Imaging Cellular Responses in Experimental Spinal Cord Injury Using SPIO and 3DFIESTA at 1.5T

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Introduction

Traumatic spinal cord injury (SCI) results in an immediate physical injury, which is frequently followed by further tissue damage that may continue over a period of days or months after the initial injury, and which has been termed secondary or 'bystander' injury. During this cellular inflammatory response there is a spatially regulated and time-dependent influx of blood-derived leukocytes into the lesion site, involving neutrophils (PMNs), hematogenous macrophages (hMacs) and resident CNS macrophages (mMacs), or microglia. There are important and unresolved questions related to how the degree of macrophage involvement, the role of distinct populations of macrophages and their spatial distribution contribute to lesion development and regeneration in SCI. Cellular imaging is a newly emerging field with the potential to allow visualization of early events in inflammation, at the cellular level, before the consequences of disease are apparent by conventional MRI. The use of iron oxide based contrast agents for cell-specific imaging by MRI has now been demonstrated in a number of different disease applications. The presence of iron oxide is indicated by abnormal signal hypointensities in T2 or T2* weighted images; regions of signal void (RSVs). The purpose of this paper was to determine if the macrophage labeling strategies could be used together with new tools and concepts for cellular and microimaging at low field, developed in our lab[7], to enable the visualization of cellular inflammatory responses in SCI.

Experiments

SCI was induced using a clip compression model. Feridex was administered i.v via the tail vein. Rats were sacrificed, perfused and spinal cords were removed for imaging. Imaging was performed at 1.5T using a custom-built high-powered gradient coil insert and a prototype FIESTA sequence optimized for single cell imaging. FIESTA imaging of cord specimens (TR/TE 10/5ms, 21 kHz BW, 100micron isotropic spatial resolution, 30 FA, 4nex) was compared with T2w FSE (3000/80) and a T1w 3DSPGR with all parameters as for 3DFIESTA and with SNR matched. Two experiments were performed. In the first experiment three groups of rats were studied: (1A, n=4) Feridex at 4hrs post SCI, imaging at day 1; (1B, n=4) Feridex at 3 days post SCI, imaging at day 4; (1C:n=2) Feridex at 4hrs post SCI, imaging at day 4. The second experiment included a macrophage depletion study. Clodronate liposomes were injected both i.v. and i.p. 24-36hrs prior to SCI. These liposomes cause apoptosis of hematogenous macrophages (hMacs) only, sparing neutrophils (PMNs) and microglia (mMacs). Two groups of rats have been studied after cell knockout: (3A, n=3) Feridex at 4hrs post SCI, imaging at day 1; (3B, n=2) Feridex at 3 days post SCI, imaging at day 4.

Results and Discussion

Figure 1 shows representative cord specimen images from Experiment 1A. In FIESTA images of cords from rats administered Feridex at 4 hours and imaged at day 1 post SCI (1A) small, discrete RSVswere detected at and near the epicenter (EC) of the injury. 3DSPGR images matched to FIESTA for resolution and SNR also show these RSVs, however the signal due to hemorrhagic necrosis at the EC is increased, GM-WM contrast is reduced and scan time is considerably longer. T2w FSE images show a diffuse region of signal loss at the EC, RSVs are more difficult to detect since hemorrhage also appears with a low SI. At day 1 small numbers of macrophages and microglia are present but the predominant cell response is of PMNs (Figure 2). Although PMNs are capable of phagocytizing particles this size our image data suggests that we are not visualizing this response. When Feridex was administered at 3 days post SCI and cord images acquired on day 4 the picture looked quite different (Figure 3). A large more diffuse region of signal loss visible at the EC in FIESTA images (3a). Axial images show that the signal loss is predominantly within gray matter that agrees with histological studies. This is also apparent in SPGR images as a low SI region and hemorrhage again appears with high SI. The T2w FSE image shows a large area of signal loss at the EC. At 3 days post SCI the numbers of hMacs is peaking. Histology shows hundreds of iron labeled cells in PPB stained sections. These images may therefore depict the infiltration of hMacs to the site of injury. If Feridex is administered just 4 hours after SCI, the images acquired on day 4 looked show a dramatic reduction in the area of signal loss with only small numbers of isolated RSVs (3a). This result may provides more evidence to support the idea that blood-derived hMacs are being labeled at day 3, in the blood, and that their invasion of cord tissue produces the large area of signal loss.

The images are dramatically different after clodronate liposome administration. Figure 4 shows FIESTA images of cords at day 1 and 4 for treated and untreated injured rats. There is a very large diffuse area of signal loss in cords imaged at day 1 post SCI when the hMacs are knocked out (3c). The picture of cords imaged at day 4 is also very different from non-depleted animals. Figure 3d shows that in this case, the previously large region of signal void is gone and instead there is a reduction in the amount of signal loss. Our original hypothesis was that blocking hMacs would cause a reduction in RSVs detected in the cord if they were the source. Our image data, however, suggests that with hMacs knocked out, other cells compensate. PMNs may be the new source of this large response. The PMNs may now be able to take up iron without competition from macrophages. It is also possible that infiltration of PMNs is exaggerated under conditions of an injury but no hMacs present. On day 4 it appears that there are not large numbers of hMacs or PMNs. The more widespread distribution of the RSVs may indicate a response from reactive microglia.

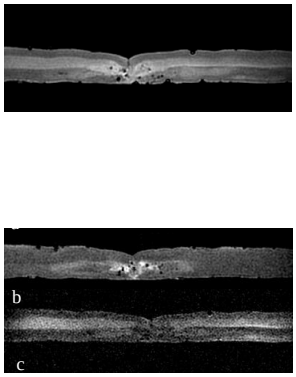

Figure 1. Images of cord specimen 1 day post SCI, Feridex was given 4 hrs after SCI. (a) 3DFIESTA (b) 3DSPGR (c) T2w FSE.

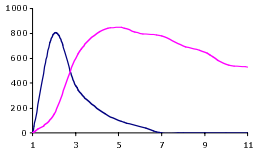

Figure 2. Timecourse of neutrophil (blue) and hematogenous macrophage (pink) influx into injured cord tissue.

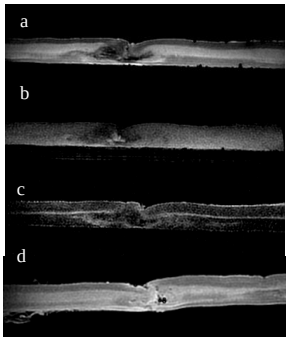

Figure 3. Images of cord specimen 4 days post SCI, Feridex was given 3 days after SCI for (a-c) and 4 hrs after SCI for (d). (a) 3DFIESTA (b) 3DSPGR (c) T2w FSE. (d) 3DFIESTA

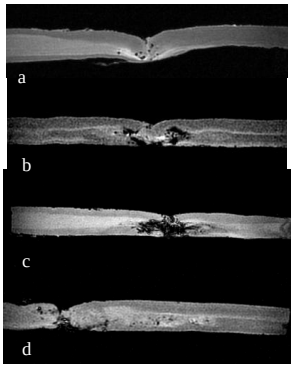

Figure 4. 3DFIESTA images of cord specimens. (a,b) 1 and 4 days post SCI, as in fig1 and 3. (d,e) liposome treated cords imaged at 1 and 4 days post SCI.