Contrast-enhanced MRI of Blood-Brain Barrier Disruption in CCL2 Transgenic Mice during Pertussis Toxin-induced Inflammation

A. E. Schellenberg[1], R. Buist[2], H. Toft-Hansen[3], T. Owens[3], and J. Peeling[1,2]

[1]Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Manitoba, Canada, [2]Radiology, University of Manitoba, Winnipeg, Manitoba, Canada,

[3]Medical Biotechnology Center, University of Southern Denmark, Odense, Denmark

Introduction: The chemokine CCL2 is believed to have a role in the recruitment of inflammatory cells into the central nervous system (CNS). CCL2 is expressed within active and chronic multiple sclerosis (MS) lesions[1,2] with decreased levels in the cerebral spinal fluid of MS patients.[3] The role of CCL2 in the initiation and progression of the disease is currently unknown. Inflammatory cells accumulate in the perivascular space between the endothelial cells and astrocytic foot processes of the blood-brain barrier (BBB) in unmanipulated CCL2 transgenic mice.[4,5] Transgenic mice that overexpress CCL2 in the CNS show weight loss and infiltration of leukocytes across the BBB following pertussis toxin (PTx) administration.[4] Unlike the PTx-induced infiltration, perivascular accumulation was not detectable by USPIO-enhanced MRI.[4] This study uses contrast-enhanced MRI to detect the extent and time course of BBB permeability in PTx and vehicle treated CCL2 transgenic and wild type mice.

Methods: *Animal Model:* All animal procedures were approved by the Institutional Animal Care Committee at the University of Manitoba. Twenty-two female and male 11-13 week old transgenic mice overexpressing CCL2 in the CNS[4] were given a single intraperitoneal injection of 10µg/kg PTx (n=8), 20µg/kg PTx (n=7), or Hanks' balanced salt solution (HBSS, n=7). Similarly, 16 female and male wild type B6D2F1 mice at 11 weeks old received either 10µg/kg PTx (n=6), 20µg/kg PTx (n=5), or HBSS (n=5). All mice were weighed and monitored daily. *MR Imaging:* MR images of the brain were obtained using a Bruker Biospec 7T/21cm spectrometer using a 2cm diameter quadrature volume coil. Eight T_2-weighted MR images spanning the brain were obtained using a multi-slice multi-echo (TE=26.8ms, TR=2500ms, matrix size=256×256, FOV=2.5×2.5cm^2, slice thickness=0.75mm, interslice gap=0.25mm, 8 echos, 2 averages) sequence and a set of T_1-weighted images (TE=13.0ms, TR=600ms, matrix size=256×256, FOV=2.5×2.5cm^2, slice thickness=0.75mm, interslice gap=0.25mm, 4 averages) were acquired. A bolus of 0.4mmol/kg Gd-DTPA was injected intravenously and a set of contrast-enhanced T_1-weighted images was obtained. The animals were imaged pre-PTx/HBSS injection and at days 1, 3, and 5 post injection. *MR Image Analysis:* The contrast enhancement on T_1-weighted images was quantified by calculating percent difference images using ((post-contrast image – pre-contrast image)/pre-contrast image)×100%. T_2-weighted images were used to define the region of interest (ROI) outlining the brain and omitting the enhancing ventricles. These ROIs were superimposed onto the calculated images of percent enhancement to obtain the number of pixels above the selected intensity threshold of 20%. *Tissue Processing:* After the last imaging session each mouse was injected with 0.25g/kg of Texas Red 70kD dextrans into the left ventricle of the heart and after one minute perfusion-fixed using phosphate buffered 10% formalin. The imaged region of the brain was embedded into paraffin and 6µm sections were examined for regions of fluorescent dextrans and stained with hematoxylin & eosin to visualize general features of inflammation.

Results: Focal regions of contrast enhancement were observed scattered throughout coronal brain slices in CCL2 transgenic mice following PTx injection, while no enhancement was found within the brains of CCL2 transgenic mice injected with HBSS (Figure 1). A significant increase in area of enhancement was seen in CCL2 transgenic mice at day 3 post-10µg/kg PTx and at days 3 and 5 post-20µg/kg PTx with greatest enhancement at day 3 (Figure 2). No enhancement was found within the brains of wild type mice injected with either dose of PTx or HBSS (Figure 1). A loss in weight was observed exclusively in transgenic mice following PTx injection, with greater losses in mice receiving the higher dose (Figure 3). Inflammatory cells were observed within the perivascular space of CCL2 transgenic mice. Following PTx injection, inflammatory cells were found within the brain parenchyma surrounding vessels and dextrans were found to leak from vessels (Figure 4). No inflammatory cells or leaking dextrans were observed in wild type mice before or after PTx/HBSS (Figure 4).

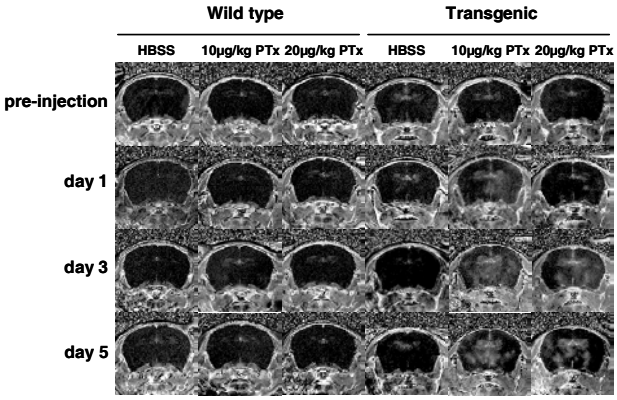


Figure 1. Representative coronal MR images (12mm×12mm) of the striatal region of wild type and transgenic mice injected with HBSS, 10µg/kg PTx, or 20µg/kg PTx. MR images were obtained pre-injection, days 1, 3 and 5 post-injection from the same animal.

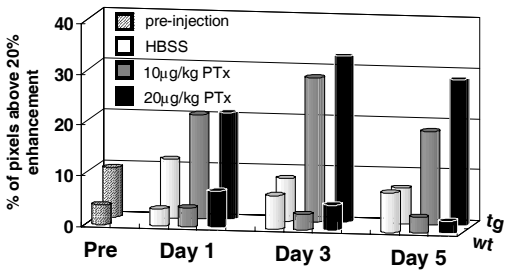
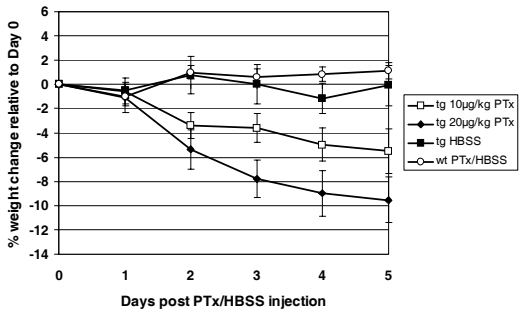


Figure 2. The percentage of pixels in the posterior 6 slices of the brain showing a 20% or greater increase in intensity on calculated percent enhancement images for wild type (wt) and transgenic (tg) mice pre-injection and days 1, 3, and 5 post-HBSS post-10µg/kg PTx, post-20µg/kg PTx.

Figure 3. The change in weight following injection of HBSS or PTx in transgenic and wild type mice over 5 days.



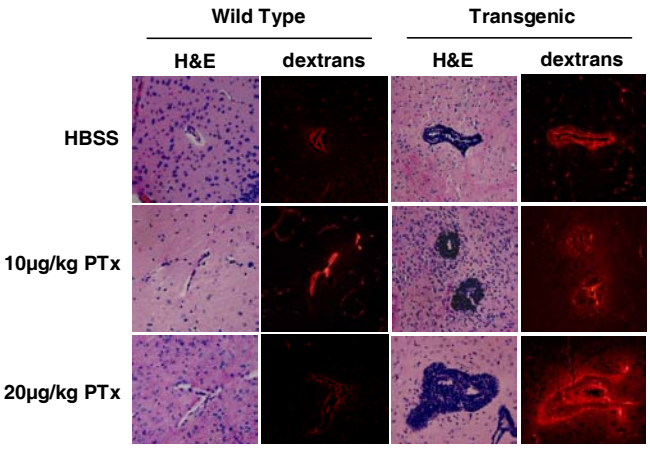


Figure 4. Histological sections stained with H&E to observe general features of inflammation and corresponding unstained sections to visualize regions of fluorescence due to dextrans surrounding blood vessels.

Discussion: Contrast-enhanced T_1-weighted images showed areas of BBB disruption in CCL2 transgenic mice following PTx administration that was maximal at day 3. The presence of dextrans in tissue surrounding vessels further confirmed increased permeability across the BBB. No indication of BBB breakdown was seen in CCL2 transgenic mice following the injection of HBSS or in wild type mice post-PTx/HBSS. Only the combination of PTx and overexpressed CCL2 in the CNS resulted in contrast enhancement and weight loss in this model suggesting both genetic and environmental roles in the initiation of BBB permeability and infiltration of inflammatory cells into the CNS in MS lesions.

References: 1. McManus, C. et al. *J Neuroimmunol* **1998**, *86*, 20-29. **2.** Van Der Voorn, P. et al. *Am J Pathol* **1999**, *154*, 45-51. **3.** Sørensen, T. L. et al. *Eur J Neurol* **2004**, *11*, 445-449. **4.** Toft-Hansen, H. et al. *J Immunol* **2006**, *177*, 7242-7249. **5.** Fuentes, M. E. et al. *J Immunol* **1995**, *155*, 5769-5776.