

Hyperbaric oxygen pre-breathe modifies the outcome of decompression sickness.

B.D. BUTLER¹, T. LITTLE¹, V. COGAN¹, M. POWELL²

¹Baromedical laboratory, Department of Anesthesiology, The University of Texas Houston Medical School, Houston, TX 77030; ² NASA-JSC Houston, TX

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Butler BD, Little T, Cogan V, Powell M. Hyperbaric oxygen pre-breathe modifies the outcome of decompression sickness. *Undersea Hyperb Med* 2006; 33(6):407-417. Deep sea divers, aviators and astronauts are at risk of decompression sickness when the ambient pressure reductions exceed a critical threshold. Venous bubbles associated with decompression sickness have the potential to react with the vascular membrane and adjacent blood products, eliciting an inflammatory cascade. Preventive measures usually involve careful decompression procedures to avoid or reduce bubble formation. De-nitrogenation with 100% oxygen pre-breathing as a preventive measure has been well established at least in altitude decompression exposures. The objective of this study was to determine the physiological and biochemical effects of Hyperbaric Oxygen Pre-breathe (HBOP) upon decompression from a hyperbaric exposure. Male Sprague-Dawley rats were randomly assigned to one of eight groups. Two experimental groups received HBOP at 1 and 18 hours prior to decompression, as compared with ground level oxygen or non-treated groups that still experienced decompression stress, and the associated non-decompressed controls. The results showed decreased extravascular lung water (pulmonary edema), bronchoalveolar lavage and pleural protein and arterial, broncho-alveolar lavage, and urine leukotriene E₄ (LKE₄) levels in both the 1Hr and 18Hr HBOP decompressed rats compared to non-oxygenated decompressed rats, as well as a decreased overall expression of signs of decompression sickness. This study indicates that HBOP-treated rats exhibit fewer signs and complications of decompression sickness compared with non-treated or ground level oxygen treated rats.

INTRODUCTION

Decompression sickness (DCS) is a significant concern for divers, pilots and astronauts when there is a decrease in ambient pressure sufficient to cause tissue and venous nitrogen bubble formation. The symptoms of DCS range from minor joint pain and skin rashes/itching to more severe cases of respiratory or circulatory complications and paralysis (1). The etiology has been attributed to both extravascular and intravascular bubbles. The hemodynamic and biochemical changes associated with venous bubbles can cause damage to the microvasculature of the lung and other organs (2, 3), and initiate an inflammatory response due to recruitment of neutrophils (4, 5) with subsequent release of bioactive mediators

including arachidonic acid metabolites in the blood and tissues (6, 7).

Hyperbaric oxygen therapy and recompression are the treatment of choice in cases of DCS where the release of gas bubbles cause physiological changes and tissue injury (8-12). The venous gas emboli (VGE) cause some of the injury and evolve from the nitrogen gases stored in the body's tissues. Methods utilizing 100% oxygen pre-breathe, as a preventive measure before decompression to altitude, facilitate the removal of nitrogen from the lungs, blood and body tissues, thereby reducing the formation of VGE, the development of DCS, and the need for hyperbaric oxygen therapy. De-nitrogenation of the tissues

before an altitude [hypobaric] decompression exposure has long been proven to lessen both the occurrence and symptoms of DCS (13, 14, 15). 100% oxygen pre-breathe has been used as a preventive measure to reduce the risk of DCS by astronauts during extravehicular activity (EVA) in space (16). Recent improvements, utilizing exercise-enhanced preoxygenation (non-hyperbaric), have shown to be beneficial in altitude DCS prevention (17).

Using oxygen pre-breathe as a preventive measure in medicine has been limited principally to anesthesia induction (preoxygenation) and surgical cases (18). Preconditioning of animals using oxygen breathing in various injury models has been reported sparsely to show cerebral and spinal ischemic tolerance in gerbils (19), rats (20) and rabbits (21). Its use in prevention against DCS from hyperbaric exposures has not been well explored (22). This paper addresses the potential protective effects of HBOP prior to hyperbaric compression and whether any such effects are limited by time.

METHODS

The institutional Animal Welfare Committee approved the procedures described in this study. Procedure: 107 Male Sprague-Dawley rats (479.21 ± 37.43 g SD) were divided into eight {8} groups:

Group 1 – No Oxygen Pre-breathe (No O₂): No Compression/Decompression (No C/DC)

Group 2 – Ground Level Oxygen (GLO): No C/DC

Group 3 – HBO₂ Pre-breathe (HBOP), 1 Hour delay: No C/DC

Group 4 – HBOP, 18 Hour delay: No C/DC

Group 5 – No O₂: Compression/Decompression (C/DC)

Group 6 – GLO: C/DC

Group 7 – HBOP, 1 Hour delay: C/DC

Group 8 – HBOP, 18 Hour delay: C/DC

All groups were kept on the same circadian time schedule. Ascent from compression was scheduled for 6 hours after lights on. Groups 1 and 5 received no oxygen pretreatment, either ground level (GLO) or hyperbaric (HBOP). Groups 2 and 6 were placed in the hyperbaric chamber on 100% O₂ (medical grade) for 45 minutes at sea level. Groups 3, 4, 7 and 8 were placed in the hyperbaric chamber, compressed with 100% O₂ to 285.10 kPa at a rate of 45.94 kPa/min (15 ft/min). The chamber was continuously flushed with oxygen throughout the dive to prevent the build-up of carbon dioxide. The rats remained at depth on 100% O₂ for 45 minutes and then were decompressed to surface at 45.94 kPa/min.

Following their respective oxygen exposures, the rats were removed from the hyperbaric chamber for a surface interval of 1 hour (Groups 2, 3, 6 and 7), or 18 hours, (Groups 4 and 8) on room air. Groups 5, 6, 7 and 8 were then compressed to 683.29 kPa on room air at a rate of 52.07 kPa/min (~17 ft/min over 11 min). The rats exposed to 683.29 kPa remained at depth for 60 minutes and then were decompressed to surface at a rate of 61.26 kPa/min (20 ft/min over 9.5 min). Groups 1, 2, 3 and 4 were not exposed to pressure. Gross signs of DCS were noted following each dive by an un-blinded observer. The 683.29 kPa exposure has been shown to produce moderate to severe signs (Table 1) of DCS and venous bubble formation (7). HBOP-induced decreases in tissue and blood nitrogen levels can effect DCS

Table 1. Occurrence of DCS Signs

Severity Scale [1-4] {Group}	No O ₂ C/DC {5}	1Hr GLO C/DC {6}	1Hr HBOP C/DC {7}	18Hr HBOP C/DC {8}
n	14	15	16	13
Tachypnea [1]	13	12	8*	9
Cyanosis [1]	13	13	3*	5*
Dyspnea [1]	11	9	7*	6
Cheyne-Stokes [1]	0	1	0	0
Weighted Respiratory Score	2.64	2.33	1.12*	1.54*
Pulmonary Arterial Gas Emboli [2]	3	3	1	2
IVC Gas Emboli [2]	8	7	2*	6
Thoracic Aortic Bubbles [3]	4	2	1	2
Weighted Air Emboli Score	2.43	1.73	0.56*	1.69
Blebs [1]	8	6	1*	3
Petechiae [2]	3	12*	10*	7
Hematomas [2]	6	12*	6	7
Perivascular Cuffing [2]	10	4*	1*	3*
Atelectasis [2]	8	1*	0*	0*
Alveolar Edema [3]	3	0	0*	0*
Weighted Pulmonary Score	5.07	4.27	2.19*	2.85*
Limb paralysis [3]	6	5	0*	2
Chokes [3]	4	1	1	1
Seizure [3]	3	3	0*	1
Death [4]	3	3	1	2
Weighted Neurological Score	3.64	2.60	0.44*	1.54*
Weighted Score Based on Severity	13.78	10.93	4.31	7.62

Weighted Scores are calculated by multiplying Severity scale value [1-4] with the number of animals showing signs and dividing by the total number of animals in group (n). * p<0.05 No O₂:C/DC vs. GLO (1Hr):C/DC, HBOP (1Hr):C/DC or HBOP (18Hr):C/DC

bubble formation. Because the rate of nitrogen uptake is such that saturation in rats occurs within 50 min. (23), the time at depth in the current studies was sufficient to avoid carry-over of the nitrogen washout effect.

Analysis

Following the postdecompression period (60 minutes), all animals were anesthetized with 5% Halothane in air and maintained on 1.5-2.5% for blood collection and euthanasia via exsanguination through the abdominal descending aorta. This time was selected for optimal response to post-decompression eicosanoid levels (6). In Groups 2 and 3 this corresponded to 3 hours post GLO or HBOP and 21 hours post HBOP for Group 4.

Once the rats were anesthetized, an abdominal incision was made and gross observation of the abdominal cavity was performed. Pathological signs of DCS were noted, including venous and arterial bubbling (See Table 1). Urine collected via direct bladder

puncture was stored at -70°C until assayed for mediator activity. An additional sample was stored at 0°C to be assayed for creatinine concentration (Sigma Chemical Company, St. Louis, MO) (24) to standardize the results. The abdominal aorta was cannulated for collection of arterial blood. This sample was used to obtain white cell counts (WBC), differential cell counts, extravascular lung water (EVLW) ratio blood corrections, arterial nitric oxide (NO) measurements and thromboxane B₂ (TxB₂) and leukotriene E₄ (LKE₄) levels. Euthanasia was performed at this time. The thorax was opened and pleural fluid collected for WBC counts, differential cell counts and protein analysis (25). The lungs were observed for blebs, petechiae, hematomas, perivascular cuffing, atelectasis and alveolar flooding (Table 1). The trachea was cannulated and the lungs removed for bronchoalveolar lavage (BAL) and lung edema assessment.

Evaluation of the lungs for BAL and lung edema assessment was performed

by obtaining a fresh lung weight and then degassing them by vacuum (3x). The airways were lavaged with 10 mls (3x) ice-cold saline (5°C). This procedure was repeated for a total of 4 washes. All washes were pooled (~40 mls) and analyzed for WBC and differential cell count, and protein and eicosanoid measurements. The lung was weighed, post-lavage to determine residual saline volume as a correction factor when determining lung edema. EVLW determination was corrected to account for residual blood volume in the lung (26). The EVLW was expressed as the ratio of blood-free extravascular lung fluid to dry lung weight.

Protein

Total protein from the bronchoalveolar lavage (BAL) and pleural fluid were determined using the Modified Lowry Protein Assay (Pierce Chemical Company, Rockford, IL) (27) adapted to a microplate reader. Samples were obtained from a cell-free supernatant and stored at 0°C until assayed. BAL concentrations are expressed as milligrams per gram dry lung weight to minimize the effects of edema on the lung protein values. Pleural fluid is expressed as milligrams per milliliter fluid.

Cell Counts

WBC counts for the arterial blood, pleural fluid and BAL were determined using a Neubauer hemocytometer. Differential cell counts for the arterial blood was determined after staining with Wright-Geimsa and for the pleural and BAL samples after a HEMA 3 stain (Fisher Scientific Company, Kalamazoo, MI), a 3-step staining process similar to Wright-Geimsa.

Mediator Assay

The concentration of vasoactive mediators taken from the biological samples (urine, plasma and BAL) of control animals

and those undergoing decompression were quantified using enzyme immunoassay (EIA) techniques (Cayman Chemical Company, Ann Arbor, MI). The EIA's analyzed were Thromboxane B₂ (TxB₂) and Leukotriene E₄ (LKE₄). TxB₂ is the stable metabolite of TxA₂, a potent vasoconstrictor, and is found primarily in the plasma. LKE₄, a potent vaso- and broncho- constrictor, is prominent in the urine. Arterial and BAL samples were purified using a C-18 Sep-Pak (Waters Corporation, Milford, MA) before performing the assays. Eicosanoid concentrations were expressed as picograms per milliliter in arterial plasma, picograms per gram dry lung weight in the BAL, and picograms per milligram creatinine in urine.

Nitric oxide levels, expressed as plasma nitrate, were quantified using a two-step process. The first step converts the nitrate to nitrite with nitrate reductase. The second step is the addition of the Griess Reagents to convert the nitrite into a purple azo compound. A photometric measurement of this compound determines nitrite concentration at 540 nm (Cayman Chemical Company, Ann Arbor, MI).

Statistics

Data were analyzed using ANOVA with Fisher multiple comparison test. All data were expressed as mean ± standard error. DCS Signs chart values (Table 1) were analyzed using a Chi-square comparison between the No O₂: C/DC group and the 1Hr GLO: C/DC, 1Hr HBOP: C/DC or 18Hr HBOP: C/DC groups. p<0.05 was considered statistically significant for both comparisons.

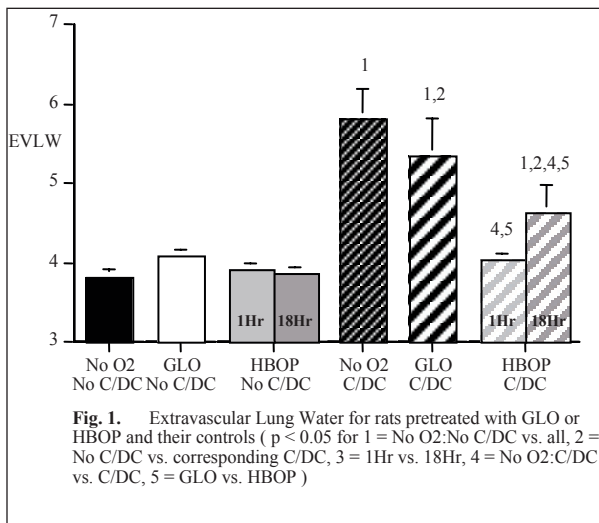
RESULTS

Signs of DCS are presented in Table 1. The weighted respiratory, air emboli, pulmonary and neurological scores for the 1Hr and 18Hr HBOP: C/DC treated groups showed

significant protection compared with the No O₂: C/DC group, in all cases except for the air emboli in 18Hr HBOP: C/DC group.

Lung Weight

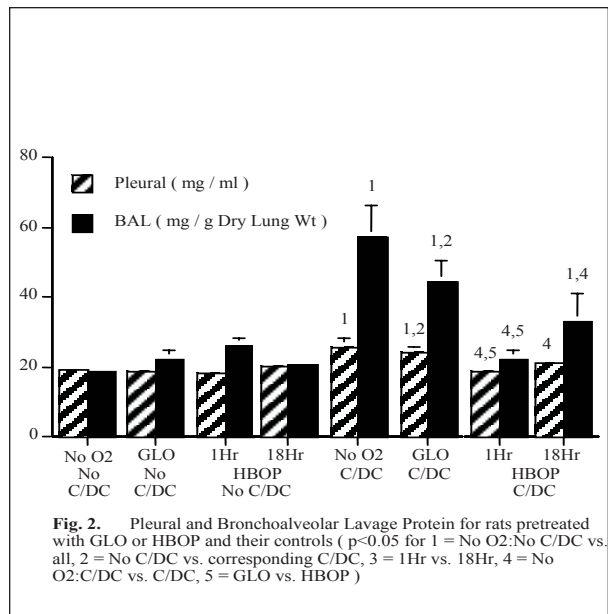
Wet:dry lung weight ratios (Fig. 1) that depict the extent of pulmonary edema (extravascular lung water) formation were significantly increased ($p < 0.05$) in the No O₂: C/DC, GLO: C/DC and 18Hr HBOP: C/DC group compared with the No O₂: No C/DC control group. The lung weight ratios of the treated 1Hr HBOP: C/DC rats remained normal, for the No O₂: No C/DC and 1Hr HBOP: No C/DC levels. Both 1Hr and 18Hr HBOP: C/DC treated groups were significantly decreased from No O₂: C/DC rats. HBOP clearly provided protection from DCS.



Bronchoalveolar lavage (BAL) and pleural protein

Protein values (Fig. 2) were significantly increased ($p < 0.05$) at 1Hr post decompression in the No O₂: C/DC and GLO:C/DC groups compared with the controls, No O₂:No C/DC for pleural and lavage. 18Hr HBOP: C/DC lavage protein levels were also significantly higher than the No O₂: No C/DC group. 1Hr HBOP: C/DC remained unchanged compared with the No O₂: No C/DC and 1Hr HBOP:

No C/DC groups for both pleural and lavage protein. 1Hr and 18Hr HBOP: C/DC protein levels were significantly lower than No O₂: DCS levels for BAL and pleural, indicating protection of the microvascular membrane.



Cell Counts

BAL WBC counts (Table 2) in all decompressed groups (C/DC) were significantly elevated over the No O₂: C/DC group. BAL neutrophils (Table 2) similarly showed significant increases over the No O₂: No C/DC control rats. The BAL neutrophils in the 1Hr and 18Hr HBOP: C/DC groups were not significantly increased over their corresponding 1Hr and 18Hr HBOP: No C/DC groups.

Arterial WBC (Table 2) counts from all other groups were elevated over the control, No O₂: No C/DC group. 18Hr HBOP: C/DC, although elevated, was not significant ($p > 0.05$). The 1Hr GLO: C/DC and 1Hr and 18Hr HBOP: C/DC groups were reduced from No O₂: C/DC, but only 18Hr HBOP: C/DC was significant. Arterial neutrophils (Table 2) were significantly increased in the GLO: No C/DC, No O₂: C/DC, GLO: C/DC and 1Hr and 18Hr HBOP: C/DC groups over the No O₂: No C/DC group.

Table 2. White Blood Cell and Neutrophils

Group {#}	Arterial		Pleural		BAL	
	WBC	Neutrophils	WBC	Neutrophils	WBC	Neutrophils
No O ₂ :No C/DC {1}	2950±157	18.7±0.9	64675±3487	17.4±1.1	330±32	0.4±0.3
1Hr GLO:No C/DC {2}	4060±300 ¹	25.7±1.8 ¹	65313±3906	21.8±1.4 ¹	410±29	0.7±0.2
1Hr HBOP:No C/DC {3}	4737±288 ¹	22.3±1.6	61409±3119	23.3±1.9 ¹	403±25	0.7±0.2
18Hr HBOP:No C/DC {4}	4281±224 ¹	16.8±1.0 ⁵	62409±2020	18.2±1.2 ³	485±43 ¹	1.0±0.1
No O ₂ :C/DC {5}	5005±637 ¹	29.9±3.6 ¹	55687±6805	22.7±1.6 ¹	710±37 ¹	1.6±0.4 ¹
1Hr GLO:C/DC {6}	4070±296 ¹	27.5±2.2 ¹	63937±4667	23.3±1.6 ¹	729±76 ^{1,2}	1.6±0.3 ^{1,2}
1Hr HBOP:C/DC {7}	4503±285 ¹	30.9±2.2 ^{1,2}	78283±6967 ^{2,4,5}	23.0±1.4 ¹	766±67 ^{1,2}	1.2±0.2 ¹
18Hr HBOP:C/DC {8}	3691±189 ⁴	26.8±2.1 ^{1,2}	69300±3949	17.8±1.2 ^{3,4,5}	595±68 ^{1,3}	1.3±0.3 ¹

Data are means ± S.E. (Significance 1-5). WBC expressed as cells/cumm, neutrophils expressed as % total WBC count. p < 0.05 for 1 = No O₂:No C/DC vs. All, 2 = No C/DC vs. C/DC, 3 = 1Hr vs. 18Hr, 4 = No O₂:C/DC vs. C/DC, 5 = GLO vs. HBOP

Pleural WBC counts (Table 2) for 1Hr HBOP: C/DC were significantly elevated over 1Hr HBOP: No C/DC, No O₂: C/DC and GLO: C/DC. Pleural WBC counts for the 18Hr HBOP: C/DC group were elevated as well, but not significantly. Pleural neutrophils (Table 2) significantly increased compared with the No O₂: No C/DC group, in GLO: No C/DC, 1Hr HBOP: No C/DC, No O₂: C/DC, GLO: C/DC and 1Hr HBOP: C/DC. The 18Hr HBOP: No C/DC and 18Hr HBOP: C/DC pleural neutrophils remained unchanged from No O₂: No C/DC.

Eicosanoid levels

Arterial TxB₂ levels (Table 3) remained unchanged from the control (No O₂: No C/DC) in all groups except for the 18Hr HBOP groups. These 2 groups were also significantly elevated over their respective GLO groups and 1Hr HBOP groups. Arterial LKE₄ levels (Fig.

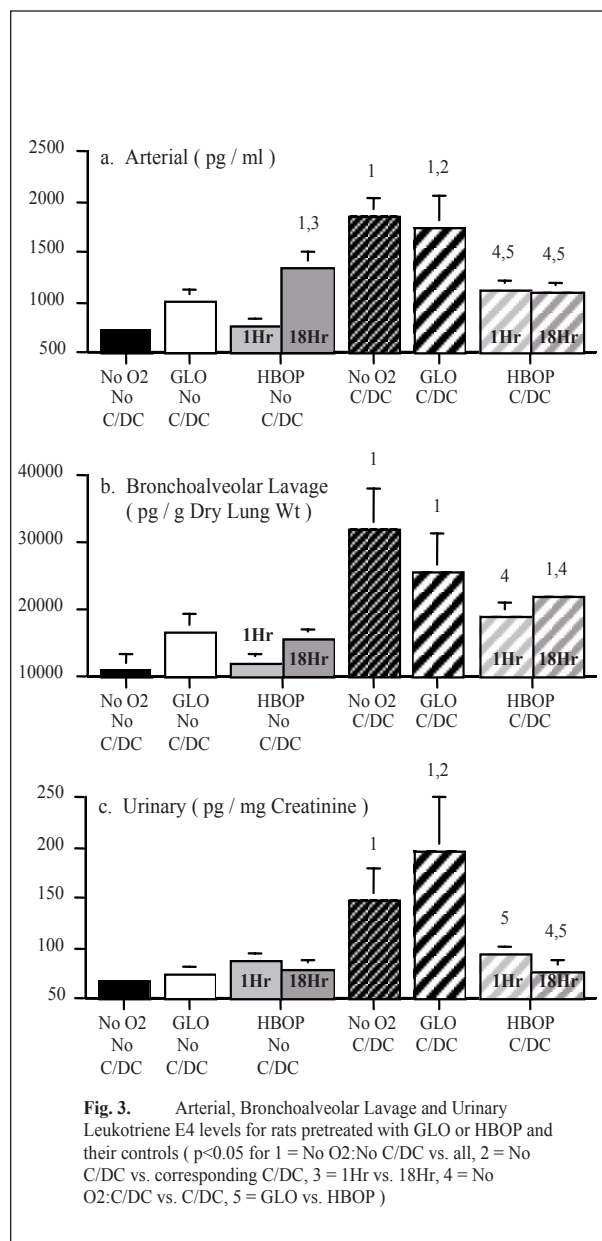
3.a.) were significantly increased in the 18Hr HBOP: No C/DC, No O₂: C/DC and GLO: C/DC groups over the No O₂: No C/DC control group. The 1Hr and 18Hr HBOP: C/DC groups remained unchanged from the control No O₂: No C/DC group as well as their corresponding 1Hr and 18Hr HBOP: No C/DC Groups. The 1Hr and 18Hr HBOP: C/DC groups were also significantly decreased from the No O₂: C/DC Group and the GLO: C/DC Group.

BAL TxB₂ levels (Table 3) were significantly elevated over No O₂: No C/DC in the 18Hr HBOP: No C/DC and GLO: C/DC groups. Both the 1 Hr HBOP groups (No C/DC and C/DC) were significantly decreased from the No O₂: No C/DC group. The BAL LKE₄ levels (Fig. 3.b.) in the No O₂: C/DC group, GLO: C/DC group and 18Hr HBOP: C/DC group were significantly increased over No O₂: No C/DC. 1Hr HBOP: C/DC although elevated was not significant. Both 1Hr and 18 HR HBOP:

Table 3. Thromboxane B₂ and Nitric Oxide

Group {#}	Thromboxane B ₂		Urine	Nitric Oxide
	Arterial	BAL		
No O ₂ :No C/DC {1}	142±20	12329±589	544±59	21.48±1.94
1Hr GLO:No C/DC {2}	178±28	14148±769	654±92	14.41±1.40 ¹
1Hr HBOP:No C/DC {3}	226±34	9687±294 ^{1,5}	905±103	14.62±0.77 ¹
18Hr HBOP:No C/DC {4}	340±62 ^{1,3,5}	14793±726 ^{1,3}	706±65	14.65±1.64 ¹
No O ₂ :C/DC {5}	215±28	11653±586	1854±612 ¹	13.12±1.11 ¹
1Hr GLO:C/DC {6}	156±33	14380±701 ^{1,4}	1245±256 ¹	13.73±0.69 ¹
1Hr HBOP:C/DC {7}	227±37	10121±360 ^{1,5}	1133±93 ⁴	14.17±1.10 ¹
18Hr HBOP:C/DC {8}	341±37 ^{1,3,4,5}	13223±920 ³	1201±156	14.60±1.07 ¹

Data are means ± S.E. (Significance 1-5). Arterial TxB₂ expressed as pg/ml, BAL TxB₂ expressed as pg/g Dry Lung Wt., and Urine TxB₂ expressed as pg/mg Creatinine. p < 0.05 for 1 = No O₂:No C/DC vs. All, 2 = No C/DC vs. C/DC, 3 = 1Hr vs. 18Hr, 4 = No O₂:C/DC vs. C/DC, 5 = GLO vs. HBOP



C/DC groups were significantly decreased from No O₂: C/DC.

Urinary TxB₂ levels (Table 3) were significantly elevated over the control group, No O₂: No C/DC, in the No O₂: C/DC and GLO: C/DC groups. 1Hr HBOP: C/DC was significantly decreased from No O₂:C/DC. 18Hr HBOP: C/DC was decreased, but not significantly. Urinary LKE₄ levels (Fig. 3.c) in the No O₂: C/DC group and the GLO: C/DC group were significantly elevated over No O₂:

No C/DC. 1Hr and 18Hr HBOP: C/DC groups were decreased from the urinary LKE₄ levels in the No O₂: C/DC group, but only significantly in 18Hr HBOP: C/DC.

Nitric Oxide levels

Nitric oxide (NO) levels (Table 3) were significantly decreased in all experimental groups compared with the control group, No O₂: No C/DC. The decreases caused by oxygen administration were not different than those caused by C/DC alone.

DISCUSSION

The results from this study indicate that HBOP (1Hr and 18Hr) provided significant protection from inflammatory DCS symptoms. The control animals (No C/DC groups) receiving either GLO or HBOP without decompression, showed no significant untoward signs of oxygen toxicity on the lungs. Other indicators, such as NO, arterial WBC and pleural neutrophils were changed.

The use of oxygen pre-breathe as a preventive measure in hyperbaric decompression has been limited, however, its use with altitude decompression has been well established since the 1940's (14). The use of HBOP in prevention against DCS following hyperbaric exposures has not been well explored (22,28). Soutiere et. al. (29) reported greater survival and reduced Type II DCS with isobaric oxygen pre-breathe under saturation conditions (2.82 ATA), when using a large animal (porcine) model simulating DCS conditions in a disabled submarine. Landolfi et al (30) reported that HBOP administered immediately prior to compression/decompression exposures reduced VGE formation in human subjects.

An initial hypothesis of the beneficial effects of HBOP was that it caused a biomechanical reduction in the size of blood

and tissue gas nuclei by Boyle's Law. Increased surface tension effects would then further reduce bubble nuclei size. This may be true at 1Hr HBOP, but the prolonged positive effects at 18Hrs after its application indicate that nuclei reduction cannot be the sole effect. Using HBOP in a cerebral rat model of DCS, Martin and Thom (31) suggested that the lack of cerebral DCS symptoms may be related to HBOP's ability to prevent leukocyte sequestration in the brain and possibly other organs. Further studies suggested that HBOP inhibited β_2 -integrin-mediated leukocyte adhesion (32). Other studies showing that dibutyryl cAMP reduced some of the respiratory symptoms and pathophysiological changes generally seen in rats' lungs following decompression (7) were also associated with the lack of neutrophil activation. In the current study, HBOP delivered 1 hour or even 18 hours prior to DCS afforded protection in most of the variables examined.

Gross pulmonary changes associated with DCS (dyspnea, cyanosis, chokes and other respiratory signs) are attributed to both obstruction of the pulmonary vasculature by bubbles as well as activation of neutrophils and the associated damage to endothelial cells (33) leading to pulmonary edema formation. In small animal models of C/DC, pulmonary edema is a key marker indicating severity of decompression sickness (6, 7, 34). In the present study we found that HBOP treated rats had significantly decreased extravascular lung water (EVLW) compared with non-treated and GLO rats that underwent C/DC. The 1Hr HBOP C/DC rats' EVLW remained unchanged from control and HBOP no C/DC rats. Elevated protein in the lavage and pleural fluids of the non-HBOP (No O₂ and GLO) treated groups compared to 1Hr and 18Hr HBOP-decompressed rats also indicated that HBOP prevented microvascular epithelial membrane permeability increases. Pleural protein levels have been shown to correlate with pulmonary interstitial levels in

rats, thereby allowing one to extrapolate the flow of fluids from the microvasculature across the interstitial space and into the alveolar sacs (25).

The results show that even before C/DC, HBOP elevates arterial and BAL WBC counts and arterial (1Hr), pleural (1Hr) and BAL (1Hr and 18Hr) neutrophils over that of Control (no O₂) rats. After decompression, however, the HBOP arterial WBC counts decreased from their respective no C/DC HBOP groups, while the non-oxygenated decompressed rats' cell counts increased. Although not as dramatic as EVLW and protein, the changes in white blood cell (WBC) counts and neutrophils give the same indication that HBOP prior to C/DC exposure is beneficial.

C/DC in all groups elevated BAL neutrophils, while HBOP decreased these values from C/DC alone, albeit non-significantly. Thom et al., (12) reported that HBOP did not change circulating neutrophil counts. The authors suggested that by having no effect on surface β_2 -integrin expression, normal surface adherence was maintained. They further concluded that HBOP may manifest its effect at the membrane surface. Increased levels of activated neutrophils have been noted as the cause of microvascular permeability and edema formation with VGE in sheep (35, 36). Hyperbaric oxygen inhibits cell adherence to the cerebral microvasculature (37) and skeletal muscles (38) during an ischemic episode. In this study, 1Hr and 18Hr HBOP prior to C/DC blocked the increases in pleural (18Hr only) and BAL neutrophils and arterial WBC counts from that of No O₂ C/DC, again suggesting that the integrity of the epithelial lining remained intact.

The release of eicosanoids due to C/DC sets about a cascade of events that involve broncho- and vaso-constriction, impaired cardiac function, increased platelet and neutrophil activity and loss of microvascular

integrity (7). Inhibiting the production of inflammatory mediators, such as thromboxanes and leukotrienes, which are by-products of arachidonic acid through the cyclooxygenase or lipoxygenase pathways (39), can reduce the negative outcome initiated by their release (40).

In the present study, we examined the levels of TxB_2 and LKE_4 in the arterial plasma, BAL and urine. TxB_2 , a by-product of TxA_2 originating from the platelets and monocytes, is a vasoconstrictor of vascular and bronchiolar smooth muscle. TxA_2 is an unstable metabolite of arachidonic acid that is produced by thromboxane synthetase (41). The enzyme is found in platelets, neutrophils, monocytes, lymphocytes, and in various organs including the kidneys, heart, brain, GI and lungs (42). TxA_2 is also an active stimulant for platelet aggregation and can cause pulmonary vasoconstriction. Measurement of TxA_2 levels in any body fluid is difficult because it is rapidly hydrolyzed to its primary stable metabolite, thromboxane B_2 . Measurement of the TxB_2 metabolite, especially in the urine, reportedly gives a good approximation of TxA_2 synthesis (43). The levels of TxB_2 in the arterial and BAL fluids remained remarkably unaffected by either HBOP or DCS, except in the HBOP (18hr) groups. In the HBOP compressed groups, urine levels were reduced from the decompressed group, suggesting HBOP prevented an increased production of TxB_2 .

It has been suggested that the 5-lipoxygenase pathway (leukotrienes) plays a greater role in pulmonary edema formation (44), even with DCS (7) compared with the cyclooxygenase pathway (thromboxanes). LKE_4 can cause increased permeability in the airways, exacerbating pulmonary edema formation (45). Leukotrienes are produced in the lungs and, when protein-bound, cause neutrophil chemotaxis, smooth muscle constriction and edema formation (46). In

their free form, leukotrienes can cause many of the same symptoms seen in DCS. Pulmonary edema and hypertension, bronchoconstriction and inflammation are not uncommon. Bernard (47) and Snapper (48) both reported elevated LKE_4 levels in the urine of adult respiratory distress syndrome patients and animal models. Many of the symptoms of adult respiratory distress syndrome are similar to those seen with respiratory DCS, such as bronchoconstriction, pulmonary hypertension, lung compliance changes, hypoxemia, and lung edema. A primary source of leukotriene production is through its selective release from neutrophils (49,50). Lagarde (51) reported that neutrophil/endothelium interactions are, also a source of circulating leukotrienes. This is a likely source for increased levels of leukotrienes found in the blood due to DCS. In previous work, we have shown that pretreatment with an analogue of cAMP, dibutyryl cAMP, reduced edema formation, protein levels in the pleural and lungs and other symptoms of DCS. The LKE_4 levels decreased significantly while the TxB_2 levels were elevated or remained unchanged with exposure to C/DC (7). HBOP appears to have similar effects. In the present study HBOP decreased the arterial LKE_4 levels of both 1 Hour and 18 Hour HBOP, BAL and urine compared with the levels of non-treated rats.

In summary, this study demonstrated that rats treated with HBOP, at 1 hour and to a lesser effect at 18 hours prior to compression had decreased levels of EVLW, protein, neutrophils in the lungs, as well as LKE_4 levels and that this offered some protection from DCS. It is concluded that operational administration of HBOP prior to a bubble-provocative decompression clearly warrants further study.

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