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Hyperbaric oxygen reduces delayed immune-mediated neuropathology in experimental carbon monoxide toxicity

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Abstract

The goal of this investigation was to determine whether exposure to hyperbaric oxygen (HBO_2) would ameliorate biochemical and functional brain abnormalities in an animal model of carbon monoxide (CO) poisoning. In this model, CO-mediated oxidative stress causes chemical alterations in myelin basic protein (MBP), which initiates an adaptive immunological response that leads to a functional deficit. CO-exposed rats do not show improvements in task performance in a radial maze. We found that HBO_2 given after CO poisoning will prevent this deficit, but not eliminate all of the CO-mediated biochemical alterations in MBP. MBP from HBO_2 treated CO-exposed rats is recognized normally by a battery of antibodies, but exhibits an abnormal charge pattern. Lymphocytes from HBO_2-treated and control rats do not become activated when incubated with MBP, immunohistological evidence of microglial activation is not apparent, and functional deficits did not occur, unlike untreated CO-exposed rats. The results indicate that HBO_2 prevents immune-mediated delayed neurological dysfunction following CO poisoning.

Keywords: Myelin basic protein; Lipid peroxidation; Microglia; CD-40

Introduction

Carbon monoxide (CO) is the leading agent of injury and death by poisoning worldwide (Raub et al., 2000). Annually in the United States, approximately 40,000 individuals are treated for CO poisoning (Cook et al., 1995; Hampson, 1998; Moolenaar et al., 1995). Neurological sequelae are the most frequent form of morbidity. Dysfunction may be present at the time of hospital presentation in a small number of survivors (Choi, 1983; Ginsberg and Myers, 1974). The risk is much greater for developing so-called delayed neurological sequelae. Impairments of concentration and learning, dementia, cog wheel rigidity, amnesia, and/or depression develop in 23 to 76% of patients from several days to approximately 4 weeks after poisoning (Mathieu et al., 1996; Raphael et al., 1989; Scheinkestel et al., 1999; Thom et al., 1995; Weaver et al., 2002).

The affinity of CO for heme proteins is well known and formation of carboxy-hemoglobin (COHb) is a recognized effect of CO exposure. An elevated COHb can precipitate tissue hypoxia, and this stress appears to be responsible for fatalities and cardiac injuries (Anderson et al., 1973; Cramlet et al., 1975). CO poisoning can also cause mitochondrial dysfunction and oxidative stress in brain. High COHb levels impair mitochondrial electron transport due to the direct effects of CO on hemoglobin O_2 delivery and because of impaired perfusion from cardiac dysfunction. These changes augment CO binding to cytochrome *c* oxidase, which inhibits ATP synthesis and causes hydroxyl radicals to be generated (Brown and Piantadosi, 1992; Chance et al., 1970; Okeda et al., 1982; Piantadosi et al., 1995; Zhang and Piantadosi, 1993). Energy production and mitochondrial function are restored after COHb levels decrease, but the transient changes may cause neuronal necrotic or apoptotic death (Brown and Piantadosi, 1992; Gilmer et al., 2002; Piantadosi et al., 1997). This mechanism appears to be responsible for the acute neurological abnormalities that are present at initial presentation in some patients and do not resolve (Choi,

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1983; Garland and Pearce, 1967; Ginsberg and Myers, 1974).

In the clinical setting, syndromes from acute and delayed neurological injuries can overlap (Choi, 1983; Gorman et al., 1992; Raub et al., 2000; Weaver et al., 2002). COHb values have consistently been shown to correlate poorly with clinical outcomes (Choi, 1983; Hampson, 1998; Raub et al., 2000). Even when CO poisoning appears to be relatively mild, there are times when delayed neurological sequelae still occur (Hampson, 1998; Raub et al., 2000; Remick and Miles, 1977; Ryan, 1990; Schulte, 1969; Thom et al., 1995; Weaver et al., 2002). Therefore, additional pathophysiological mechanisms beyond COHb-mediated hypoxia are thought to exist.

In a recent study involving a rat model, a mechanism for CO-mediated brain injury other than acute hypoxia was identified. CO exposure was shown to precipitate abnormalities in myelin basic protein (MBP) due to reactions with lipid peroxidation products, and adaptive immunological responses to modified MBP caused neurological dysfunction (Thom et al., 2004a). Lymphocytes from CO-exposed rats proliferate when exposed to MBP, and microglia become activated in brains of CO-exposed rats. Rats made immunologically tolerant to MBP before CO exposure exhibited acute biochemical changes in MBP due to reactions with lipid peroxidation products, but no proliferative lymphocyte response or brain microglial activation. CO-exposed rats exhibited a decrement in learning to maneuver in a maze, but this was not observed in immunologically tolerant rats.

Clinical management of acute CO poisoning involves supportive care and administration of supplemental oxygen. Hyperbaric oxygen (HBO_2) treatment is sometimes considered for CO poisoning. Several randomized, controlled clinical trials have demonstrated its efficacy for reducing the incidence of delayed neurological sequelae, but not all investigations have found benefit (Ducasse et al., 1995; Mathieu et al., 1996; Raphael et al., 1989; Scheinkestel et al., 1999; Thom et al., 1995; Weaver et al., 2002). Similarly, in an animal model, HBO_2 failed to exhibit benefit for reducing neurological sequelae due to CO poisoning (Gilmer et al., 2002). Given the complexity of the alternative pathway for CO-mediated neurological sequelae, the immunological cascade, the goal of our investigation was to determine whether HBO_2 altered the progression of immunological responses and functional deficits in experimental CO poisoning.

Methods

Animals and reagents. Wistar male rats (Charles River Laboratories, Wilmington, Massachusetts) weighing 200–240 g were fed a standard diet and water ad libitum. Unless otherwise stated, chemicals were purchased from Sigma-Aldrich (St. Louis, Missouri). Commercial antibody suppliers were as follows: monoclonal anti-MHC II (Accurate Chemical, Westbury, N.Y.), polyclonal anti-CD40 (Santa Cruz Biotechnology, Santa Cruz, California), monoclonal anti-GLUT3 (Calbiochem, La Jolla, California), monoclonal anti-actin (Sigma, St. Louis, MO), monoclonal anti-MBP [to amino acids 67–74, 82–87, and 129–138], and polyclonal anti-MBP (Chemicon, Temecula, California).

Animal manipulations. CO exposure was performed according to published protocol in a 7-l Plexiglas chamber (Thom, 1990). Rats breathed 1000 parts per million (ppm) CO for 40 min, then 3000 ppm for up to 20 min, until they lost consciousness, and then they were removed to breathe room air and regain consciousness. Where indicated, rats were treated with hyperbaric oxygen at 2.8 atmospheres absolute (ATA) for 45 min starting 45 min after CO exposure following published methods (Thom, 1993). Alternative interventions included 45 min exposures to 100% O_2 at ambient pressure or as a pressure control, 2.8 ATA pressure using a gas containing 7.5% O_2 so that O_2 partial pressure was the same as ambient air (0.21 ATA O_2).

Tissue preparation for chemical assays. Rats were anesthetized and brain tissue from some animals was processed for Western blotting as described previously (Thom et al., 2002). Blots were probed with 1:1000 dilutions of antibody. Other brains were processed for column chromatography as described in a recent publication (Thom et al., 2004a). In brief, isolated brains were frozen in liquid nitrogen, homogenized in 5 ml chloroform:methanol (2:1 v/v), and then diluted with chloroform:methanol to obtain a ratio of 1 g brain: 19 ml ice-cold chloroform:methanol. This volume of solvent was defined as "one volume" for subsequent steps. Following centrifugation (2000 × *g* for 5 min) and washing twice in one volume of fresh chloroform:methanol, the residue was washed once in ice-cold acetone. Following centrifugation (2000 × *g* for 5 min), the pellet was washed three times with 10 ml water, and then suspended in 10 volumes of 0.03 N HCl. The suspension was incubated for 1 h at 4 °C while stirring, centrifuged at 44,000 × *g*, and material in the clear aqueous-acid extract obtained by overnight vacuum evaporation. The lyophilized sample was dissolved in 500 μl 0.1 M acetic acid and applied to a carboxymethylcellulose (CM) column equilibrated with 0.1 M acetic acid. Protein was eluted using a linear gradient of sodium chloride (0–0.75 M) in 0.1 M acetic acid and collecting as 300 μl fractions. Protein concentration in the fractions was determined by absorbance at 280 nm. Fractions of pooled protein were desalted on a Sephadex G-25 column prepared with 7% formic acid. The pooled material was lyophilized and stored at −80 °C until specified fractions were used in the lymphocyte proliferation assay.

Rat MBP isolation for lymphocyte activation assays. Procedures followed were exactly as previously described (Thom et al., 2004a). Brain homogenates from control rats and those killed 90 min after CO exposure were subjected to SDS-PAGE and transferred to nitrocellulose paper. Bands were visualized using Aurodye reagent (Amersham), the 18.5 kDa band corresponding to MBP was cut out and dissolved in DMSO. These samples were precipitated by adding 50 mM carbonate/bicarbonate buffer (pH 9.6), centrifuged as 10,000 × *g* for 10 min, resuspended in RPMI 1640, divided into aliquots and frozen at −20 °C until use.

Lymphocyte proliferation. Lymphocytes were harvested from axillary and cervical lymph nodes following published procedures (Thom et al., 2004a). Nodes were pulverized using a sterile glass syringe plunger and wire mesh screen, the released individual cells were aspirated into a syringe through a 25-guage needle to release clumps, washed in RPMI medium + 10% heat-inactivated fetal bovine serum + 50 μg/ml gentamicin, and counted. Individual wells of a 96 well microtiter plate were inoculated with 300,000 cells, stimulatory agents added to each well, and then plates were incubated at 37 °C in a CO_2 incubator. After 48 h, 0.5 μCi [^{3}H] thymidine was added to each well and plates incubated for an additional 21 h. Cells were then removed, washed, and radioactivity assessed in a scintillation counter. Cultures were run in triplicate and the mean taken as the proliferative response for cells from each rat. Cells were incubated with either 5 μg Concanavalin A (ConA), 5 μg 18.5 kDa MBP, or chromatography fraction 5 obtained from control rats and rats killed 90 min after CO exposure.

Histochemistry. Immunohistochemistry was performed on paraffin-embedded sections after perfusion/fixation of anesthetized rats following published methods (Ischiropoulos et al., 1996; Thom et al., 2004a). Coronal 8 μm thin sections were prepared from brains starting 3.8 mm posterior to the bregma and extended in a posterior direction in order to include hippocampal structures. Two consecutive sections were placed on individual poly-L-lysine coated slides. De-paraffinized slides were stained overnight with primary antibody (1:100 dilutions of anti-MHC II or CD-40) followed by counterstaining with 1:1000 dilution of the appropriate anti-IgG conjugated to Cy3 for 2 h. Staining with

FITC-conjugated isolectin B_4 (1:500) was also performed for 2 h. Photomicrographs were taken with a BioRad Radiance 2000 attached to a Nikon TE 300 inverted stage confocal microscope that was operated with a red diode laser at 638 nm, and krypton lasers at 488 nm and 543 nm. Microglia were identified across an entire brain section by isolectin B_4 staining and activated microglia as those cells positive for MHC II or CD-40. To compare treatment groups, the number of activated microglia was taken as the average count from two slides with brain sections separated by at least 60 μm to avoid double-counting of cells. These studies were carried out with a Nikon Diaphot-TND epifluorescence inverted microscope at ×200 magnification. The examiner was blinded to which rats were poisoned and to treatment. Consecutive sections on each slide were stained for either MHC II or CD-40, and to identify microglia, isolectin B_4, so that two sets of sections 60 μm apart were analyzed. Because cells would only appear yellow (dual-positive cells) when antigens in each section were in the same focal plane, stereological counting according to optical dissector methodology was achieved (Coggeshall and Lekan, 1996). That is, activated microglia were counted between two focal planes in a ~16 μm portion of brain (two consecutive sections). Moreover, we could discount concern that cells might have slightly different sizes or shapes because isolectin B_4 was used to identify the microglia. Our choice to survey for MHC II and for CD-40 was based on observations that both should be expressed on activated microglia (Becher et al., 2001; Sedgwick et al., 1991; Streit and Kreutzberg, 1987).

Maze studies. Performance function was examined using an 8-arm wooden radial maze as previously described (Thom et al., 2004a), following procedures first described by Levin et al. (1993). In brief, familiarization and testing procedures were performed every other day, 3 days per week. CO exposure occurred after the third familiarization session on Friday afternoon and rats were left undisturbed for 2 days. Testing started the following Monday and was performed for 4 weeks (12 test sessions) by a tester blinded to which rats were poisoned and to treatment. Rats were deprived of rat chow for 23 h prior to each test and each maze arm was baited with food reinforcement (a Kellogg's Froot Loop) located 2 cm from the end. Rats were held by the tester for 5 min, fed 1/2 of a Froot Loop, and then placed in the maze central octagon. After 10 s, a stopwatch was started and rats maneuvered down maze arms to eat the Froot Loop. An arm choice was recorded when all four paws were placed into an arm. If a rat reentered an arm, it was recorded as an error. The testing session continued until the rat entered all 8 arms or 5 min had elapsed. Data recorded were number of entries until a repeat occurred (choice accuracy), reentries, and the time to complete the session (in seconds) divided by the total number of arm entries (latency or response duration). The maze was wiped with 1% acetic acid solution before each session to remove scents left by a previously tested rat.

Statistics. Statistical analysis of maze scores was determined by repeated measures ANOVA followed by the Tukey test (SigmaSTAT, Systat, Point

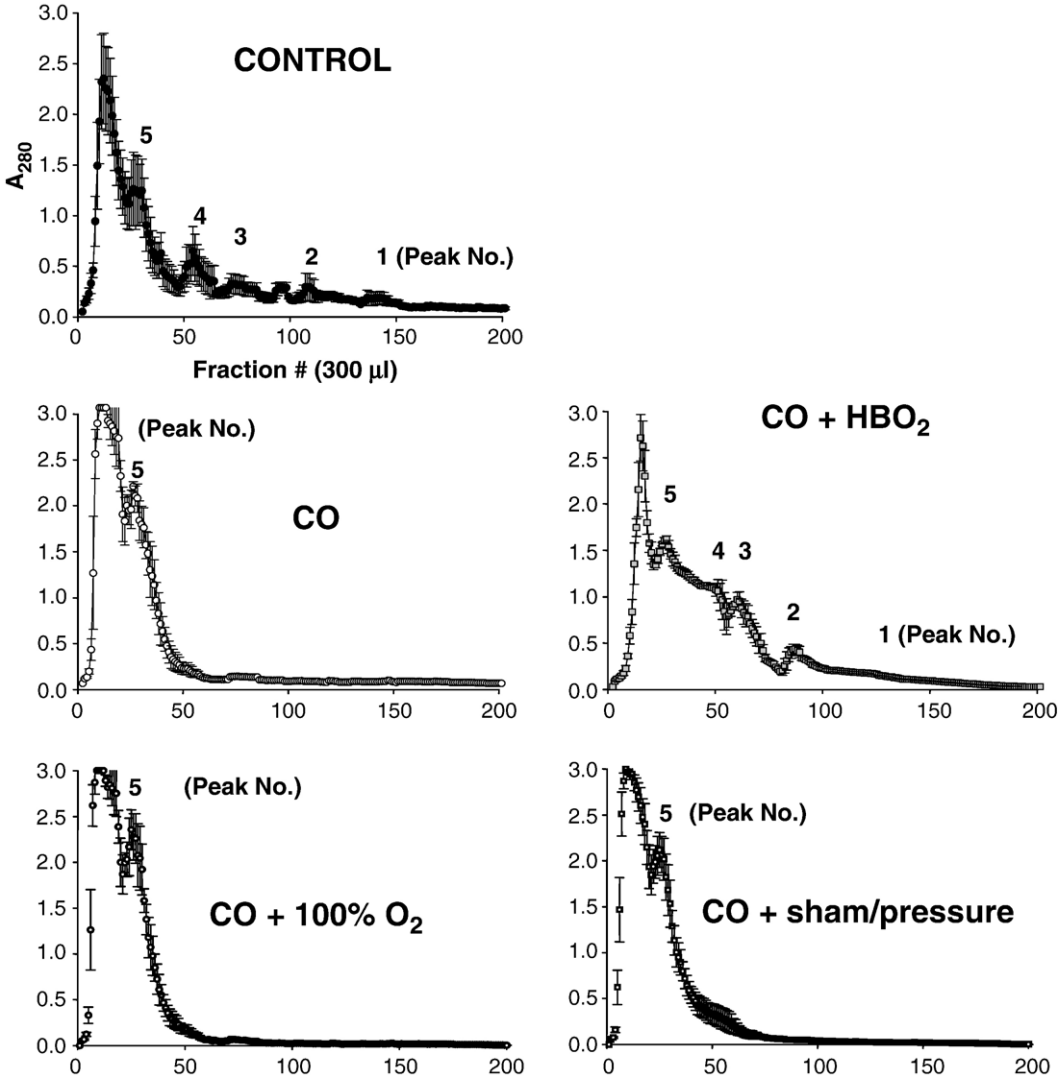

Fig. 1. Column chromatography analysis of acid soluble material from brain. Figures show the pattern for control rats, rats killed 90 min after CO exposure, and for rats killed 90 min after CO but treated for the 45 min prior to death with either 100% O_2 at ambient pressure, HBO_2 at 2.8 ATA or a gas mixture to achieve 0.2 ATA O_2 at a total pressure of 2.8 ATA (pressure control). Results are mean ± SE for 4 rats in each group.

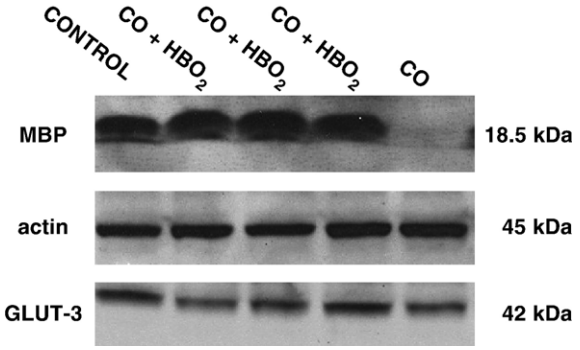

Fig. 2. Western blots using brain homogenates from a control rat, a rat killed 90 min after CO exposure, and from rats exposed to CO followed by 45 min of 2.8 ATA O_2 beginning 45 min after CO exposure. Blots were probed with a monoclonal antibody that recognizes the MBP epitope between amino acids 82–87, glucose transport protein 3 (GLUT3) and actin.

Richmond, CA). The level of significance was taken as $P < 0.05$. Results are expressed as mean ± SE.

Results

Myelin basic protein alterations

The first goal of this investigation was to assess whether HBO_2 altered CO-induced changes in myelin basic protein (MBP). Acid-soluble material from brain homogenates was fractionated by CM cation-exchange column chromatography as shown in Fig. 1. Whereas five peaks were resolved in control samples, the material obtained at 90 min after CO exposure exhibited only one peak and the majority of the protein eluted in the void volume. The material from CO-exposed rats subsequently treated with HBO_2 was not entirely normal, but showed a pattern intermediate between samples from control and the CO-exposed rats. Also shown are results with material isolated from rats exposed with CO and then to either 1 ATA O_2 or 2.8 ATA pressure (see Methods). The charge patterns of these materials were identical to that seen with material from untreated CO-exposed rats, indicating that it was the high oxygen partial pressure and not just pressure itself, that ameliorated the CO-mediated changes.

As an additional probe for alterations in protein structure, we assessed antibody recognition of MBP on Western blots. Blots were probed using a polyclonal antibody and three monoclonal antibodies that recognize epitopes between amino acids 67–74, 82–87, and 129–138 (see Methods). Fig. 2 shows staining using the antibody recognizing epitopes between amino acids 82 and 87. Very similar images were obtained with all the other antibodies (not shown). Recognition of MBP was markedly reduced in samples from rats taken at 90 min post-CO, and if rats were treated with just 1 ATA O_2 or 2.8 ATA pressure (just the CO-only sample is shown in the figure). If rats had been treated with HBO_2 following CO exposure, recognition was preserved. This indicated that although the charge pattern of MBP in HBO_2-treated animals was not normal, antibody recognition was preserved.

Reduced antibody recognition of MBP from CO poisoned rats was assessed quantitatively by comparing the MBP band density with the band density of glucose transporter 3 [GLUT 3] and actin (Fig. 3). GLUT 3 was chosen because it is abundantly expressed within neurons (Nagamatsu et al., 1993). Similar analyses were possible using a less specific protein, actin. A reduction in band density expressed as the ratio of MBP or actin to GLUT 3 was present at 90 min through 5 days after CO exposure, but this was not observed with material from HBO_2-treated CO-exposed rats.

Lymphocyte activation

Because the MBP from HBO_2-treated CO-exposed rats was not normal, as shown in Fig. 1, we questioned whether the lymphocytes in these rats become sensitized to MBP as do lymphocytes in untreated CO-exposed rats (Thom et al., 2004a). To test this, lymphocytes were harvested from control rats and rats killed 4 days after CO exposure (without or with HBO_2 treatment), and they were screened for an in vitro proliferative response to MBP. Two stimuli from control rats and from rats killed 90 min after CO exposure were used for this study; the 18.5 kDa MBP (see Methods for extraction details) and fraction 5 protein collected from CM chromatography elutions (as shown in Fig. 1). A proliferative response to these materials was observed with lymphocytes from CO-exposed rats, but not lymphocytes from control rats or from rats exposed to CO and treated with HBO_2 (Fig. 4).

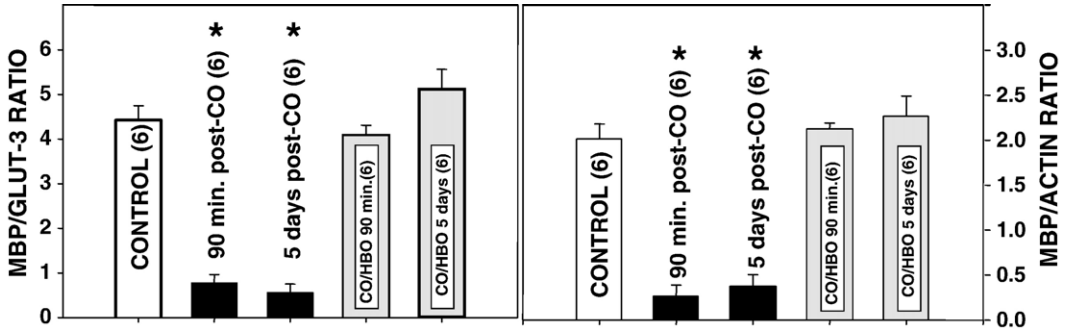

Fig. 3. Quantification of Western blot MBP staining expressed as the ratio of band densities for MBP using the antibody that recognizes the 82–87 amino acid epitope versus GLUT 3 or actin. Homogenates from 6 different rats were used for all blots probed. Values are mean ± SE, *$P < 0.05$.

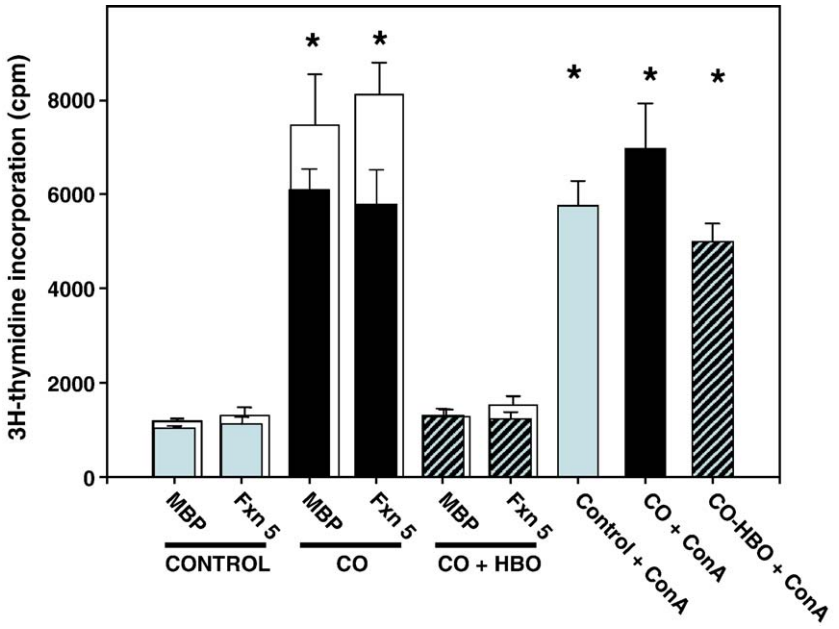

Fig. 4. Lymphocyte proliferation in response to MBP. Groups show proliferative responses of lymphocytes from control rats, rats killed 4 days after CO exposure, and rats killed 4 days after being subjected to CO exposure and then treated with 2.8 ATA O_2 for 45 min beginning 45 min after CO. Cells were exposed to 5 μg of 18.5 kDa MBP or CM chromatography fraction 5 eluates from the brains of control rats (white bars) and rats killed 90 min after CO exposure (shaded bars), or 5 μg Concanavalin A. Values are mean ± SE, $n = 6$ per group, $^*P < 0.05$.

Microglial activation

Microglia were identified on brain sections by their binding of isolectin B_4 and cell activation determined by expression of MHC II antigens and CD-40, the glycoprotein ligand for the T cell receptor. In control brains, microglia exhibited only faint staining for MHC II and CD-40 in comparison to observations in brains from CO-exposed rats (MHC II staining is shown in Fig. 5). Sections were analyzed encompassing the hippocampus, but activated microglia were distributed throughout the

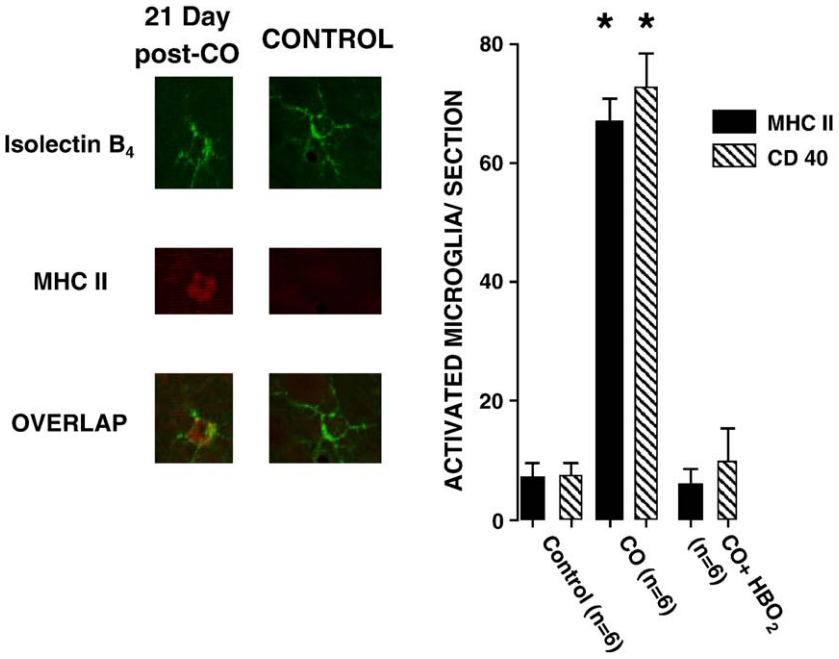

Fig. 5. Microglia activation 21 days after CO exposure. The left side of the figure shows examples of cells exhibiting dual staining for FITC-conjugated isolectin B_4 (green) and MHC II counterstained with Cy3 (red) in brain sections from a rat killed 21 days after CO exposure and a control rat. The right side of the figure shows results when activated microglia were counted as cells expressing isolectin B_4 and MHC II on brain sections, and on other sections, isolectin B_4 and CD 40. The label CO indicates rats killed and brains fixed 21 days after CO exposure; CO-HBO indicates rats killed 21 days after being subjected to CO exposure and then treated with 2.8 ATA O_2 for 45 min beginning 45 min after CO. Data are mean ± SE, six rats used in each group, $^*P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

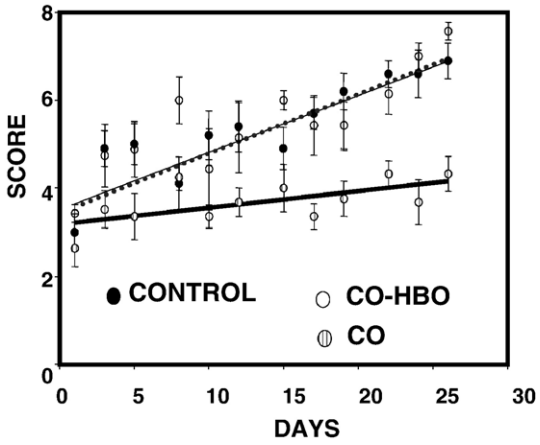

Fig. 6. Maze maneuvering scores. Entry-to-repeat scores for control (closed circles), CO-exposed rats (hatched circles), and CO-exposed rats treated with 2.8 ATA O_2 for 45 min beginning 45 min after CO exposure (open circles and dashed line) ($n = 7$ rats in each group). Scores for the last week of testing were significantly greater than the initial score for control rats and CO-exposed rats treated with HBO_2 ($P < 0.05$, repeated measures ANOVA). Scores for untreated CO-exposed rats were not significantly different over the 4 week testing period.

grey and white matter in no discernible anatomic pattern. When activated microglia were counted across the entire thin section of brains, there were significantly more after CO exposure, but activation was not evident in brain sections from CO-exposed rats treated with HBO_2 (Fig. 5).

Radial maze performance

We assessed the ability of rats to maneuver in a baited 8-arm radial maze. Control rats exhibited progressive improvement in 'entry-to-repeat' scores, reflected by comparing the scores on individual days and by the slope of the linear regression line (Fig. 6). There were no significant differences in the 'entry-to-repeat' scores of CO-exposed rats, and the regression line showed no significant trend. Significant improvements in 'entry-to-repeat' scores were observed among rats exposed to HBO_2 after CO, and the linear regression line for treated rats overlapped that seen with control. Rats in the control and CO-exposed, HBO_2-treatment groups exhibited fewer errors (arm reentries) than did the untreated CO-exposed rats (Fig. 7). Control and HBO_2-treated rats also had significantly shorter session durations in the last week of testing, as the test terminated when all eight arms had been entered. As shown in Fig. 7, the session duration was insignificantly different over the entire 4-week testing period for untreated, CO-exposed rats. Because these rats still entered many arms, however, there was no significant difference among the three groups of rats in the response-duration parameter or latency measurement.

Discussion

Results from this study indicate that HBO_2 treatment after CO exposure can prevent lymphocyte sensitization to MBP, secondary microglial activation, and impaired radial maze performance. In previous studies, we have shown that HBO_2 will inhibit β_2 integrin-mediated neutrophil adhesion to brain microvasculature, and that this action prevents CO-mediated brain lipid peroxidation (Thom, 1993). This mechanism of action is consistent with results in the present trial, but HBO_2 was not completely effective at blocking biochemical changes. The charge pattern of MBP based on cation exchange chromatography was intermediate between control and that observed

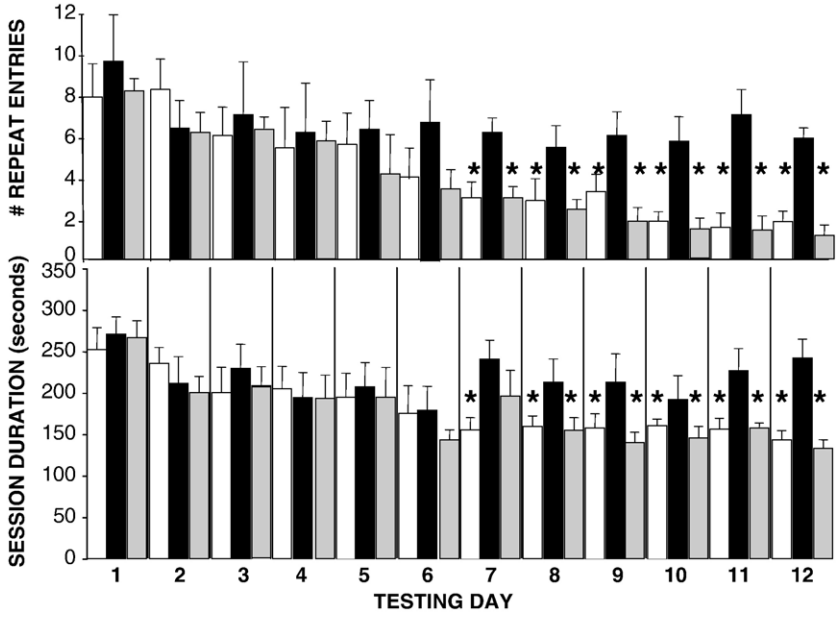

Fig. 7. Maze errors and session duration. Upper panel of bar graphs shows the number of repeat entries and the lower panel shows session duration for control (white bars), CO-exposed rats (black bars), and CO-exposed rats treated with 2.8 ATA O_2 for 45 min beginning 45 min after CO exposure (grey bars, $n = 7$ rats in each group). *Significantly lower value ($P < 0.05$, repeated measures ANOVA) versus initial score for each group of rats. Scores for CO-exposed rats were not significantly different over the 4 week testing period.

with material from untreated, CO-exposed rats. This may indicate that a degree of lipid peroxidation occurred in the brains of HBO_2-treated rats which was below the detection limits of assays utilized in previous trials, or that there are several pathways for MBP modification.

As a separate method to examine MBP alterations that occur following CO exposure, we assessed antibody recognition on Western blots. Whereas antibodies did not recognize MBP obtained from brains of rats for 5 days following CO, MBP from HBO_2-treated, CO-exposed rats was recognized by the battery of antibodies. From this, we conclude that HBO_2 treatment prevented gross alterations in MBP protein structure.

Lymphocytes from CO-exposed rats proliferate when exposed to MBP and to the fraction 5 MBP column chromatography eluate. That responsive lymphocytes exhibit cross-reactivity to materials from control and CO-exposed rats is consistent with our previous report (Thom et al., 2004a). Responsive lymphocytes were not found in the HBO_2-treated, CO-exposed rats. From this observation, we conclude that the MBP alterations that occurred in the HBO_2-treated rats were insufficient to trigger recognition of MBP as a foreign protein. Because lymphocytes responded normally to ConA, HBO_2 does not appear to have a primary immunosuppressive effect in this model.

Entry of inflammatory cells to the CNS and microglial activation is correlated with progression of brain injury in a variety of pathophysiological processes (Fan et al., 2005; McGeer et al., 1988; Heneka et al., 2002). The consequences of brain inflammation due to infection have been extensively studied, but inflammation is also believed to play a role in the pathogenesis of chronic neurodegenerative disorders (Ballestas and Benveniste, 1995; Fan et al., 2005; Moor et al., 1994; Wu et al., 2002). How immune cells gain entry to the brain is unclear. Studies with chimeric mice have shown that expression of the T cell receptor (CD-40) by microglia controls the migration and retention of self-reactive T cells, and if interactions are inhibited; CD-40 expression and chemokine secretion are diminished (Becher et al., 2001; Eliopoulos et al., 2003). This sequence of events may be occurring as a consequence of CO poisoning. Microglial activation in response to CO fails to occur if lymphocytes are de-sensitized from responding to chemically altered MBP (Thom et al., 2004a). Given that lymphocyte activation did not occur in CO-exposed rats treated with HBO_2, the absence of microglial activation offers additional support that this pathway may be associated with CO-mediated neuropathology.

Clinical CO poisoning causes global neuropathology (Raub et al., 2000). We have not found a discrete neuroanatomical pattern for activated microglia in this study, or our prior investigation (Thom et al., 2004a). Inflammation has been shown to impair learning by inhibiting hippocampal neurogenesis (Ekdahl et al., 2003). In our radial maze experiments, we did not carry out studies to discretely identify whether the performance decrement associated with CO exposure was due cognitive dysfunction per se, versus one or more of the more basic systems that influence cognition. Whether disturbed neurogenesis plays a role in neurological dysfunction following CO poisoning requires additional investigation.

HBO_2 was not found effective for preventing hippocampal neuronal losses and neurological dysfunction in a recent mouse model (Gilmer et al., 2002). Dysfunction in our rat model is correlated with an immunological response, and we have not observed significant neuronal losses assessed by standard histological techniques or the TUNEL assay (Thom et al., 2004a, and unpublished observations). The results with the mouse model indicate that HBO_2 was not efficacious for reducing cortical dysfunction associated with neuronal losses that are presumably related to CO-mediated hypoxic stress. Mice were exposed for a period of time to 50,000 ppm CO (Gilmer et al., 2002). Previous studies with our model have shown that cerebral blood flow increases by 54% within minutes of the 1000 ppm CO exposure and it remains elevated until loss of consciousness during the exposure to 3000 ppm CO when, due to transient cardiac compromise, blood pressure drops to 50% below normal for ~4 min (Thom, 1990, Thom et al., 2004b). There is never a time when blood flow ceases, and brain mitochondrial function is only mildly disturbed during unconsciousness. Assessed by reflectance spectroscopy, CO poisoning causes a transient 11% increase in the NADH/NAD ratio (Mayevsky et al., 1994, 1995; Meilin et al., 1996). Thus, hypoxic stress is nominal and the immunological pathway for neuronal dysfunction predominates.

Results from this study offer a perspective that may explain discrepant results pertaining to the efficacy of HBO_2 treatment in animal models, and perhaps also in clinical CO poisoning. HBO_2 will temporarily inhibit human neutrophil β_2 integrin function, just as we have shown in rats (Thom et al., 1997). We conclude that HBO_2 prevents immune-mediated neurological deterioration after CO poisoning because it partially prevents the acute biochemical alterations in MBP.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.taap.2005.10.006.

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