Novel Mn-Porphyrin Contrast Probe for Molecular MR Imaging of Glial Reactivity in the Rat Brain

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Background:

Commonly employed contrast agents in clinical MR imaging, such as the lanthanide chelates (e.g. gadopentate dimeglumine), localize to areas of pathology characterized by hypervascularity and/or blood vessel permeability. Their mechanism is therefore inherently nonspecific. Additional limitations are evident in neurologic imaging where the tight junctions of the blood-brain-barrier (BBB) preclude contrast agent accumulation in regions of brain tissue even in the presence of known pathology.

Microglial activation and the concomitant production of reactive oxygen species have been implicated in numerous neuropathology models including stroke, Alzheimer's disease, and multiple sclerosis. Spreading depression is a well-established model for microglial activation in the absence of detectable neuronal injury and is known to preserve the BBB[1]. Further, PK11195 is a potent ligand of peripheral-type benzodiazepine receptors (PBR) specific to activated microglia[2].

The paramagnetic superoxide dismutase (SOD) mimetric cationic Mn(III) *ortho*-*N*-alkylpyridylporphyrins have previously been investigated as potential agents for neuroprotection and cancer therapy secondary to their industrious catalytic activity as reactive oxygen species scavengers. Mn(III) *meso*-tetrakis (*N*-n-hexylpyridinium- 2-yl) porphyrin (MnTnHex-2-PyP5+) and its analogues demonstrate potent *in vivo* SOD-like catalytic activity and have the benefit of adjustable lipophilicity achieved through variations in their molecular structure. This latter characteristic allows for their previously demonstrated accumulation in brain tissue despite an intact BBB.

We performed a series of experiments designed to determine if the lipophilic MnTnHex-2-PyP5+ could be used as an MR contrast agent sensitive to microglial activation in the setting of an intact BBB. Our efforts also began an exploration of the molecular basis of their reactive glial specificity.

Methods:

All imaging experiments were performed on a Bruker Biospec 7T horizontal MR scanner. Examination of *in vitro* T1 relaxation times was performed via creation of standard phantoms with variable concentrations of the hexyl porphyrin in solutions of PBS (1x containing 1mM of $MgCl_2$ and $CaCl_2$), PBS with Glycerol (2.13:1 ratio), and PBS with 20% BSA, respectively. The rat spreading depression model was created as previously described. Spreading depression was induced throughout the right hemisphere, leaving the left hemisphere as an internal control. Experimental rats were subcutaneously administered 2mg/kg of MnTnHex-2-PyP5+ at 24 hours after the completion of the spreading depression procedure. *In vivo* images with T1 relaxivity measurements were acquired at 48 hours, 72 hours, and 1 week after spreading depression induction. The T1 relaxation time measurements were carried out using a saturation-recovery variable TR RARE sequence (TE = 11ms; TR = 300, 600, 1500, 4000, 9000 ms). Competitive blockade experiments were performed in an analogous manner with the addition of intraperitoneal injections of PK11195 dissolved in DMSO (20 mg/kg at 24 hours followed by 5 mg/kg at days 2 through 7).

Results:

MnTnHex-2-PyP5+ exhibits strong T1 relaxivity in vitro with demonstrable changes at low micromolar concentrations in phantom experiments (Fig 2 and 3). Images acquired of the rat brain at 24 hours after induction of spreading depression (with concomitant creation of diffuse hemispheric microglial activation) and prior to the administration of porphyrin revealed an initial decrease in T1 relaxivity within the experimental hemisphere that corresponded to areas of T2 hyperintensity (suggestive of inflammation). After administration of MnTnHex-2-PyP5+, 24-hour images (48-hours after spreading depression induction) exhibited increased T1 relaxivity in comparison to the contralateral control hemisphere (2002 and 2204 msec, respectively). This effect was prevented via the administration of PK11195 (Fig 5).

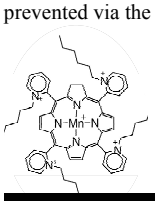

Figure 1:
MnTnHex-2-PyP5+

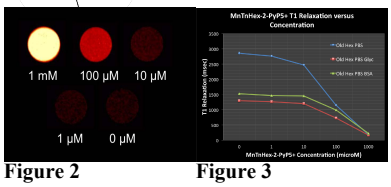

Figure 2 **Figure 3**

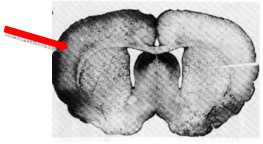

Figure 4:
Immunohistochemistry specific for gliosis.

References:
1. Kraig RP et al. J Neurosci. 1991 Jul;11(7):2187-98.
2. Ji et al. J Neurosci. 2008 Nov;28(47):12255-12267.

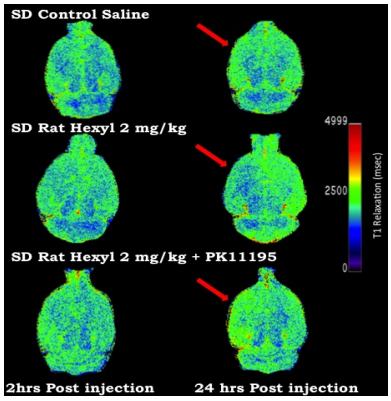

Figure 5

Conclusion:

The efficacy of standard contrast agents in MR imaging of neuropathology is limited by their non-specific nature as well as their inability to penetrate the BBB. Initial experiments demonstrate successful induction of increased T1 relaxivity after the administration of MnTnHex-2-PyP5+ within an experimental rat model of brain injury and microglial activity known to preserve the BBB. Further, this effect was successfully inhibited via the administration of PK11195, a potent ligand of peripheral benzodiazepine receptors specific to activated microglia. The findings suggest that MnTnHex-2-PyP5+ and its analogues may be useful as imaging agents specific for the detection of microglial activation, an early finding common to many forms of brain pathology, even in the presence of an intact BBB. Further, they suggest that peripheral benzodiazepine receptor binding may be the molecular basis for MnTnHex-2-PyP5+ activated glial specificity.