conclusion: After a single dose of MnTE-2-PyP⁵⁺, contrast changes in prostate tumors are up to sixfold greater than in surrounding, noncancerous tissues, suggesting the potential use of this metalloporphyrin as a novel diagnostic probe for detecting prostate malignancy using MRI.

Introduction

TO DATE, NO METHODOLOGY EXISTS that enables prostate cancer (PCa) imaging with sufficient sensitivity and specificity for diagnostic utility. As a consequence, urologic oncologists proceed with treatment strategies based on random biopsy tissue sampling and limited diagnostic evaluations using conventional MRI and transrectal ultrasonography.^{1,2} Molecular imaging techniques hold promise for improving prostate cancer imaging, yet currently approved radionuclear imaging methods such as fluoro-deoxy-glucose positron emission tomography do not achieve the requisite

sensitivity or specificity for clinical application, nor provide detailed anatomic or functional information essential for accurate diagnosis and treatment. The development of a high-resolution, noninvasive imaging technique that combines detailed anatomy with molecular specificity would therefore represent a major advance in noninvasive prostate cancer assessment.

In this study, we have explored a new class of therapeutic metalloporphyrins as molecular MRI probes for PCa detection. Although porphyrins have long been known to have high affinity for malignant cells, and, indeed, metal-based porphyrins have been investigated previously as potential

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MR contrast probes, the use of metalloporphyrins as molecular contrast agents has heretofore been limited by poor solubility and unacceptable toxicity.

Over the past several years, our group has developed a novel series of Mn porphyrin-based mimics of superoxide dismutase (SOD) enzyme with markedly improved solubility and toxicity profiles.^{3–5} A lead compound, Mn(III) *meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin (MnTE-2-PyP⁵⁺), with SOD activity equal to the native enzyme, and impressive adjunctive chemotherapeutic activity, was further modified to Mn(III) *meso*-tetrakis(*N*-n-hexylpyridinium-2-yl)porphyrin (MnTnHex-2-PyP⁵⁺), increasing lipophilicity by ~4 orders of magnitude and yielding much greater mitochondrial accumulations (Fig. 1).^{3–5} While MnTE-2-PyP⁵⁺ localizes to yeast mitochondria twofold to threefold more than cytosol, greater than 90% of MnTnHex-2-PyP⁵⁺ is found in mitochondria.⁶ Further, in animal studies, the more lipophilic MnTnHex-2-PyP⁵⁺ accumulates ~20-fold more in heart mitochondria than MnTE-2-PyP⁵⁺.^{3–5} Accumulation in mitochondria facilitates MnSOD mimicry, contributing to the remarkable efficacy of these compounds as both chemotherapeutic adjuncts and neuroprotectants.^{3–5,7}

We report the results related to the use of these compounds as MRI probes in phantoms and *in-vivo* murine model of PCA.

Methods

MRI experiments were performed at 7.0T on a Bruker Biospec horizontal bore scanner. *In-vitro* experiments were performed with different concentrations of porphyrins, Magnevist, ProHance, and MultiHance in Ringer solution. All *in-vivo* experiments were performed on 12 C57 black mice aged 4 to 6 weeks, implanted with RM-9 prostate cancer cells on the hind-limb location, as described previously. The size of the tumor at the time of injection of the contrast agent was no less than 1 cm³ and was usually observed 7 to 10 days after inoculation, corresponding to our intended time frame for tumor imaging during an early (localized) stage of disease. Before imaging, 2 mg/kg of hexyl analogue, MnTnHex-2-PyP⁵⁺ (n=6) and 8 mg/kg of ethyl analogue MnTE-2-PyP⁵⁺ (n=6) were administered intraperitoneally (IP) in a single dose.

Imaging studies were performed before and at 90 minutes after metalloporphyrin administration while the animals re-

FIG. 1. Structures of ortho Mn(III) *N*-alkylpyridylporphyrins (alkyl being ethyl, E, with 2 carbon-atom pyridyl substituents, and hexyl, Hex, with 6 carbon-atom pyridyl substituents), MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺.

FIG. 2. Phantom Studies: RARE T1-weighted images are shown for MnTE-2-PyP⁵⁺ at different concentrations in Ringers solution. The observable detection limits of MnTE-2-PyP⁵⁺ by 7T MRI are in the low micromolar range *in vitro* with conventional T1-weighted acquisitions.

mained in the scanner under light isoflurane anesthesia. T2 images were collected using a RARE based sequence, with a TE/TR=12/4200, thickness=1 mm, FOV=4 cm×4 cm, RARE factor=2. Quantitative T1 maps were performed with a RARE sequence with TE=9.7 ms, and variable TRs=300 ms, 800 ms, 1800 ms, 3000 ms, 5000 ms, 7500 ms. All images were collected using a send/receive volume coil. For phantoms, T1-weighted images were acquired with a RARE sequence with TE=7.5 and TR=1300 ms. All images were processed using Paravision 4.0.

Results

Phantom studies revealed remarkably high T1 relaxivity changes for both MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺ when compared with commercially available gadolinium chelates. T1-weighted imaging of MnTE-2-PyP⁵⁺ in phantoms is shown in Figure 2. Both Mn porphyrins were detectable at concentrations as low as 1 μM.

In vivo, we readily observe MR relaxation changes in prostate tumor xenografts after a single IP dose of either ethyl or hexyl analogues, although T1 shortening is the highest after hexyl administration (Table 1). Figure 3 shows superposition of T1 shortening merged onto T2 anatomic images. “T1 mapping” allowed absolute quantitation of Mn porphyrin accumulation in tumors over time.

TABLE 1. T1 RELAXIVITY OF DIFFERENT CONTRAST AGENTS AT 7T MRI (20°C).

Compound	Relaxivity, T1 (mM ⁻¹ s ⁻¹)
Magnevist	2.85
ProHance	2.37
MultiHance	3.19
MnTE-2-PyP ⁵⁺	5.09
MnTnHex-2-PyP ⁵⁺	5.34

FIG. 3. T2-weighted image of PCa tumor with hind flank muscle (top). Overlay of T1-weighted image over T2-weighted image (bottom left, predose and bottom right, post intraperitoneal injection) shows contrast enhancement within the tumor (*in-vivo* RM-9 model) after MnTE-2-PyP⁵⁺ intraperitoneal administration.

Figure 4 (A, B) demonstrates tumor delineation before and at 90 minutes after IP injection of MnTE-2-PyP⁵⁺ using quantitative T1 mapping, where absolute measurements of longitudinal shortening are depicted as a decrease in signal intensity rather than as an increase with conventional T1-weighted images in Figure 3. Table 2 shows comparison of observed tumor/tissue contrast for MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺.

There were no recorded adverse reactions or side effects of Mn porphyrins on the study animals. All 12 mice tolerated MRI studies well and were sacrificed on completion of the experiment. The gross anatomy and histologic staining of the tumor site confirmed the high aggressiveness of RM-9 cell line tumor that has been reported previously.

FIG. 4. *In-vivo* quantitative T1 mapping in the RM-9 tumor model: (A) before injection and (B) 90 minutes after 8 mg/kg intraperitoneal injection of MnTE-2-PyP⁵⁺.

Discussion

The search for novel prostate cancer molecular imaging agents is an active area of interest for the urologic oncology community with the goals of achieving better diagnostic specificity, detailed tumor localization and extension (ie, staging), and providing a means for monitoring tumor response to less invasive treatment options, including cryotherapy, radiation, and chemotherapy.

Although MRI currently achieves the best tissue contrast among available clinical imaging modalities, its specificity for cancer detection remains limited, especially in the prostate. Existing MRI contrast agents in clinical practice based on lanthanide chelates (eg, gadopentetate dimeglumine, gadobenate dimeglumine) do little to improve specificity, especially in early stage disease. Lanthanide chelates localize primarily by extravasation through blood vessels, and their differential accumulation in various tissues is primarily a function of the relative “leakiness” of local vasculature. Thus, currently approved MRI contrast agents have limited application in PCa imaging where concomitant changes in vascular integrity are not a dominant feature.^{8–10} Potential toxicity (ie, nephrogenic systemic fibrosis) of gadolinium compounds, although extremely rare, further limits their application.^{11–13}

In the present study, we have examined a novel class of water-soluble Mn(III) *N*-alkylpyridylporphyrins for their potential as selective MRI probes for PCa imaging. The pentacationic structure of these porphyrins enables their high SOD-like potency and water solubility. Further modification of the starting metalloporphyrin scaffold led to the design of more lipophilic, but nonetheless water-soluble compounds that more easily cross plasma and mitochondrial membranes.^{3–5} MnTnHex-2-PyP⁵⁺ is a potent SOD mimic and is significantly more lipophilic than its corresponding ethyl analog, MnTE-2-PyP⁵⁺.^{4,14} The ability of both MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺ to mimic site and action of essential endogenous antioxidant defense enzyme, mitochondrial MnSOD, contributes to their high *in-vivo* efficacy as SOD mimics.^{3–6}

We have shown earlier that both compounds accumulate in tumors.^{15,16} Further, MnTnHex-2-PyP⁵⁺ accumulates ~five-fold more in a tumor grown on a mouse right flank than in the muscle from the left leg in a 4T1 mouse study, either when injected alone or coadministered with ascorbate.^{16,17} Relative accumulation within tumors is at least in part believed to be secondary to increased tumor perfusion, leaky vasculature, larger cancer cell surface area, and net surface negative charge relative to normal cells.¹⁷

In MRI experiments at 7T, phantom studies reveal high T1 relaxivity changes for both hexyl and ethyl analogues, two-fold to threefold higher than the relaxivity observed with commercially available gadolinium chelates. Observable detection limits are in the low micromolar range, one to two orders of magnitude lower than of the conventional chelates using routine T1-weighted imaging sequences. *In vivo*, we readily observe MRI relaxation changes in prostate tumor xenografts after a single IP dose of either MnTE-2-PyP⁵⁺ or MnTnHex-2-PyP⁵⁺, although T1 shortening is highest after MnTnHex-2-PyP⁵⁺ administration. After a single dose of MnTnHex-2-PyP⁵⁺, the drop in relaxation (T1) is 6.3% in tumor compared with 9% in adjacent tissue (Table 2). With MnTE-2-PyP⁵⁺, the drop in relaxation in tumor is 2.7%, and no drop in tissue was detected. Although the drop in tumor

TABLE 2. OBSERVED *IN VIVO* TUMOR/TISSUE CONTRAST FOR MnTnHex-2-PyP⁵⁺ AND MnTE-2-PyP⁵⁺ GIVEN INTRAPERITONEALLY AT 2 MG/KG AND 8 MG/KG, RESPECTIVELY

Drug	Prostate tumor T1 map (ms)		Tissue T1 map (ms)		% Drop		
	Before	After	Before	After	Tumor	Tissue	(Tumor-tissue)
MnTnHex-2-PyP ⁵⁺	3708 ± 147	3475 ± 213	4491 ± 171	4083 ± 288	6.3	9.1	−2.8
MnTE-2-PyP ⁵⁺	3591 ± 155	3488 ± 22	3915 ± 342	4051 ± 434	2.7	−3.3	6.0

Before refers to no drug and After refers to 90 minutes after single intraperitoneal injection of Mn porphyrins.

for MnTE-2-PyP⁵⁺ is smaller, preferential tumor *vs* background tissue accumulation is greater than that observed with MnTnHex-2-PyP⁵⁺, and therefore suggests MnTE-2-PyP⁵⁺ may have more potential as a selective diagnostic contrast agent for MRI of PCa.

Note should be made that this initial investigation studied one cell line, RM-9, which is relatively aggressive, and therefore selective targeting and bioaccumulation of metalloporphyrins may vary considerably in more differentiated and quiescent tumor cells. In addition, the tumor implants investigated were subcutaneously implanted in mouse hind limbs, possibly altering the apparent pharmacokinetics and biodistribution of metalloporphyrins as compared to tumors *in situ*.

Conclusion

We have developed a novel series of metalloporphyrins, MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺, with chemotherapeutic activity, strong paramagnetic contrast enhancement, and strong tumor avidity. The strength of contrast enhancement *in vivo*, their specificity for cancer cells, water solubility, and improved toxicity profile suggest their potential as diagnostic MRI probes for PCa. Both MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺ were detectable at micromolar concentrations *in vitro* at 7T. Given that paramagnetic contrast from longitudinal relaxation increases with decreasing field strength, the unusual contrast strength of these compounds would be expected to only improve further at clinical MRI field strengths (ie, 1.5T and 3.0T). When compared with gadolinium-based chelates at 7T, these compounds also show two- to threefold higher relaxivity.

Ongoing studies are exploring the possible use of a broader range of Mn(III) *N*-substituted pyridylporphyrins with different bioavailabilities. Most recently, a new lipophilic analogue, Mn(III) *meso*-tetrakis(*N*-*n*-butoxyethylpyridinium-2-yl)porphyrin¹⁸ demonstrates still greater improvement in toxicity profile and will serve as our lead compound in follow-on diagnostic studies.

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Disclosure Statement

No competing financial interests exist.

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Abbreviations

IP = intraperitoneal
MnTE-2-PyP⁵⁺ = Mn(III) *meso*-tetrakis
(*N*-ethylpyridinium-2-yl)porphyrin
MnTnHex-2-PyP⁵⁺ = Mn(III) *meso*-tetrakis
(*N*-n-hexylpyridinium-2-yl)porphyrin
MRI = magnetic resonance imaging
PCa = prostate cancer
SOD = superoxide dismutase