Detection of Pre-Symptomatic Prion Infection in the Mouse Spleen by MRI

Y. Zaim Wadghiri[1], M. Sadowski[2], D. Brown[3], C. Y. Tang[4], D. H. Turnbull[2], T. Wisniewski[2]

[1]Skirball Institute, NYU School of Medicine, New York, United States, [2]NYU School of Medicine, New York, NY, United States, [3]University of Bath, Bath, United Kingdom, [4]Mt Sinai School of Medicine, New York, NY, United States

Introduction

Prion diseases are transmissible, invariably fatal neurodegenerative diseases manifesting as a rapidly progressing dementia[1]. In infected subjects the very brief clinically symptomatic period, which rapidly leads to death, is preceded by months to years of pre-clinical prion replication in the spleen[2]. Currently there is no reliable non-invasive test to identify prion-infected carriers who are a potential source of infection through blood transfusions and organ transplantation[3]. The key element in the pathogenesis of prionoses is the conversion of normal host protein PrP^{Sc} to the toxic and infectious PrP^{Sc} characterized by a high β-sheet content. PrP^{Sc} accumulates in the extracellular space of lymphatic organs in asymptomatic carriers and in the brain of symptomatic patients[4]. PrP binds with high affinity to PrP^{Sc}. We propose to use Gadolinium (Gd) labeled short synthetic, non-toxic peptides homologues to PrP as specific ligands for MRI detection of PrP^{Sc} accumulation in the spleen as a method to identify asymptomatic carriers.

Design and Methods

Six week-old CD-1 mice were infected with 139A mouse adapted scrapie strain[5]. Between 60 to 90 days post inoculation, animals were clinically asymptomatic but harbored a significant level of PrP^{Sc} in lymphatic organs. Studies were initially done with full length recombinant PrP (rPrP) to assess the *in vivo* binding between of rPrP to PrPsc (Fig 1). Among the potential ligands screened for PrP^{Sc} detection, we choose PrP145-174 because of its high binding affinity to PrP^{Sc} assessed *in vitro* by solid phase binding assay and *in vivo* by radioactive ^{125}I-PrP145-174 labeling of the spleen six hours after injection (Fig 2).

For MRI, the ligand was synthesized with diethylene-triamine-penta-acetic acid (DTPA) attached to its N-terminus. Gd was then chelated to DTPA-PrP145-174 by overnight incubation. Mass spectrometry was performed before and after chelation. Gd-DTPA-PrP145-174 was injected intravenously (11.4mg/kg) into CD-1 infected and normal mice. Animals were sacrificed and perfused with paraformaldehyde 6 hours later. Extracted spleens were embedded in 3% agarose. MRI was performed on a SMIS console interfaced to a 7T horizontal magnet with 250-mT/m actively shielded gradients (Magnex) and a homemade 22-mm (ID) saddle coil. Two image resolutions were explored using a 3D gradient echo sequence (TE/TR/FA = 5-ms/50-ms/20°): 50µm isotropic (Imaging Time=14h35') to analyze the spleen with the highest detail and 100µm isotropic (Imaging Time=1h50') for future *in vivo* studies.

Results

Autoradiography revealed a greater than twelve fold increase of ^{125}I-rPrP signal from spleens of infected mice compared to non-infected mice (Fig 1), demonstrating that labeled rPrP can be used as a vector to detect accumulation of PrP^{Sc} in lymphatic organs. MR images following intravenous injection of Gd-DTPA-PrP145-174 (K_D of binding to rPrP=202nM) showed a significant reduction in signal intensity in spleens of infected mice (Fig 2A, B) but not in spleens of control mice (Fig 2C, D). The Gd-induced susceptibility effect altered the spleen's white pulp predominantly (Fig 2B, D) which is known to accumulate the toxic and infectious PrP^{Sc}.

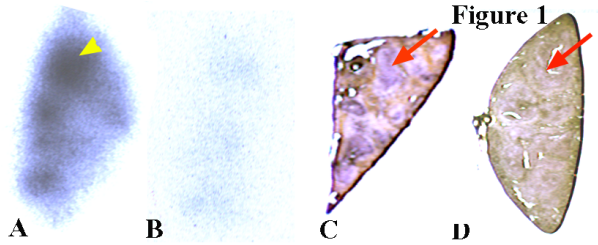

Fig 1: Autoradiography after intravenous injection of ^{125}I-rPrP demonstrated increased uptake in the infected mouse (A) compared to control (B). ^{125}I-rPrP accumulated in a lobular fashion (A, yellow arrowhead) corresponding to accumulation of PrP^{Sc} in the spleen's white pulp. Immunohistochemistry revealed accumulation of PrP^{Sc} (blue) in the white pulp (red arrow) in infected mouse (C) compared to control (D).

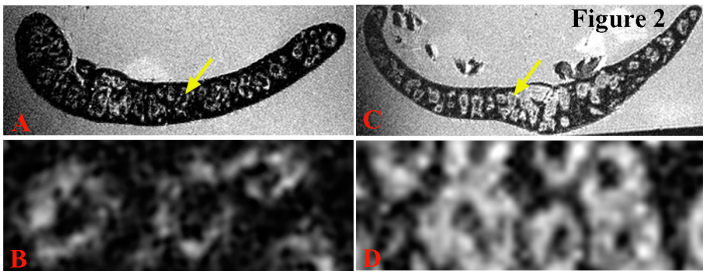

Fig 2: Ex-vivo MRI of mouse spleen. (A) Following intravenous injection of Gd-DTPA-PrP145-174, a significant susceptibility effect was observed predominantly in the white pulp (yellow arrow) of infected mouse (A, and a magnified view in B) compared to non-infected mouse injected with the same construct (C, and a magnified view in D). No differences were observed in infected or non-infected mice that did not receive the labeled ligand (data not shown). Resolution in these images is 100 µm isotropic (imaging time, 1h 50 min).

Conclusions:

Autoradiography demonstrated that ^{125}I-rPrP has a significantly higher uptake in the spleens of prion infected animals which accumulate PrP^{Sc}. However rPrP is a potential substance for conversion to PrP^{Sc} and this possible toxicity prevents it from being used as an imaging ligand. The synthetic, non-toxic peptide PrP145-174 shows high binding affinity to PrP^{Sc} and the magnetically labeled form, Gd-DTPA-PrP145-174, efficiently and exclusively enhances the spleens of infected mice. These results demonstrate the feasibility of PrP^{Sc} detection in lymphatic organs of asymptomatic prion infection carriers using MRI with specific magnetically-labeled peptide ligands in under 2 hours imaging time. *In vivo* experiments are currently underway.

References:

[1] S. B. Prusiner, *N.Eng.J.Med.* 344, 1516-1526 (2001); [2] K. L. Brown et al., *Nature Med.* 5, 1308-1312 (1999); [3] P. Aucouturier et al., *J.Clin.Invest.* 108, 703-708 (2001); [4] T. Wisniewski et al., in *Molecular and Cellular Pathology in Prion Disease*, H. F. Baker, Ed. (Humana Press, Totowa, New Jersey, 2001), Ch. 13; [5] M. Sadowski et al., *Neurosci.Lett.* 345:1-4 (2003).