EFFECTS OF DIVING ON SPINAL CORD INJURY-ASSOCIATED DECOMPRESSION SICKNESS

Richard Sové

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INTRODUCTION

Decompression sickness (DCS) is a common injury associated with scuba diving and other activities associated with depressurisation, such as space flight and aviation (Doolette and Mitchell, 2001). There are approximately nine million recreational divers in the U.S. alone, 900 to 1000 of which are treated for severe DCS each year (Newton, 2001). DCS can be classified into two clinical patterns, Type I and Type II. The symptoms of Type I include musculoskeletal pain, cutaneous and lymphatic manifestations, anorexia and fatigue; whereas Type II is primarily associated with central nervous system dysfunction, cardiopulmonary and peripheral neuropathy (Francis and Mitchell, 2004). From 1990 to 1997, of 1,170 DCS cases in the United Kingdom, 77% had neurological dysfunctions, 66% of which were associated with a sensory abnormality (Francis and Mitchell, 2003). Sensory abnormalities are thought to be associated with spinal cord defects (Francis and Mitchell, 2003); this occurrence is referred as spinal cord injury-associated decompression sickness (SCI-DCS).

DCS is a result of inert gas bubbles forming in the blood during re-pressurization, causing damage to blood vessels and surrounding tissue (Newton, 2001). The origins of the gas bubble formations arise from either dissolved gases leaving solution, or from inspired gases entering the arterial circulation following barotraumatic lung damage; both origins result in similar symptoms (Doolette and Mitchell, 2001).

Spinal cord injury (SCI) is often studied in rodent models due to the variety of methods available to induce the injury (Scheff et al., 2003). In order to assess new treatment strategies for SCI, it is important to understand how lesions in the spinal cord develop and change over time as well as to better understand the causes of SCI (Gonzalez-Lara et al., 2009). Specific indications of spinal cord pathologies are not well understood (Blatteau et al., 2010); however, previous research suggests that SCI may be associated with lesions in the white matter associated with haemorrhaging and embolism (Blatteau et al., 2010). SCI-DCS often results in sensory loss, pain or motor weakness; this affects multiple regions along the neuraxis (Greer, 2004).

Histology is, currently, the most common technique used for studying SCI pathologies (Gonzalez-Lara et al., 2009). This technique is performed by examining a thin slice of fixed tissue under a microscope. However, when studying pathological processes involving the entire spinal cord, this technique is inefficient and invasive as it requires the analysis of only thin
portions of the tissue at one time (Gonzalez-Lara et al., 2009). Thus it would be impractical to acquire samples from entire cord, reducing the utility of this technique as important signs of pathology could go undetected. Histology also requires tissue fixation which could cause undesirable deformations to the tissues which could result in subtle lesions to be unseen. In addition, histology yields a two-dimensional image which also reduces its ability to detect desired pathologies (Gonzalez-Lara et al, 2009).

Magnetic Resonance Imaging (MRI) is an effective modality for studying SCI due to several characteristics. MRI has the capability of sampling the entire spinal cord in three dimensions, is non-invasive and does not require pre-imaging tissue fixation (Slucky and Potter, 1998). MRI also provides high spatial resolution and the ability for sensitive contrast in soft tissues (Slucky and Potter, 1998). It has been proven to be particularly sensitive to spinal cord soft tissue injuries (Slucky and Potter, 1998). Spinal tissue damage often results in haemorrhage or edema, making it visible by MRI due to the increased water content in the tissue (Slucky and Potter, 1998). Embolism associated with DCS can also be detected; embolism appears as dark lesions or signal voids in the spinal cord (Slucky and Potter, 1998).

The objective of this study was to determine whether MRI can be used to identify regions of pathology associated with SCI-DCS. The first hypothesis is that there will be a difference in grey matter to white matter contrast-to-noise ratio (CNR) in the spinal cords of rats subjected to a 6.3 ATA dive when compared to those who did not dive. The second hypothesis is that there will be difference in the number of signal voids in the spinal cords of rats subjected to a 6.3 ATA dive when compared to those who did not dive.

THEORY

Gases in Solution

Pressure becomes of concern for humans when diving as pressure is proportional to the depth in a fluid. Divers must be able to overcome the ambient pressure in the water so that their lungs can expand allowing them to breathe. The static pressure of a fluid can be defined by equation 1,

\[ P = P_{\text{atm}} + \rho gh \] (equation 1),

where \( P \) is the pressure at depth \( h \), \( P_{\text{atm}} \) is atmospheric pressure, \( \rho \) is the density of the fluid, and \( g \) is the acceleration of gravity (\( g = 9.8 \text{ m/s}^2 \) at the surface of the Earth). Pressure is commonly referred to in the units of atmospheres (ATM) where one atmosphere is the approximate pressure at the surface of the Earth. Divers often refer to pressure in atmospheres absolute (ATA) which is the pressure (in ATM) due to the fluid and atmospheric pressure (in ATM) (Blatteau et al., 2010). Conveniently, every ten meters under water is approximately one ATM of pressure caused by the fluid, and one ATM due to the atmosphere, so divers often report their depths in ATA (e.g. 20 m
under water, P = 3 ATA). Although it is important to note that this relationship deviates from linearity at larger depths due to the compressibility of water (Brylske, 1997).

One of the ways divers can overcome fluid pressure is to breathe in pressurized gases where the gas supply is delivered at the same pressure as the surrounding ambient pressure (Berghage, 1978); this ensures that the lungs do not have to work against the forces exerted by the water. However, breathing in pressurized gases can lead to other problems such as decompression sickness (Newton, 2001).

The kinetics of the inert gases can be described by applying the fundamentals of Dalton’s law and Henry’s law. Dalton’s law states that the total pressure of a gas is equal to the sum of the partial pressures of the individual gas components (Brylske, 1997). Thus as the ambient pressure increases due to depth, the partial pressures of the inhaled gases increase as well. According to Henry’s law, the partial pressure of a gas, for a constant temperature, is proportional to its solubility (Brylske, 1997). Therefore, as ambient pressure increases, so does the solubility of the inhaled gases. In the case of compressed air, the inhaled oxygen is quickly metabolised, however, the inert nitrogen becomes dissolved until saturation; this is evident by Fick’s law of diffusion (Newton, 2001). Fick’s law describes the flux of material by equation 2,

\[
\frac{dM}{dt} = -DkAdP/dx \text{ (equation 2),}
\]

where \( dM/dt \) is the time flux of material, \( D \) is the diffusion constant, \( k \) is the solubility constant, \( A \) is area over which diffusion occurs and \( dP/dx \) is the partial pressure gradient. By Fick’s law it is evident that as solubility increases, so does the rate of diffusion. At sea level, the solubility of nitrogen is too low for it to diffuse into the blood; however, at depth the solubility of nitrogen becomes significant (Brylske, 1997).

Gas mixtures other than air can be used while diving to improve the rate at which divers can ascend (the rate of decompression) (Arieli et al., 2006). The most common is the use of Heliox; this is a mixture of helium and oxygen (Hydegaard and Madsen, 1994). Another gas mixture commonly used is Trimix; a mixture of helium, nitrogen and oxygen (Arieli et al., 2006). Mixed gases have been shown to decrease the prevalence of DCS significantly (Arieli et al, 2006). The main advantage of helium is that it has a much faster rate of tissue saturation by Graham’s law which states that the rate of effusion is inversely proportional to the square root of the gas’s molecular mass (Berghage et al., 1978). Previous studies demonstrate that the rate of tissue saturation for helium is 2.65 times greater than that for nitrogen suggesting that heavier gases will require more time for decompression (Berghage et al., 1978).

Magnetic Resonance Imaging

MRI is an imaging modality used to visualize the magnetic nuclear resonance properties of atom nuclei (Slucky, 1998). The signal strength is proportional to the concentration of nuclei in the sample and the strength of the magnetic field (Slucky, 1998). MRI is only able to detect
atoms with nuclear spin (e.g. $^1H$, $^{13}C$, $^{31}P$). In the brain, grey matter contains a higher proportion of water than white matter (Slucky, 1998), so the signal intensity from grey matter will be higher than that of white matter (i.e. grey matter will appear brighter than white matter).

When measuring differences between white matter and grey matter properties, MRI can be useful due to its ability to detect differences in water content. MRI can also be useful for detecting haemorrhage in white matter because the blood saturates the white matter tissue making the signal from the white matter larger due to increased water (Slucky, 1998); this results in a lower grey matter to white matter contrast-to-noise ratio (CNR). CNR can be calculated using equation 3,

$$\text{CNR} = \frac{(GW - WM)}{SD}$$

where $GM$ is the mean signal from the grey matter, $WM$ is the mean signal from the white matter and $SD$ is the standard deviation, or noise of the background. MRI can even detect gas bubbles in the spinal cord; this results in the appearance of a dark lesion or void in signal due to the water deficit (Slucky, 1998).

**METHODS**

**Animal Model**

SCI-DCS was induced in adult male *Wistar* rats by simulating a 6.3 ATA dive on Heliox gas. Adult male *Wistar* rats of similar age did not dive and were used as controls. The intact spinal columns (spinal cord and vertebrae) of both groups were isolated for analysis using *ex vivo* magnetic resonance imaging to identify the regions of pathology.

**Imaging Model**

The spinal cords were scanned using a 9.4 T small animal MRI scanner. The parameters for the long scans were; resolution: 70x70x70 $\mu$m, TR/TE = 9.4/4.7, scan time: 11 hours. The parameters for the short scans were; resolution: 75x75x75 $\mu$m, TR/TE = 9.4/5.5, scan time: 3 hours.

**Image Analysis**

The grey matter to white matter CNR was measured for both the control rats and the rats subjected to a 6.3 ATA dive for the long and short scan using ImageJ software. Regions of interest were drawn in the grey matter and white matter of sagittal slices of the rats’ spinal cords to find the mean signal from the white matter and grey matter. A region of interest was also drawn in the background (where there appears to be no signal) to measure the standard deviation, which represents the image noise. These data were used to calculate the CNR of the images. The number of signal voids was recorded from axial slices of both the control group and the 6.3 ATA
group for the long scans. Data analysis was done using Prism software and Microsoft Excel. Statistical tests were conducted using a non-parametric one-way ANOVA (Prism).

RESULTS

The mean grey matter to white matter CNR for the long scan time of the rats subjected to a 6.3 ATA dive was 15.13 ± 3.46 (mean ± SD; Figure 1A: n = 4) compared to 20.90 ± 4.32 (Figure 1B: n = 4) for the long scan time of the control rats. The mean grey matter to white matter CNR for the short scan time of the rats subjected to a 6.3 ATA dive was 11.90 ± 2.81 (Figure 1C: n = 3) compared to 23.36 ± 10.03 (Figure 1D: n = 4) for the short scan time of the control rats.

There were no significant differences in the grey matter to white matter CNR between the images of the rats subjected to a 6.3 ATA dive and the control rats for the long scan time. There were also no significant differences in grey matter to white matter CNR between the images of the rats subjected to a 6.3 ATA dive and the control rats for the short scan time. Furthermore, there were no significant differences between the grey matter to white matter CNR between the short and long scan times (Figure 2).

The mean number of signal voids in the white matter of the images of the rats subjected to a 6.3 ATA dive was 10.8 ± 4.82 (Figure 3A: n = 5) compared to 5.0 ± 4.24 (Figure 3B: n = 5) in the white matter of the images of the control rats. The mean number of signal voids in the grey matter of the images of the rats subjected to a 6.3 ATA dive was 6.2 ± 3.11 (Figure 3C: n = 5) compared to 2.0 ± 2.35 (Figure 3D: n = 5) in the grey matter of the images of the control rats.

There were no significant differences in the number of signal voids in the white matter of the rats subjected to a 6.3 ATA dive when compared to the number of signal voids in the white matter of the control rats. There were also no significant differences in the number of signal voids in the grey matter of the rats subjected to a 6.3 ATA dive when compared to the number of signal voids in the grey matter of the control rats. Furthermore, there were no significant differences between the number of signal voids in the white matter and the number of signal voids in the grey matter of either group (Figure 4).
Figure 1: [A] Sagittal slice of the spinal cord of a control rat using the long scan time. [B] Sagittal slice of the spinal cord of a rat subjected to a 6.3 ATA dive using the long scan time. [C] Sagittal slice of the spinal cord of a control rat using the short scan time. [D] Sagittal slice of the spinal cord of a rat subjected to a 6.3 ATA dive using the short scan time.
Figure 2: Grey matter to white matter contrast-to-noise ratio (CNR) from the spinal cord images of control rats (short scan, n = 4; long scan n = 4) and rats subjected to a 6.3 ATA dive (short scan n = 3; long scan n = 4) for both the short scan time and long scan time. There are no significant differences between any of the groups.
Figure 3: [A] Axial slice of the spinal cord of a control rat using the long scan time. [B] Axial slice of a rat subjected to a 6.3 ATA dive using the long scan time. A large lesion can be seen in the grey matter of the spinal cord of a rat subjected to a 6.3 ATA dive.

Figure 4: The number of signal voids in the white matter and grey matter of control rats (grey matter, n = 5; white matter, n = 5) and rats subjected to a 6.3 ATA dive (grey matter, n = 5; white matter, n = 5). There are no significant differences between any of the groups.

DISCUSSION

Despite the lack in significant difference of the grey matter to white matter CNR between the rats subjected to a 6.3 ATA dive and the control rats, there appears to be a trend to lower CNR in the rats that dove. Future studies should include a larger sample size in order to re-evaluate the significance of this trend.

The lack in significant difference between the short scan time and the long scan time indicates that a shorter scan time may be used to yield the same results as a longer scan. A decreased scan time would be useful in the potential clinical visualization of SCI-DCS using MRI, since subjecting patients to an overnight scan would be impractical.

There seems to be a trend to greater number of signal voids in both the grey matter and white matter of the rats subjected to a 6.3 ATA dive when compared to the control rats despite
the lack of significant difference. Future studies using a larger sample size may show significance. According to past literature, the spinal cords of the rats that did not dive should not have any signal voids due to embolism (Blatteau et al., 2010). Since the spinal columns were removed and transported to the imaging facility, the appearance of signal voids in the control rats may be due to air bubbles in the tissue caused by pre-imaging manipulation. Furthermore, isolated spinal columns may be more sensitive to manipulation because they are exposed to air to a greater extent. Previous literature also suggests that signal voids due to embolism usually occur in the white matter of the spinal column rather than in the grey matter; reasons behind this are still not clear (Francis, 1998). The appearance of signal voids in the grey matter suggests that the embolism may also be due to the manipulation of the spinal cord prior to the MRI scans. Future studies that image spinal cords in vivo, rather than removing them, may provide increased sensitivity to SCI-DCS induced lesions. These studies would provide further insight into the possibilities of using in vivo MRI in the clinic to visualize SCI-DCS in patients.

CONCLUSION

In conclusion, there was no significant difference in grey matter to white matter CNR between the rats subjected to a 6.3 ATA dive when compared the rats that did not dive for both the long and short scan times. There was also no significant difference between the CNR in the images of the long scan time and short scan time. MRI was able to detect signal voids in both the white matter and grey matter of the spinal cords. There was no significant difference in the number of signal voids between the rats subjected to a 6.3 ATA dive when compared to the rats that did not dive. Thus, MRI may be a promising candidate to study the effects of SCI-DCS in vivo.

REFERENCES


