Manipulation of Oxygen and Endoplasmic Reticulum Stress Factors as Possible Interventions for Treatment of Multiple Sclerosis: Evidence for and Against

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Abstract

Multiple sclerosis (MS) is normally considered a chronic inflammatory disease of the central nervous system (CNS), where T-cells breaching the blood brain barrier react against proteins of the axonal myelin sheaths, leading to focal plaques and demyelination in the brain and spinal cord. Many current therapies are immunosuppressive in nature and are designed to target the immune system at an early stage of the disease. But there is no cure and MS may evolve into a neurodegenerative disease, where immunomodulatory treatments appear less effective. Neurodegeneration is influenced by oxidative and endoplasmic reticulum (ER) mediated stress which can be induced independently of immune processes. Since 1970, MS patients have been self-managing their long term symptoms using hyperbaric oxygen and reporting improvement in their symptoms, especially bladder control. In contrast, the majority of clinical trial evidence does not support the views of patients. Therefore does oxygen under pressure affect brain tissue by modulating oxidative or ER stress at the cellular level resulting in CNS tissue repair or deterioration? This chapter reviews our understanding and the role of oxidative and ER stress in the context of employing hyperoxia treatments to treat MS and evaluate its effects on neural cells.

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Multiple sclerosis (MS) is a demyelinating autoimmune disease whereby damage to cells of the central nervous system (CNS) results in the generation of lesions that results in loss of neurological function. The disease is categorized to various degrees of severity beginning with preclinical, followed by in most cases relapsing remitting, primary progressive and finally secondary progressive, suggesting a chronic onslaught of inflammation which leads to an increase in neurodegeneration to the CNS. The greatest genetic risk factor comes from carrying the class II HLA-DRB1*1501 allele which can increase susceptibility by 2–4-fold (Odds ratio 3.06; 95% CI, 2.30–4.08), while Epstein-Barr virus infection has a similar risk association (Odds ratio 2.60; 95% CI, 1.48–4.59) (Xiao et al. 2015) Geographical latitude (Kinoshita et al. 2015) and ethnic considerations (Langer-Gould et al. 2013) also contribute to the overall chance of developing MS. It is well established that the adaptive immune system plays a role in MS pathology, especially pro-inflammatory T-cells (Cao et al. 2015; Hong et al. 2009). Autoreactive T-cells can be found in the peripheral blood of autoimmune patients and healthy control subjects, but such cells appear to be more resistant to apoptosis and reactive against myelin proteins in MS patients (Mandel et al. 2009; Vergelli et al. 2001). The cause of development of peripheral blood autoreactive T-cells against CNS tissue derived myelin, prior to T-cell exposure to such tissue is largely unknown. In MS, the transmigration of autoreactive T-cells across the blood brain barrier (BBB) can ultimately lead to an escalation of pro-inflammatory damage to myelin-producing oligodendrocytes in close proximity to neuronal axons, leading to major damage and cell death. The oxidative damage and endoplasmic reticulum (ER) stress that ensues (Mhaille et al. 2008), requires the cells of the CNS to either undergo apoptosis or repair, which is controlled to a large extent by the unfolded protein response (UPR) (Stone and Lin 2015). Moreover, the UPR can also influence the ability of various cells to resist apoptosis and influence their cytokine phenotypes (Chan et al. 2011; Kim et al. 2006). Therefore pathways such as the UPR that regulate many aspects of cell survival and repair might be a fruitful area of research in developing therapeutics to alleviate or prevent MS pathology, and are already being investigated for other neurodegenerative diseases (Rozpedek et al. 2015; Torres et al. 2015).

We and others have shown that cells in vitro exposed to 100 % oxygen under hyperbaric pressure (HBO) alter the expression of a wide variety of genes involved in immunity and inflammation (Kendall et al. 2011, 2012, 2013a; Thom 2011). Consequently, HBO might work as a therapy by promoting or suppressing selective genes and
their products in a non-invasive manner. But, how HBO works downstream, at the cellular and biochemical level remains largely unknown and more work is required, but it does not appear to damage DNA in the longer term (Yuan et al. 2011). Hyperbaric oxygen therapy (HBOT), which involves breathing pure oxygen under pressure is used to treat a number of clinical conditions including non-healing wounds (Eggleton et al. 2015) and to ameliorate the side-effects of radiation therapy (Clarke et al. 2008; Glover et al. 2015). However HBOT as a treatment for MS is highly contentious and does not have approval from the USA Food and Drug Administration (US Food and Drug Administration 2013) or The National Institute for Health and Care Excellence (The Guideline Development Group NICE 2014). Despite the non-recommendation by health governance authorities, many patients continue to use HBOT to treat their symptoms and frequently report symptomatic improvement. In the late 1970s and 1980s when HBOT began to be trialled, some clinicians supported the use of HBOT for MS sufferers (Boschetty et al. 1970; Fischer 1983; Fischer et al. 1983; James 1984; James 1983; Neubauer 1978, 1980; Neubauer et al. 2005), while others did not (Barnes et al. 1985b; Neiman et al. 1985; Wiles et al. 1986). This has led to confusion for both patients and clinicians alike. Here we evaluate the pros and cons of HBO treatment in the context of oxidative and ER stress, the unfolded protein response and the changes that occur in cells and their genes under hyperbaric conditions.

2.2 Oxidative and ER Stress in MS Pathology

The cellular damage induced in the CNS of MS patients directly accounts for many of the dysfunctional changes observed in the well-being, mobility and motor processes of individual MS sufferers. Within all nucleated cells, a number of cell repair molecules, the UPR sensor molecules, are sensitive to changes in their environment, and this is particularly so of CNS cells (Giovannoni and Ebers 2007; Hedstrom et al. 2015). Inflammatory cells and the molecules they release can attack oligodendrocytes and the neuronal axons and signal to these cells to shut down and die by apoptotic death. Under the appropriate conditions the UPR can attempt to repair the cell. Whenever the UPR response signal is one of repair, regeneration and remyelination of axons can occur (Gow and Wrabetz 2009). One potential way of driving the decision to repair rather than destroy a cell is to manipulate the ER-mediated UPR stress response. There are several diverse environment factors that can trigger cellular and ultimately ER-stress, namely virus, microbial toxins, oxidative stress and nutrient deficiency (Mkhikian et al. 2011). These stimuli can all trigger additional rapid protein production within the ER to help maintain the status quo of the cell. The rapidity of this process can lead to errors in amino acid biosynthesis, protein folding and glycosylation, inducing degradation factors to deal with the disruption in cellular homeostasis and triggering reactive oxidative (ROS) and nitrosoative species (RNS) production. Similarly, activation of ROS and RNS can also activate the UPR, and the UPR has been shown to be elevated in myelin-generating oligodendrocytes of the CNS, as well as other cells of the peripheral nervous system (Lin and Popko 2009).

It is established that oxidative stress plays a role in cellular damage and particularly so in MS neuropathology, where the cerebro spinal fluid (CSF) and plasma are observed to have increased amounts of lipid peroxidation (Calabrese et al. 1998). During lesion formation activated microglia cells release superoxide, which in part can be defended by the antioxidant systems of the brain such as superoxide dismutases (SOD) and reduced glutathione. Free iron can promote CNS damage by catalyzing the production of hydroxyl and peroxyl-based free radicals from hydrogen peroxide and lipid peroxides (Halliwell 2001). The balance between free radical and antioxidant production undoubtedly plays a role in whether inflammation subsides or progresses, leading to lesion development (Gilgun-Sherki et al. 2004; Syburra and Passi 1999), although it has been
questioned whether the formation of ROS in MS is in fact deleterious (Koch et al. 2006). At the cellular level in vitro, the myelin producing oligodendrocytes are thought to be more susceptible to damage by ROS/RNS compared to astrocytes and microglia possibly due to higher iron content and diminished antioxidant defenses (Smith et al. 1999). The molecular events that lead to oligodendrocyte loss and lesion formation are not fully understood, but are known to involve signaling pathways associated with both the ER (Kraus and Michalak 2011) and mitochondria organelles (Aboul-Enein and Lassmann 2005; Dutta et al. 2006; Gilgun-Sherki et al. 2004; Lu et al. 2000). Furthermore, dysfunctional mitochondria are an additional source of ROS production (Mahad et al. 2009; Nickel et al. 2014). Ultimately, inflammation, oxidative stress, demyelination of axons and the lack of remyelination and restoration of axonal function will be partially dependent on the cellular activation of the UPR to these various insults. The response can manifest itself in various ways including accumulation of unfolded or misfolded proteins in the ER. The main UPR sensor pathways are regulated by three proteins inositol requiring kinase 1 (IRE1), activating transcription factor 6 (ATF6), and PKR-like ER kinase (PERK) (Fig. 2.1). The signalling path-

![Fig. 2.1](image)

**Fig. 2.1** Oxidative stress induces ER stress than can activate the unfolded protein response (UPR) pathways. (a) Oxidative stress can arise from localized activated inflammatory cells, secretion of proinflammatory cytokines and induction of ROS and NOS by activated macrophages and microglia in the brain. (b) The resulting oxidative stress can lead to damage of lipid, DNA and protein. This in turn can disrupt lipid and protein biosynthesis, resulting in the accumulation of misfolded proteins in the ER and ER stress. In turn, ER stress activates one or more of the three ER-transmembrane transducers of the UPR. Individual stressed cells are then programmed to survive or undergo cell death. Localized regions of the brain where oxidative and ER stress are present can result in the formation of lesions in the CNS white matter.
ways that these sensors regulate have been well documented and described in detail with regards to MS (Getts et al. 2008; Stone and Lin 2015), but are triggered initially when B-cell immunoglobulin heavy chain binding protein (BiP) detaches from PERK. Key components downstream of the UPR initiating signal are phosphorylated eukaryotic initiation factor alpha (p-eIF2α) and C/EBP homologous protein (CHOP) which drive cells toward survival (Walter and Ron 2011) or apoptosis (Szegedzi et al. 2006) respectively.

Over a period of time the chronic inflammation, oxidative and ER-stress leads to visible MS pathology in the CNS. Affecting predominantly white matter, demyelinating lesions become clearly distinguishable from the surrounding normal appearing white matter (NAWM) tissue (Fig. 2.2a–c). Evidence of myelin-specific T-cell accumulation leads to the development of lesions which can be acute or sub-acute (sometimes referred to as chronic active), in which myelin is progressively stripped from the axon sheaths of neurons and is engulfed by macrophages and microglial cells. An additional type of lesion is the chronic lesion (sometimes referred to as chronic silent) in which inflammation has abated and scarred lesions devoid of myelin present within the CNS. Lesions can be seen on MRI scans (Fig. 2.2 d–f). MS lesions defined in terms of inflammatory destruction and neurodegeneration are useful for studies designed to identify differences in gene expression at the DNA and mRNA level of diverse cellular and molecular biomarkers of pathology at distinctive stages of disease progression. As shown in Fig. 2.2a, during the acute phase of lesion formation there is a gradation of infiltrating inflammatory (microglia and macrophages) cells, with more cells close to the lesion border engorged with oil red O stained myelin, providing evidence of demyelination of axons. In the sub-acute stage (Fig. 2.2b) the lesion border appears more distinct, with the central region of the lesion becoming devoid of myelin and oligodendrocytes. The chronic lesions have little evidence of inflammatory cells, typically appear hypocellular and are devoid of a visible inflammatory border with the NAWM and represent a scarred region of irreversible demylation (Fig. 2.2c). However we have recently identified a novel proinflammatory subset of T-cells (CD20+/IL17+) associated with the chronic and acute lesions of MS patients (Holley et al. 2014). The NAWM tissue in MS differs from that of white matter in non-MS brain, in that greater numbers of T-cell infiltrates are detected (Allen et al. 2001; Kutzelnigg et al. 2005), indicative of pre-lesion inflammation and breach of the blood brain barrier (BBB).

Through analysis of significant changes in UPR genes in various MS lesions, a better understanding of the cell response to oxidative and ER stress with respect to MS pathology can be established. A number of microarray studies have identified elevated levels of expression of certain genes including UPR pathway genes in biopsy material obtained from the demyelinating lesions in the CNS of MS patients (Cwiklinska et al. 2003; Lock and Heller 2003; Mycko et al. 2003, 2004; Tajouri et al. 2003). Mycko and colleagues examined differences in gene expression from cell extracts from the border and centres of active lesions, with varying degrees of inflammatory infiltrates. Not surprisingly more genes were upregulated at the DNA level in active lesions compared to inactive lesions both at the lesion borders and centres (87 vs. 69 genes and 65 vs. 22 genes) respectively, which included a number of intracellular signalling and transcription factors (Mycko et al. 2003). The same group went on to look at mRNA gene expression in the same tissue regions and observed a number of ER-stress and heat shock protein genes upregulated in both active and inactive regions of MS lesions including activated transcription factor (ATF4) and heat shock protein 70 (HSP70) (Cwiklinska et al. 2003; Mycko et al. 2004). Tajouri and co-workers also examined NAWM and chronic and acute lesion material from five MS patients with secondary progressive disease and non-MS subjects (Tajouri et al. 2003). The authors observed 139 genes that were differentially regulated >1.5 fold in the five MS lesions compared with NAWM. Several of the genes upregulated were associated with tissue damage and oxidative stress including transferrin (TF), superoxide dismutase 1 (SOD1), glutathione peroxidase
(GPX1) and glutathione S-transferase (GSTP1), which are all expressed during free radical formation and in some cases as antioxidants to counteract oxidative stress. More recently, Cunnea and associates have detected elevated expression levels at the mRNA level of a number of ER and hypoxic stress genes in actively demyelinating lesions of MS patients with primary or secondary progressive disease compared to control white matter (Cunnea et al. 2011). Specifically they observed a 2–8 fold elevated expression of BiP, CHOP and ATF4. Interestingly the elevation of these classical ER-stress proteins were not restricted to lesions but also in the NAWM of MS patients, indicative of ER stress occurring prior to lesion formation. Increases in UPR gene products are not restricted to the white matter of MS patients and various grey matter lesions have been shown to have significantly increased levels of CHOP compared to normal grey matter. The increased CHOP appeared to be predominantly associated with microglial cells. Whether increased CHOP in microglial cells predestines such inflammatory cells to undergo apoptosis remains to be elucidated (McMahon et al. 2012). The function and

**Fig. 2.2** Classification and imaging of MS lesions. Oil red ‘O’/hematoxylin staining of 10 μm sections of MS brain tissue, showing lesion areas at the top of each image and NAWM at the bottom, demarcated by a dashed lined. (a) Depicts an acute lesion with increasing numbers of oil red ‘O’ positive macrophages containing myelin and more densely packed towards the lesion border. (b) Illustrates oil red ‘O’ positive macrophages located mainly at the lesion border (black arrow heads) and a demyelinated area of the lesion. (c) Shows a chronic lesion, devoid of myelin and oil red ‘O’ positive macrophages. All images are at 100x magnification. (d) Normal brain axial T2 weighted MRI scan. (e) Axial T2-weighted MRI in a patient with MS demonstrating several white matter hyper-intense lesions. (f) Coronal fluid-attenuated inversion recovery (FLAIR) MRI in a patient with MS demonstrating high-signal intensity lesions in the deep white matter and the periventricular regions. Key: R right, L left, A anterior, P posterior, S superior, I inferior. White arrows depict lesions.
over expression of CHOP and other UPR genes should be considered on an individual cellular basis, especially in the knowledge that elevated CHOP protects oligodendrocytes from cell death (Gow and Wrabetz 2009).

Oxidative and ER stress appears to have a dynamic affect and differential sensitivity on various UPR response genes and the proteins they encode in human CNS biopsy tissue of specific lesions. Specifically many UPR genes appear to be elevated in MS. However, the underlying mechanisms through which UPR genes act in individual cell types (e.g. oligodendrocytes, neurons, microglial cells) or individual MS patients requires more work. The knowledge gained from such studies might aid the development of therapeutic strategies that protect both oligodendrocytes and neurons in patients with MS. One overall impression is that the UPR appears to be ‘over activated’ in MS lesions and mechanisms that can suppress the UPR or at least alter it may be of benefit.

2.3  HBOT and MS: Clinical and Patient Perspectives

The data above describes a number of human studies post-mortem, in which evidence of oxidative and ER-stress is clearly implicated in altering CNS tissue cell survival and degeneration. So the logical question is how does environmental oxygen affect MS patients? In 1970, 26 MS patients were treated with 100 % O₂ under hyperbaric pressure (HBOT) at 2 ATA (Boschetty and Cernoch 1970) and fifteen patients symptoms were observed to improve. Over the past 45 years, both clinicians and patients have reported or observed improvements in MS symptoms after HBOT treatment, often as anecdotal reports or in randomized control trials. But the use of HBOT as a treatment for MS remains highly contentious. Indeed HBOT has been regarded by some as no better than other ‘alternative’ treatments such as oral arsenic, intrathecal injections of tuberculin, oral seaweed and snake venom (Bates 1986). The early history and controversy in using HBOT to treat MS patients has been eloquently described by an advocate pioneer in the field, RA Neubauer (Neubauer et al. 2005). The main conclusion of his and his colleagues report was that HBOT is not a cure, but does stabilize the symptoms in the majority of patients and slows progression in 17–33 % of patients. They also recommend that additional treatments might be required as treatment is transient and the effect of HBOT diminishes over time. The first formal small placebo-controlled, double-blinded study conducted in 1983 produced positive results for HBOT treatment of MS with ‘objective’ improvement in 12/17 patients compared with 1/20 patients treated with a placebo (Fischer et al. 1983). This was despite using 100 % O₂ at 2 ATA (pure oxygen at 1.5–1.75 ATA has generally been recommended since this initial study) for 90 min. An age-sex placebo group of match MS patients were exposed to 10 % O₂/90 % N₂ for the same time period. To assess MS disability as a whole disease severity must be monitored. A number of clinical scales have been developed to this end, the most well established is the Kurtzke’s Expanded Disability Status Score (EDSS) which was originally described in 1955 (Kurtzke 1955) and has been modified through the following decades (Kurtzke 1965, 1970, 1983, 1989, 2000, 2008). Clinical parameters can also be monitored using the Multiple Sclerosis Functional Composite (MSFC), Symbol Digit Modality Test (SDMT) and low contrast visual acuity. In the original Fischer trial in 1983, the successfully treated patients showed improvements in a number of features on the EDSS scale by 1–2 points in mobility, coordination, bladder control and fatigability. Historically, this study was significant as it also provided evidence that MS may be an autoimmune disease whereby oxygen might have immunosuppressive properties. At the time of the study MS was thought by some to consist of venous infarction in the CNS (James 1983) which was disputed by Mertin (Mertin and Mcdonald 1984).

After the Fischer trial of 1983, at least 14 additional double blinded control studies were conducted (Barnes et al. 1985a, 1987; Confavreux et al. 1986; Harpur et al. 1986; Lhermitte et al. 1986; Oriani et al. 1990; Wiles et al. 1986; Wood...
et al. 1985), and many of these refuted the original findings. It is fair to say that in hindsight these studies were poorly controlled. In some of these studies all MS patients irrespective of their disease severity as judged by their EDSS were given the same number of treatments, and therefore this was not treating ‘like with like’. This has led to inconsistency in results and confusion in the clinical community as to whether HBOT is useful (Adamson 1985; Bates 1986; Jacoby 2001; Kleijnen and Knipschild 1995; Monks 1988; Wynne and Monks 1989). One consistency is that HBOT treatment at pressures below 2.0 ATA for short periods of time are not detrimental to patients, indeed O2 used at higher ATA have been indicated to improve recovery from brain trauma in patients (Rockswold et al. 2010). To address the controversy two meta-analysis reports have addressed the use of HBOT for MS. In 2004, a Cochrane report evaluated nine randomized control trials comprising of 504 participants. Only two of the nine trials showed a reduction in EDSS score at 12 months (−0.85 compared to sham). The conclusions suggested better well-designed trials would be required to confirm this improvement but overall did not recommend such trials to be performed (Bennett and Heard 2004). The same authors reevaluated the use of HBOT in 2010, in trials they had previously analyzed between 1983 and 1987 and came to the same conclusions that HBOT for MS was ineffective. They suggested that ‘only staunch advocates would be willing to pursue such investigations’, (Bennett and Heard 2010).

Such ‘staunch advocates’ come in the form of MS individuals. The internet is full of testimonials from MS subjects (http://www.oxygenunder-pressure.com/category/multiple-sclerosis/) and advocate clinicians (Maxfield 2005) who have personally used or employed HBOT, patients have reported feeling better in terms of pain relief, gait, bladder control and overall mobility. The justification for using HBOT for MS is most likely governed by the ability of the treatment to suppress disease symptoms for long periods of time. Despite the resistance and skepticism from many clinicians to prescribe HBOT for MS, several thousand MS individuals use such treatment in the UK and elsewhere every year and report positive outcomes, including decreased fatigue and depression. There are more than 50 hyperbaric centers throughout the UK (Fig. 2.3), where individuals can book a HBOT session, either in a monoplace or multiplace chamber that can accommodate up to 12 people (Fig. 2.3b). MS subjects who are more mobile and in the early stages of the disease have anecdotally reported the use of HBOT to be beneficial. Ideally it would be useful to gauge the effectiveness of HBOT on MS individuals with different stages of the disease (e.g. relapsing remitting vs. secondary chronic progressive with and without conventional medication), but no such data exists. The word used frequently by MS individuals is ‘stabilize’. Again qualitative testimonials from MS subjects who self-administer HBOT, commonly report a stabilization of their symptoms or mild improvement. One difference between the clinical trials and the practical use of HBO by MS subjects is the frequency of HBOT use by the individuals themselves. Whereas trial protocols in the past, used HBOT on MS patients suffering from differing degrees of severity on ~20 occasions over a period of a month (Fig. 2.4), many of these protocols have not been used over longer periods. In reality MS subjects voluntarily use HBOT more frequently and over a longer period of time. Perrins and James reported on 1384 MS subjects employing long-term treatment of HBOT (Perrins and James 2005). About 9 % were regularly treated with HBOT for 5–15 years and 11 % were treated for 17 years or more. Better stabilization and retardation of MS progression was reported if treatment was used soon after MS was diagnosed and before irreversible lesions developed. As HBOT treatment is not offered by the UK National Health Service (NHS) there are no official numbers of clients or treatments in the public domain. However there are a large number of MS therapy centers located around the UK (Fig. 2.3). One such center in Exeter, Devon, UK opened in 1982. The Exeter Center recommends that ‘MS clients’ begin with 15 session (5 daily sessions/week for 3 weeks at between 1.5 and 2.0 ATA) and then ‘top up’ with HBO on a weekly basis, depending on how the
The client is responding. The client is placed in a three or seven seater chamber and gently placed under pressure at a rate of 1 m/min until the appropriate pressure is reached. Clients breathe 100 % oxygen for 60 min and then the pressure is reversed at 1 m/min until normobaric pressure is reached. Some clients at this center have used the facility for decades and feel it prevents their condition deteriorating (personal communication - Esme Gibbins, Therapies Manager). In 2011, Professor Philip B. James (Emeritus Professor of Medicine, University of Dundee, UK) wrote an open letter (http://www.hjernebarnet.dk/fileadmin/_temp_/Philip_James_-110405.pdf) suggesting over 2.5 million HBOT sessions have been safely provided to over 20,000 individuals in MS National Therapy centers since they began to operate in 1982 (figures up to 2011).

**Fig. 2.3** Frequency of multiple sclerosis and quantity and location of HBO chambers for MS patients in the UK. (a) The frequency of MS increases with latitude; England & Wales < Northern Island < Scotland. (b) Patients frequently use single and multiplace HBO chambers to help alleviate their symptoms. (c) There are HBOT chambers located in many major cities and regions around the UK, including the islands of the Scottish coast, where incidences of MS are some of the highest in the world: 420/100,000 in the Orkney Islands and 295/100,000 in the Shetland Islands.

### 2.4 ER Targeted Therapeutics and MS

#### 2.4.1 Effect of HBOT on Cell Function and Gene Expression

Neurologists may agree or disagree with the merits of using HBOT for MS, but HBOT is used successfully to treat many other conditions, and more information as to the effect of HBOT at the cellular and molecular level is required to aid in the further understanding of the mechanism of action of HBOT. We have investigated the role of hyperoxia on various cells under hyperbaric pressures (HBOT) at 2.4 ATA, including platelets (Shaw et al. 2009), endothelial cells and neutrophils (Almzaiel et al. 2013, 2015; Kendall et al. 2013a; Kendall et al. 2012; Kendall et al. 2013b)
and bone tissue (Al Hadi et al. 2015; Al Hadi et al. 2013). We developed a chronic wound model to study neutrophil-endothelial interactions to study the effect of HBO on individual cell types in chronic wounds (Kendall et al. 2011). The culmination of these and other studies suggested HBO reduces the surface expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells and reduces neutrophil adhesion. Although we did not observe changes in neutrophil adhesion molecule expression CD18, CD11b, CD62L, CD31, we proposed HBO inhibited neutrophil adhesion to endothelial cells by S-nitrosation (Kendall et al. 2013b). In the context of MS, similar effects of HBO could possibly inhibit T-cell interaction with brain vascular endothelial cells.

Chronic wounds that are normally exposed to 2 % O₂ are frequently treated with HBO at a pressure of 2.4 ATA. In contrast, MS patients whose brain tissue is normally exposed to 4 % O₂ are normally treated with HBO at 1.5 – 2.0 ATA (Fig. 2.4). Recently we examined how oxidative and inflammatory gene expression alters under different pressures. We have cultured human endothelial cells under hypoxic conditions (2 % O₂) as a model because they are important in both wound healing and immune cell interaction in the BBB. We studied the effect of a single 90 min time (60 min) and pressure protocols (1.5–2 ATA). (c) The HBO protocols used to successfully treat chronic wounds, commonly employs the use of oxygen at ≥2 ATA for longer periods ~90 min, with air breaks.

Fig. 2.4 Examples of MS HBO protocols in comparison to wound treatment HBO. (a) & (b) Examples of protocols to treat MS patients with oxygen under pressure. The protocols over the past 25–35 years have evolved but have retained consistently the same treatment exposure of HBO on a number of categories of genes, including adhesion molecules, apoptosis, angiogenesis and tissue remodeling, inflammation, intracellular signaling and oxygen responses and redox signaling (Kendall et al. 2013a). In these studies a number of genes were sensitive to HBO at both 1.5 and 2.4 ATA compared to cells treated under pressure at 1 ATA and showed reduced levels of expression at the mRNA level that was sustained for at least 22.5 h (the time RNA was extracted from the cells). Notably, 1–4-fold decreases in adhesion molecules; Platelet endothelial cell adhesion molecule1, fibronectin1, angiogenesis factors; angiopoietin 2, connective tissue growth factor, vascular endothelial growth factor receptor 2, endothelial tyrosine kinase, tissue inhibitor of metalloproteinases 3, the chemokine; Interleukin 8, and oxygen response genes; endothelial PAS domain protein 1- HIF-2α and glutathione peroxidase 1. In most cases treatment of endothelial cells with 96.7 % O₂ at 1.5 ATA produced greater reductions in the above genes than when treated with 97.9 % O₂ at 2.4 ATA. When mRNA was quantified from the same endothelial cells, 5 h post HBO treatment a whole serious of oxygen response genes were downregulated 2–3 fold at both 1.5 and 2.4 ATA, but more so when exposed to O₂ at 1.5 ATA compared with cells treated with O₂ at 1ATA or 2.4 ATA pressure. These included HIF-1α and −2α,
peroxiredoxin 2 and 6, glutathione peroxidase 1, superoxide dismutase 1 and 2, catalase, thioredoxin and glyoxalase 1. We have observed similar increases in antioxidants of the peroxiredoxin family at the protein levels in chronic lesions of MS patients (Holley et al. 2007). Interestingly, HBOT at 1.5 ATA reduced the expression of the ER stress chaperone calreticulin by two fold and in MS, calreticulin levels being known to increase as part of the UPR (Mhaille et al. 2008). This illustrated that several antioxidants and chaperones are sensitive to rapid changes to oxygen levels and adjust their expression accordingly. Perhaps of more interest is that O2 administered at 1.5 ATA altered the expression of many more genes more effectively than 2.4 ATA. The reason for this is unknown, but raises the possibility that at least for MS, HBOT treatment, oxygen used at 1.5 ATA, that has been adopted over the past 30–40 years by patients, is more effective at altering gene expression in a number of oxidative and ER stress conditions. One question not answered by the above experiments is how long are changes in gene expression retained post HBOT? Our work suggests at least in vitro that the effect is transient and might require multiple and regular exposures to have any long term beneficial effect on oxidative response, ER stress, UPR and inflammatory gene products. This would support the recommendations of Perrins and James, who suggest MS patients should have regular HBOT treatments for it to have a significant effect in improving EDSS scores or preventing further deterioration (Perrins and James 2005).

2.4.2 Neural UPR Sensor Genes

As HBOT can alter gene expression, it would be of great interest to be able to alter gene expression in neural cells in a non-invasive manner. More specifically to arrest or activate UPR and ER-stress regulatory genes that are involved in clearing misfolded proteins, cell repair and death (Fig. 2.5a). These processes and their regulation are known to be important in neurodegenerative diseases (Oyadomari and Mori 2004; Soto and Estrada 2008). Oxidative stress in the form of lipid peroxidation (Wang et al. 2014), oxygen consumption to form ROS during myelin sheath attack and mitochondrial injury (Haider 2015) and nitrosative stress (Kallaur et al. 2015) have all been observed to precede the inflammatory response in MS patients.

We therefore examined the effect of HBOT exposure specifically on both differentiated neuron-like SH-SY5Y cells and myelin-producing human oligodendrocytes (HOGs). These neural cells were cultured under the appropriate optimal growth conditions for differentiation and maturation in the presence of 4 % oxygen (Normoxia; 4.0 % O2/CO2 at 1.0 ATA) for 4–6 days (neural cells normally exist in a low-oxygen environment) (Ndubuizu and LaManna 2007). Next the cells were treated with HBO (96.7%O2/CO2 at 1.5 ATA), or pressure control (2.67 % O2/CO2 at 1.5 ATA) treatments for 90 min (Fig. 2.5b). All of the gas mixtures contained CO2 at a level to give a final pCO2 of 5 kPa, representing the respiration-derived CO2 at the cellular level. The cells were then placed in their former culture conditions for 5 h or 22.5 h. RNA was isolated from the cells for quantitative real time PCR and analyzed for differences in unfolded protein response (UPR) gene expression pre- and post-exposure to HBO or pressure control (PC) treatment. mRNA expression was analyzed by our previously described methods (Eggleton et al. 2010; Kendall et al. 2011; Kendall et al. 2013a; Kendall et al. 2012). The results revealed that mRNA expression in the major UPR regulatory genes PERK, IRE1α and ATF6α were largely unaffected by HBO or PC treatment 5 h after treatments in SH-SY5Y cells. But 22.5 h post-treatment the PERK and ATF6α mRNA expression levels had reduced by 50 % in cells treated with HBO or hyperbaric pressure (Fig. 2.5c, left panel). Similar reductions in PERK and ATF6α genes were seen in HOG cells after 22.5 h post-treatment with HBO. However, in contrast, to SH-SY5Y cells, IRE1α mRNA levels were reduced by over 50 % following HBO treatment at 1.5 ATA 5 h post-treatment (Fig. 2.5c, right panel). In general, hyperbaric pressure and not oxygen accounted for some but not all of the reduced gene expression in the UPR sensor genes, but did appear to act synergistically in down regulating the UPR genes tested. This
Fig. 2.5 Effect of Oxidative Stress on ER stress regulators in human CNS cells. (a) Changes in cellular oxidative status is one condition that can lead to ER stress. The three recognized UPR sensor pathways PERK, IRE1α and ATF6α induce a number of downstream genes that stimulate changes of a number of important enzyme, oxidoreductase and chaperone pathways that aid in the regulated cell death or survival of individual cell types. (b) The mechanisms by which changes in oxidative stress induced by hyperoxia effect demyelinating diseases such as MS as induced by HBOT treatment remain unknown. We assessed the effect of hyperoxia under pressure (HBO) and pressure alone (PC) on UPR gene pathways in human oligodendrocytes (HOGs) and neuronal cells (SH-SY5Y). (c) The effect of either HBO or PC affected the expression of UPR pathway sensors differently in neuronal or oligodendrocyte cells.
might in part explain why in clinical trials of HBOT for MS patients in which a pressure control is used as a placebo, some placebo treated patients report a benefit for the treatment. In the Cochrane analysis of HBO trials conducted in 2004 (Bennett and Heard 2004), all of the trials evaluated administered oxygen to patients at between 1.75 ATA and 2.5 ATA for 90 min. This is despite the recommended protocols suggesting 1.5 ATA should be initially used (Fig. 2.4). In our gene expression study on neural cells we chose 1.5 ATA because this pressure is used to treat brain injury (Stoller 2011, 2015) and we have seen greater reductions in inflammatory gene expression in cells exposed to oxygen at 1.5 ATA compared to 2.4 ATA (Kendall et al. 2013a). These results are encouraging and demonstrate the use of oxygen at a relatively low hyperbaric pressure can markedly reduce the regulatory genes of the UPR pathways responsible for controlling cell death and repair, which are known to be over-expressed in lesions of MS patients as described above.

2.5 Conclusion

There is growing evidence that both ER (Cunnea et al. 2011; Mhaille et al. 2008) and oxidative stress (Guan et al. 2015; Karlik et al. 2015; Lassmann and van Horssen 2015; Ohl et al. 2015; Pasquali et al. 2015) play a role in the pathology of MS. These stress pathways are also the focus of attention to down-regulate inflammation and aid remyelination within the CNS of MS patients. (Getts et al. 2008). A number of pharmacological agents and small molecule therapeutics have or are being trialed in an attempt to reduce ER and/ or oxidative stress in MS. (Bahamonde et al. 2014; Khalili et al. 2014; Miller et al. 2013; Naziroglu et al. 2014; Ramirez-Ramirez et al. 2013; Sanoobar et al. 2013; Seven et al. 2013). The problem with all drugs is their ability to target specific cells, and this is made more difficult when attempting to target pharmacological agents across the BBB. Despite this problem, a number of agents are being developed to suppress ROS/RNS and ER stress in the CNS (Chiurchiu 2014). While drug development continues and MS patients await new treatments, many other MS patients continue to seek solace in HBOT treatment. The fact that so many HBOT treatment centers exist worldwide and are used regularly by MS patients is a testament to their usefulness. Despite HBOT treatment not being officially approved for the treatment of MS by the clinical community, the lack of approval is probably of no consequence to individuals who use HBOT and feel they benefit from its effects.

The debate on the pros and cons of using HBOT as a MS therapy will continue ad infinitum until proper regulated trials are conducted, but this may never happen due to lack of patent protection, low financial gains and importantly a lack of understanding as to the precise mechanisms of how oxygen under hyperbaric pressure can reduce the symptoms of MS. For example the paradox that oxidative stress is detrimental to CNS tissue, but brain tissue may benefit from being exposed to 100 % oxygen under pressure warrants a cautious approach. Further studies investigating the effect of hyperoxia under normobaric and hyperbaric conditions at the cellular level may help us understand what some patients with MS already believe and feel - that HBOT is an efficacious therapy.

Acknowledgements The authors thank Drs. Debra Green and Lorna W Harries for assistance in the generation and analysis of the UPR gene expression work. We also thank Dr. Anthony Campagnoni (Brain Research Institute, UCLA, USA) for providing HOGs. This work was supported by grants from DDRC Healthcare, Royal Devon and Exeter Foundation Trust and Medical Research Council UK. Additional thanks are extended to the NeuroResource Tissue Bank, UCL Institute of Neurology, London and UK MS Tissue Bank London, U.K. We also thank Buzzy Eggleton for the design and construction of some of the figures.

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