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Synthesis and in vitro and in vivo evaluation of manganese(III) porphyrin–dextran as a novel MRI contrast agent

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ABSTRACT

5-(4-Aminophenyl)-10,15,20-tris(4-sulfonatophenyl) manganese(III) porphyrin conjugated with dextran was synthesized. Its potential of being used as a tumor-targeting magnetic resonance imaging contrast agent was evaluated in vitro and in vivo. The results demonstrated that the compound has a longitudinal relaxivity (R_1) higher than Gd-DTPA, low cytotoxicity and binding specificity to tumor cell membrane.

Magnetic resonance imaging (MRI) is one of the most popular diagnostic techniques used in clinic, and allows imaging of the body in a noninvasive manner.[1,2] Exploiting the differences in longitudinal or transverse relaxation times (T_1 or T_2) among different tissues, MRI provides not only morphological, but also physiological and functional information about the subject being imaged.[3] Currently, about one-third of the clinical MRI scans are accompanied by administration of a contrast agent.[4]

Gadolinium and manganese complexes are widely used as MRI contrast agents. The commercially-available contrast agent Gd-DTPA undergoes rapid renal excretion and has no selectivity for tumors.[5,6] Porphyrins are a unique class of metal chelating agents, and show selective affinity for a variety of tumors.[7] Although gadolinium porphyrin has a high relaxivity of 22.8 mM^{-1} s^{-1}, it dissociates rapidly in solution,[8] making the compound less useful in practice. The reason for this is that gadolinium ion is too large to fit into the porphyrin core and only binds to the chelate loosely.

Manganese, existing in human body by nature, plays an important role in human biochemistry and could chelate with porphyrin ring tightly.[9,10] In this work, we report the synthesis of 5-(4-aminophenyl)-10,15,20-tris(4-sulfonatophenyl) manganese(III) porphyrin conjugated with dextran (**2**). The aim is to investigate whether or not conjugation with the dextran moiety could improve the properties of porphyrin **2** as a tumor-targeting MRI contrast agent.

The synthesis of porphyrin **1**, the 5-(4-aminophe-nyl)-10,15,20-tris(4-sulfonato-phenyl) porphyrin was the same as previously described.[11] Porphyrin **1** was refluxed with manganese acetate in methanol for 1.5 h. The product was purified on a silica gel column.[12]

To synthesize porphyrin **2** (Scheme 1), oxidized dextran (MW: 30000) by $NaIO_4$ was conjugated to porphyrin **1** in water, and the final product was obtained by dialysis and gel filtration chromatography on Sephacryl-300.[13]

The effects of porphyrin **1** and **2** on the viability of two human cancer cell lines (Hela and HepG2) were assessed by MTT assay (Fig. 1).[14] Generally no significant cytotoxic effects was observed for porphyrin **1** and **2 on** these two cancer cell lines. However, at a high concentration of 400 μm, Porphyrin **1** appeared to be toxic to HepG2 cells.

The longitudinal relaxivity (R_1) of porphyrin **1**, **2**, and Gd-DTPA in aqueous solution were determined in vitro (Table 1).[15] The concentrations of Mn and Gd in the solutions for these measurements were determined with ICP-AES.[16] Both porphyrin **1** and **2** showed higher R_1 than Gd-DTPA. Electrostatic interaction has been found to be the dominant attractive force between water-soluble porphyrins and the surrounding water molecules.[17] Thus the rigid porphyrin structure can potentially modulate the internuclear distance

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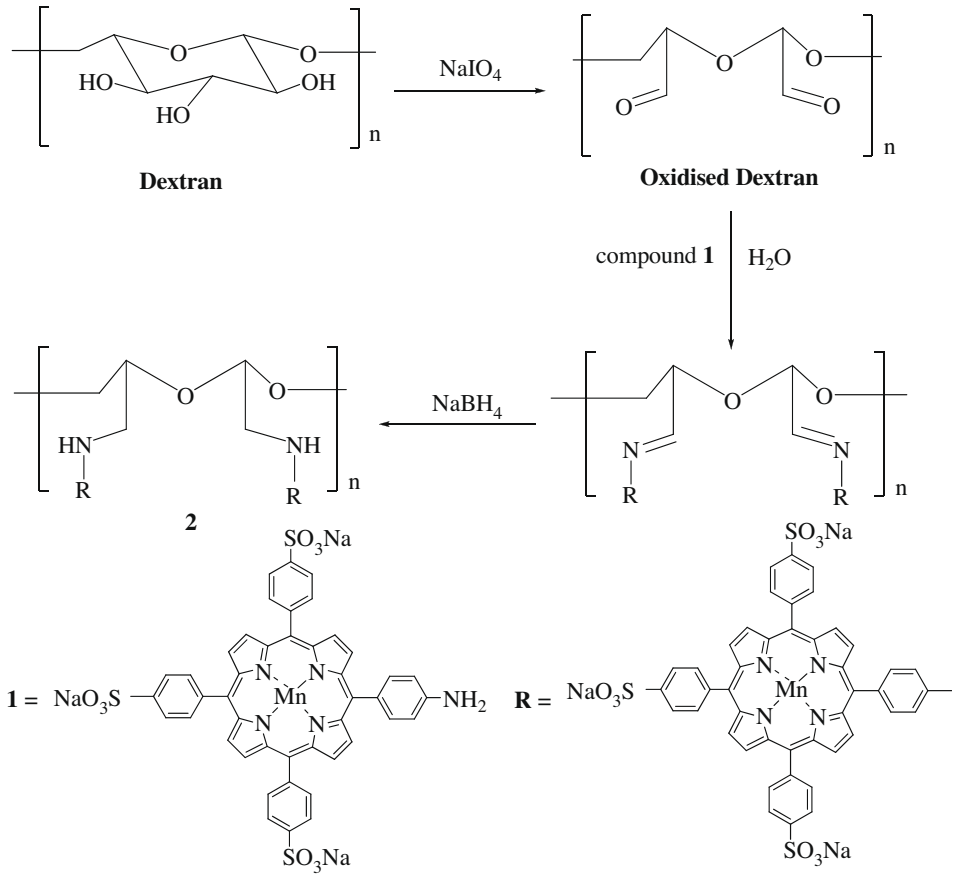

Scheme 1. Synthetic route.

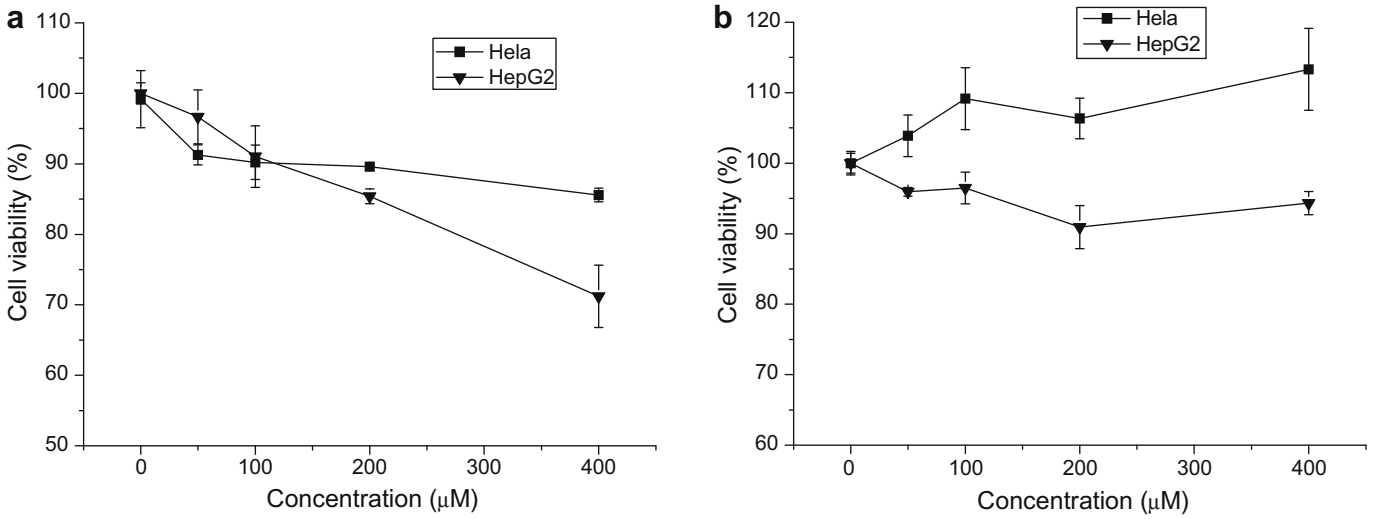

Figure 1. Cytotoxicity of porphyrin **1** (a) and **2** (b) to two tumor cell lines at different concentrations.

between the paramagnetic metal center and the hydrogen atoms in the surrounding water molecules. This modulation could have contributed to the high relaxivity of porphyrin **1** and **2**.[18–20] Among the three contrast agents, porphyrin **2** exhibited the highest relaxivity. This was likely due to its high molecular weight. Comparing to the un-conjugated porphyrin, the porphyrin–dextran complex could have a longer molecular rotational correlation time (τ_r) and a paramagnetic center having higher relaxation efficiency.

The abilities of porphyrin **2** and Gd-DTPA to enhance solid tumor was evaluated with in vivo MRI on ICR male mice implanted with H22 hepatoma tumors (Fig. 2).[21] Intravenous injection of

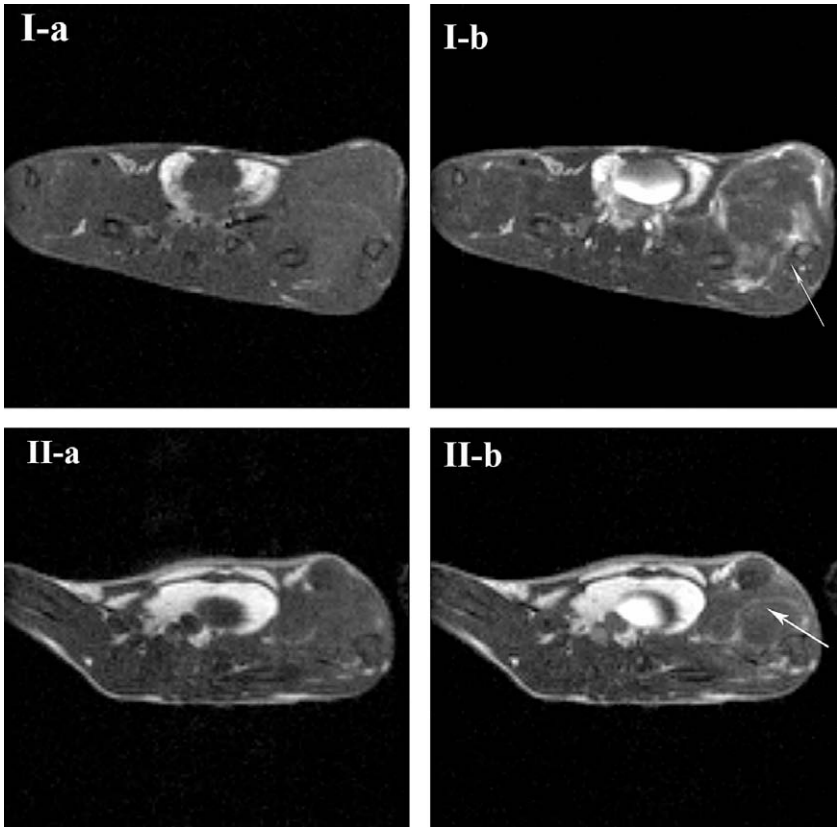

Figure 2. T_1-weighted magnetic resonance images tumor-bearing mice before and after intravenous administration of porphyrin **2** (I) and Gd-DTPA (II): (a) prior to injection; (b) post-injection, 30 min. Arrows indicate the enhanced boundary of the tumors.

Table 1
Longitudinal relaxivity (R_1) of porphyrin **1**, **2** and Gd-DTPA in aqueous solution measured at 200 MHz

Compounds	R_1 (mM^{-1} S^{-1})
Porphyrin **1**	7.36
Porphyrin **2**	8.90
Gd-DTPA	5.12

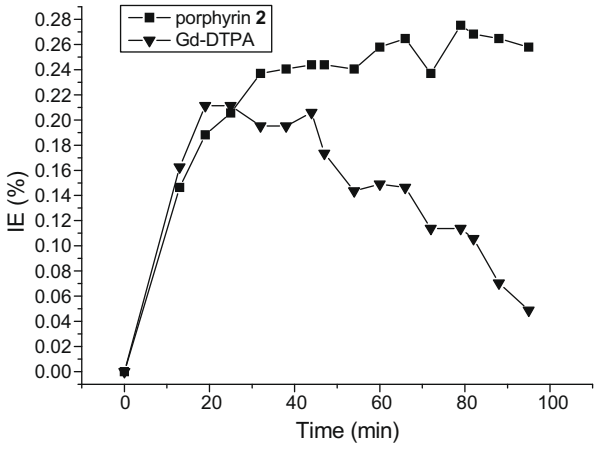

Figure 3. Time dependence of porphyrin **2** and Gd-DTPA induced intensity enhancement in tumor.

porphyrin **2** (0.050 mmol kg^{-1} Mn) or Gd-DTPA (0.130 mmol kg^{-1} Gd) resulted in significant signal intensity enhancements (IE) in part of the tumors.

The changes in IE of the tumor with time were plotted in Figure 3. Porphyrin **2**-induced signal enhancement increased gradually during the observation period, while that of Gd-DTPA peaked at about 20 min after injection and started to decrease at 40 min post-injection. Relative to Gd-DTPA, porphyrin **2** produced higher and longer signal enhancement of the tumor, probably due to the combination of two effects: (1) higher R_1 of the complex; (2) specific binding of the porphyrin complex to the tumor.

The location of porphyrin **2** in the HepG2 cell was evaluated by confocal fluorescence microscopy (Fig. 4). HepG2 cell culture was incubated in a DMEM solution[22] containing 100 μM porphyrin **2** for 24 h, and washed twice with PBS before observation. The culture was excited with a blue light at 467 nm. Red fluorescence was observed on the cell membranes, demonstrating the localization of the porphyrin moieties. The finding that porphyrin was localized to the cell membranes is consistent with previous reports.[23] Conjugation to dextran could have potentially facilitated the localization of the porphyrin complex to the membranes. The plasma membrane is composed of lipid, protein and carbohydrate, which arranges in a fluid mosaic structure. Glycolipids locate in the external part of the plasma membrane.[24,25] Dextran could assemble with the glycolipids and have facilitated targeting of porphyrin **2** to the tumor cells.

In conclusion, 5-(4-aminophenyl)-10,15,20-tris(4-sulfonatophenyl) manganese(III) porphyrin conjugated with dextran was prepared. The complex exhibited high relaxivity and targeting specificity to tumor.

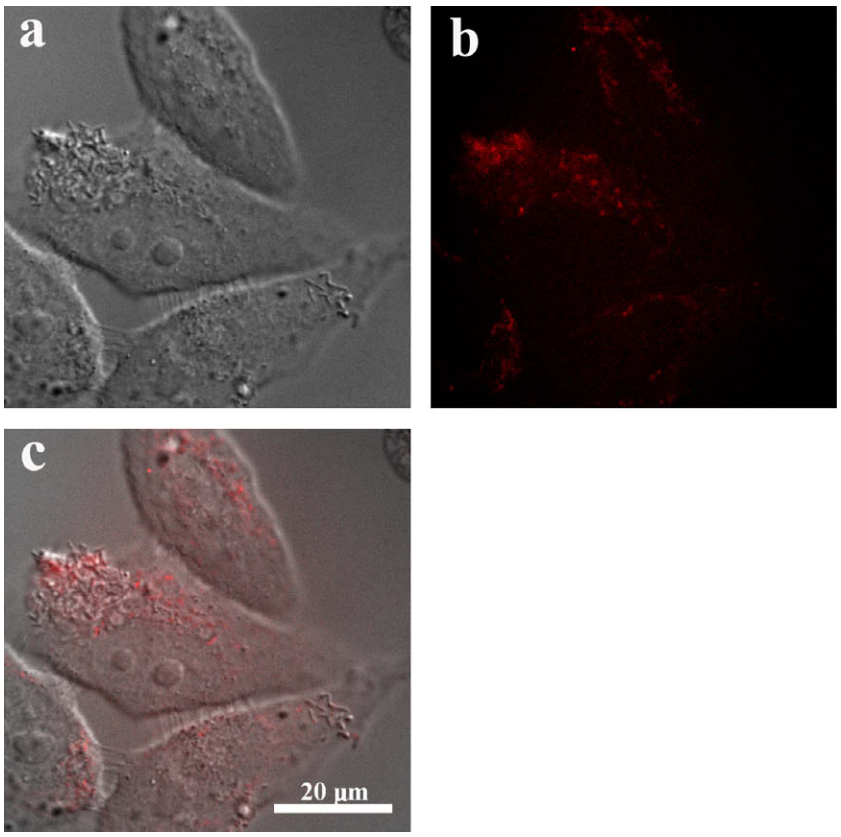

Figure 4. Confocal fluorescence microscope images of HepG2 incubated porphyrin **2** (II): (a) transmission light microscopy image, (b) confocal fluorescence microscopy image, and (c) overlay of the light microscopy and confocal fluorescence microscopy images.

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References and notes

1. Dujardin, M.; Vandenbroucke, F.; Boulet, C.; Op de Beeck, B.; de Mey *J. Eur. J. Radiol.* **2008**, *65*, 214.
2. Damadian, R. *Science* **1971**, *171*, 1151.
3. Curtet, C.; Tellier, C.; Bohy, J.; Conti, M. L.; Saccavini, J. C.; Thedrez, P.; Douillard, J. Y.; Chatal, J. F.; Koprowski, H. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4277.
4. Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293.
5. Gries, H. *Top. Curr. Chem.* **2002**, *221*, 1.
6. Feng, J. H.; Sun, G. Y.; Pei, F. K.; Liu, M. L. *Bioorg. Med. Chem.* **2003**, *11*, 3359.
7. Ogan, M. D.; Revel, D.; Brasch, R. C. *Invest. Radiol.* **1987**, *22*, 822.
8. Lyon, R. C.; Faustino, P. J.; Cohen, J. S.; Katz, A.; Mornex, F.; Colcher, D.; Baglin, C.; Koenig, S. H.; Hambright, P. *Magn. Reson. Med.* **1987**, *4*, 24.
9. Megnin, F.; Faustino, P. J.; Lyon, R. C.; Lelkes, P. I.; Cohen, J. S. *Biochim. Biophys. Acta* **1987**, *929*, 173.
10. Chen, C. Y.; Zhang, P. Q.; Lu, X. L.; Hou, X. L.; Chai, Z. F. *Fresenius J. Anal. Chem.* **1999**, *363*, 512.
11. Luguya, R.; Jaquinod, L.; Fronczek, F. R.; Vicente, M. G. H.; Smith, K. M. *Tetrahedron* **2004**, *60*, 2757.
12. Spectral characteristics of porphyrin **1**: UV–vis (H_2O): λ_{max}, nm 467 nm (Soret band), 570 nm, 612 nm. IR (KBr, v, cm^{-1}): 3226 (NH), 2916 (CH). ESI-MS: 989 (M^+).
13. Spectral characteristics of porphyrin **2**: UV–vis (H_2O): λ_{max}, nm 242 nm (OH), 468 nm (Soret band), 566 nm, 602 nm; IR (KBr, v, cm^{-1}): 3200 (NH), 2925 (CH), 1188 (C–N).
14. Mosmann, T. *J. Immunol.* **1983**, *65*, 55.
15. Experiment was carried out on a 4.7 T/30 cm Bruker Biospec scanner (Ettlingen, Germany) equipped with actively shielded gradients at 25 °C and 4.7 T. R_1 was measured by inversion-recovery experiments with following parameters: repetition time (TR) = 10 s, echo time (TE) = 13.5 ms, FOV = 3 cm × 3 cm, matrix size = 64 × 64 and slice thickness (SLT) = 1 mm.
16. ICP-AES: Inductively coupled plasma atomic emission spectrometry. An Intrepid XSP Rasial ICP-AES instrument (Thermo, USA) with a concentric nebulizer and a cinnabar model spray chamber was used.
17. Åkesson, R.; Pettersson, L. G. M.; Sandström, W. U. *J. Am. Chem. Soc.* **1994**, *116*, 8691.
18. Mercier, G. A., Jr. *Reson. Imaging* **1998**, *16*, 811.
19. Aime, S.; Botta, M.; Fasano, M.; Paoletti, S.; Anelli, P. L.; Uggeri, F.; Virtuani, M. *Inorg. Chem.* **1994**, *33*, 4707.
20. Powell, D. H.; Ni Dhubhghaill, O. M.; Pubanz, D.; Helm, L.; Lebedev, Y. S.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333.
21. T_1-weighted images were acquired with a saturation-recovery sequence, TR = 300 ms, TE = 13.6 ms, FOV = 5 cm × 5 cm, matrix size = 128 × 128 and SLT = 1 mm.
22. DMEM: Dulbecco's modified eagles's medium.
23. Sailer, R.; Strauss, W. S. L.; Emmert, H.; Stock, K.; Steiner, R.; Schneckenburger, H. *Photochem. Photobiol.* **2000**, *71*, 460.
24. Taylor, D. L.; Wang, Y. L. *Nature* **1980**, *284*, 405.
25. Hara-Yokoyama, M. *Curr. Med. Chem.* **2006**, *13*, 2233.