Biological Modifiers of Novel Theranostic Metalloporphyrins

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Purpose / Introduction: Metalloporpyrins have been investigated previously for their potential as both diagnostic and therapeutic agents. Translation, however, has been limited by poor solubility and toxicity. We have recently developed a novel class of water-soluble, paramagnetic manganese-based porphyrins (MnPs) with markedly improved biocompatibility, and that also demonstrate theranostic activity *in vivo* as both powerful chemotherapeutics and tissue-selective molecular MRI probes [1,2]. Active redox cycling is an essential mechanism underlying the therapeutic actions of these MnPs as SOD mimetics [3], however dynamic changes in oxidation state of the central metal are also likely to influence relaxation properties, rendering more complex the interpretation of *in vivo* quantitative MR assessments. As with other paramagnetic chelates, relative protein binding of these intracellular MnPs may also influence relaxation. This study investigates the potential impact of common biological moieties on the relaxation properties of our two lead candidate MnPs. These include the most abundant intracellular redox mediator, ascorbate; (2) the metabolically relevant counter-anion, citrate; (3) and serum protein, albumin.

Materials and Methods: Phantoms: MnPs were prepared as previously described [4]. The MnP concentration range examined was 500µM, 125µM, 62.5µM, 32.25µM. Ascorbic acid and Na- Citrate were prepared at 10 mM in PBS. For Human Serum Albumin (HSA) phantoms, stock HSA (Talecris BioTheropeutics) was diluted to 4% and 20% in PBS. MRI Measurements: All MRI experiments were carried out at 7.0T on a 70/30 Bruker Biospec (Bruker, Billerica, MA) system with quadrature volume coil at 20°C. T_1 experiments were performed using a saturation-recovery sequence with the following parameters: matrix 256 × 256, FOV = 6×6, TE = 9.5 ms, TR = 60, 300, 750, 1500, 4000 and 8000 msec. T_2 experiments were performed at FOV= 6×6, matrix 256 × 256; TR = 2400 ms; TE = 20, 40, 60, 80, 100, 120, 140 and 160 msecs. T_1 & T_2 maps were post-processed using Paravision 5.1 (Bruker, Billerica, MA).

Results and Discussion: The structure of our two lead MnPs are shown in **Fig 1**. **Table 1** summarizes r_1 and r_2 relaxivities of MnPs at 0%, 4% and 20% HSA, showing no significant influence from protein binding on relaxation properties. **Table 2** compares relaxivities in PBS, ascorbic acid and citric acid. In the presence of ascorbic acid, r_1 relaxivity decreases by 41% for MnTE2PyP5+ and 26% for MnTnHex2PyP. In the presence of citric acid, however, a 29% increase in relaxivity is observed for MnTE2PyP5+ and a 4% increase is measured for MnTnHex2PyP.

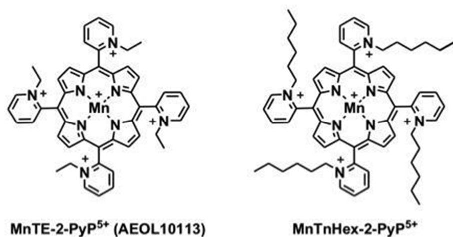

Fig 1: Structure of MnPs: MnTE-2-PyP^{5+} (left) and MnTnHex-2-PyP^{5+} (right)

Compound	**0% HSA**			**4% HSA**			**20% HSA**		
	r_1	**r_2**	**r_2/r_1**	**r_1**	**r_2**	**r_2/r_1**	**r_1**	**r_2**	**r_2/r_1**
MnTE-2-PyP^{5+}	4.1	6.9	1.68	4.48	7.45	1.66	5.4	8.76	1.62
MnTnHex-2-PyP^{5+}	4.75	9.53	2.0	4.98	9.85	1.98	4.87	8.40	1.72

Table 1: Longitudinal (r_1) and Transverse (r_2) relaxivities [$mM^{-1}Sec^{-1}$] of MnPs in 0%, 4% and 20% HSA.

Compound	**PBS**			**AA**			**Citric Acid**		
	r_1	**r_2**	**r_2/r_1**	**r_1**	**r_2**	**r_2/r_1**	**r_1**	**r_2**	**r_2/r_1**
MnTE2PyP5+	**4.1**	**6.9**	**1.68**	**2.4**	**3.4**	**1.41**	**5.3**	**7.7**	**1.48**
MnTnHex2PyP5+	**4.7**	**9.53**	**2.22**	**3.5**	**7.4**	**2.11**	**4.87**	**8.41**	**1.72**

Table 2: Longitudinal (r_1) and Transverse (r_2) relaxivities [mM^{-1} sec^{-1}] in the presence of Ascorbic Acid and Citric Acid

Conclusions: Transverse and longitudinal relaxivities of MnTE2PyP^{5+} and MnTnHex2PyP^{5+} were investigated with respect to the influence of potential biological modifiers such as protein binding, oxidation state, and anion association. Although a subtle systematic increase in longitudinal (r_1) relaxivity was observed for the ortho form of MnP in the presence of albumin, r_2/r_1 otherwise remained virtually independent of HSA concentration, in keeping with previous data also consistent with weak protein binding of MnPs. With respect to likely biological redox modifiers, both MnP isoforms showed a systematic reduction in relaxivity in the presence of ascorbate, consistent with the hypothesis that metal center oxidation state will have a significant impact on relaxation properties of MnPs *in vivo*. Intriguingly, citrate anion does not negatively impact relaxation, and in the case of MnTE2PyP^{5+}, may actually enhance contrast enhancement. Thus, citrate does not appear to compete with inner sphere water exchange with MnPs, but may instead facilitate this mechanism. Regardless of oxidation state, however, these two isoforms of MnP demonstrate impressive relaxation properties that enable MR detection *in vivo*. Further characterization of potential biological modifiers will facilitate future use of these therapeutically active MnPs as quantitative MR probes, as well as possibly including their use as selective *in vivo* MR indicators of intracellular redox status in diseased and normal tissues.

References: [1] Batinic-Haberle I *et al* Antioxid Redox Signal 2010;13:877-918. [2] Mouraviev V *et al* J Endourol 2012;11:1420-4. [3] Spasojevic I *et al* Free Radical Research 2011;45:188-200. [4] Batinic-Haberle I et al J Chem Soc Dalton Trans 2002;2689-2696.