Visualisation of phagocytic cells in a mouse brain after traumatic brain injury with micron-sized iron-oxide particles: A preliminary report

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Introduction

Cell specific imaging has become an important field in MRI. The increased use of superparamagnetic iron oxide particles as contrast agents has allowed this field to grow. Direct *in vivo* injection of these agents, such as ultra-small particle iron oxide (USPIO) and more recently micron-sized particle iron oxide (MPIO) accumulate in the mononuclear phagocyte (MP) system and induce a local region of hypointensity in MR images due to the large magnetic susceptibility of the iron particles [1]. In the brain there are two sources of macrophagic cells, bone-marrow derived monocytes and microglia that are activated and transform into phagocytic cells. The MP system plays an important role with phagocytic, antigen-presenting and secretory functions, thus tracking the response of these cells with MRI can lead to greater understanding of the pathophysiology of numerous diseases [2]. To develop effective therapies for the treatment of traumatic brain injury (TBI) a thorough appreciation of early pathophysiological events is required. This study used MPIO to detect the MP system response after experimentally induced TBI in a mouse model.

Materials and Methods

Male C57Black/6J mice aged between 11-15 weeks were used throughout these studies. Mice were anesthetized with isoflurane in N_2O:O_2 (1:1), intubated and mechanically ventilated; a femoral venous catheter was then surgically placed for MPIO injection (4.5mg Fe/Kg). The mouse controlled cortical impact (CCI) model was used as previously described [3] with minor modifications [4]. Animals were placed in a stereotaxic holder and a temperature probe was inserted through a burr hole into the left frontal cortex and the parietal bone was removed for trauma. Once brain temperature reached 37˚C and was maintained at this temperature for 5 minutes, a vertically directed CCI was delivered at 4.0m/sec with a depth of 1.0mm. The bone flap was replaced, sealed with dental cement and the incision closed. At the end of the experiment the tissue was perfused with 4% paraformaldehyde and the brain excised for *ex vivo* imaging studies.

MR studies were performed on a 7-Tesla/21 cm Bruker AVANCE DRX spectrometer using a 35 mm mouse birdcage coil. High resolution T_2*-weighted 3D images were obtained with the following parameters: TR = 500 ms, TE = 8 ms, 256x256x128 matrix and a 1.2x1.2x1.5 FOV.

Results and Discussion

The ability to track MP activity is important especially after TBI. Post TBI cerebral blood flow (CBF) is significantly lowered and this hypoperfusion is thought to play a role in secondary injury. The MPS system has the ability to release cytokines and cause activation of vasodilatory agents such inducible nitric oxide synthase [5]. Our results show labeled macrophages throughout the brain of a mouse that underwent CCI injury 24 hours before MPIO injection and the brain was excised 24 hours after injection. Figure 1B has distinct areas of hypointensity (sample indicated by arrows) which are the labeled macrophages and microglia. The naïve brain even after MPIO injection does not display these areas hypointensity (Figure 1A), which may mean that the iron-oxide particles cannot pass through the blood-brain-barrier. Another question is whether the MP's are endogenous and they had access to the particles after the membrane was disrupted post trauma or are of systemic origin. Mice were injected 4 days prior to CCI injury and the brains were removed two days post CCI. The results are seen in Figure 1C, dark spots of hypointensity are seen within the trauma site (indicated with arrows). The blood half-life of MPIO is not known but other iron oxide contrast agents have blood half-lives on the order of minutes to a day, so it is assumed that MPIO should be similar. Therefore the macrophages seen in Figure 1C are likely of systemic origin because the particles would be out of the blood pool before the trauma disrupted the membrane. *In vivo* labeling of MP's and the ability to detect their response to TBI could have great potential in understanding the cellular mechanisms behind secondary injury and its correlation with CBF.

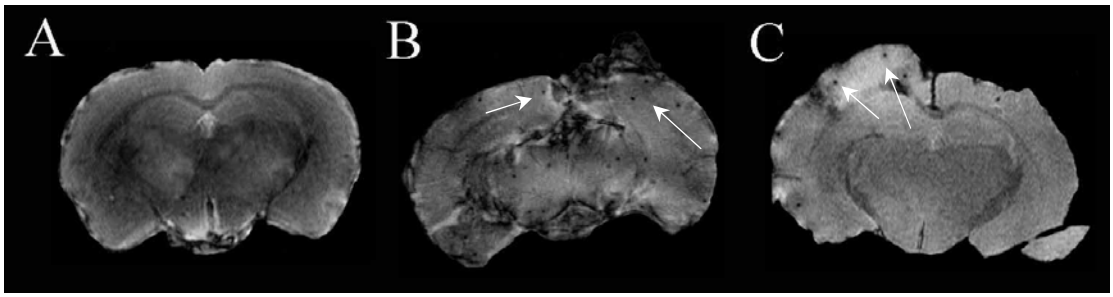

Figure 1: Representative axial T_2*-weighted images of mouse brains. (A) Naïve, 24 hours after MPIO injection. (B) 48 hours post CCI and 24 hours after MPIO injection. (C) 6 days after MPIO injection and 48 hours post CCI.

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