DTI abnormalities in sheep spinal decompression sickness: anisotropy differences and histological comparison

E. B. Hutchinson[1], I. Rowland[2], A. Sobakin[3], N. Kleman[4], D. Sun[4], P. Ferrazzano[5], M. Eldridge[5], and M. E. Meyerand[6]

[1]Neuroscience Training Program, University of Wisconsin, Madison, WI, United States, [2]Radiology, University of Wisconsin, Madison, WI, United States, [3]Veteranary Medicine, University of Wisconsin, [4]Neurosurgery, University of Wisconsin, Madison, WI, United States, [5]Pediatrics, University of Wisconsin, Madison, WI, United States, [6]Medical Physics, University of Wisconsin, Madison, WI, United States

Introduction

Diffusion tensor imaging (DTI) continues to grow in its application to biological questions, however the nature of microstructural substrates that underlie abnormal DTI indices is not well understood and different mechanisms of damage may have the same quantitative effect on index changes such as fractional anisotropy (FA). The focus of this study is to identify DTI abnormalities and their histological correlates in a model of spinal cord injury due to decompression sickness (DCS) in sheep. This model is particularly informative as neurological DCS is thought to preferentially affect spinal cord white matter. The results indicate that white matter FA is altered in both chronic and acute spinal cord damage and the latter is coincident with apoptosis.

Methods

Adult ewes were exposed to different "dive" paradigms (see Table 1) in a dry hyperbaric chamber. Animals were acclimated to a given depth (feet of seawater, fsw) for 24 hours and then rapidly decompressed over 2 minutes to surface pressure. Before decompression animals received variable duration, 100% oxygen pre-breathing treatment. Animals surviving the dive were treated for decompression sickness and allowed to recover for 6 weeks at which point they were sacrificed and the CNS tissue was excised and fixed in formalin. DTI was performed on cervical spinal cord tissue in a 4.7T Varian scanner. A multislice spin echo sequence with 3 reference and 30 diffusion weighted image volumes was used and images were processed offline using custom Matlab code to generate fractional anisotropy (FA) and mean diffusion (MD) maps. Histology was performed on a subset of the spinal cord samples to assess apoptosis using FluoroJade staining visualized with bright field fluorescence.

Group	Number of Ewes	Dive Depth	O2 treatment	Survival
1	2	-	-	yes
2	2	60 fsw	0	no
3	4	60 fsw	15	yes
4	2	60 fsw	180	yes
5	2	90 fsw	180	no

Table 1. Sample information for sheep undergoing hyperbaric exposure.

Results and Discussion

Experimental groups 3 and 4 survived the dive, while groups 2 and 5 did not (Table 1). A subset of samples from both survivors and non-survivors showed a decrease in FA (figure 2). Spinal cord injury in the non-survivors was considered to be an acute injury, while injury found in the survivors was considered to result from a more chronic mechanism. FA differences were observed in both types of injury, suggesting that DTI is a sensitive measure of DCS related tissue change. Furthermore, the results at 60fsw suggest that oxygen pre-breathing may be protective. Interestingly, hyperoxic mechanisms may contribute to tissue damage at greater depths, where more O_2 can be dissolved in tissue.

FluoroJade staining (figure 1) showed apoptotic cells in the white matter of tissue from acutely injured sheep surfacing from 90 fsw, with 180 minutes of O_2 pre-breathing (n=2), but no positive cells were found in control tissue (n=2). This may reflect oligodendrocyte apoptosis as an underlying mechanism for decreased FA in acute DCS damaged spinal cords.

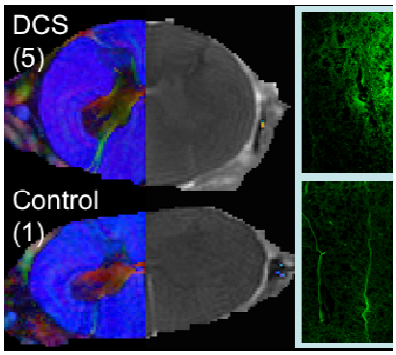

Figure 1. DTI maps and histology u
FluoroJade for apoptosis staining. control

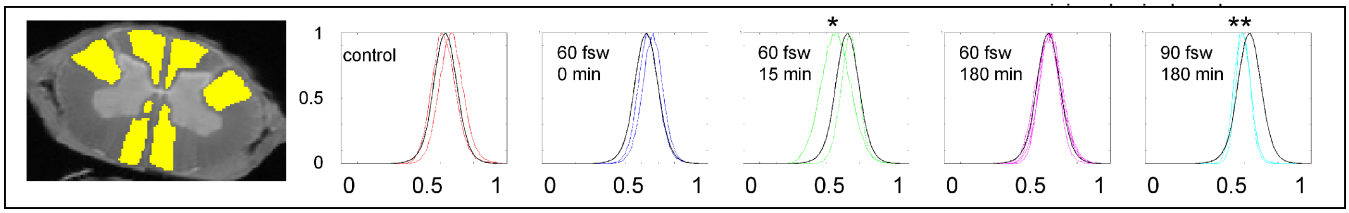

Figure 2. White matter FA profiles for spinal cord DTI from sheep in different dive groups. Black line is the average histogram for all samples and dive profile information is given. * indicates that the mean was >2 standard deviations away from the mean of all pooled data. ** indicates this for both samples