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Research Article

HBOT2 Preconditioning Prolonged Inflammation After Decompression Diving

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ABSTRACT

Background: The mechanism involved in HBO_2 preconditioning in preventing inflammation in diving is still unclear. Syndecan-1, which is an important part of glycocalyx, has never been studied for its involvement in HBO_2 preconditioning to prevent inflammation in decompression diving. This study aims to determine how HBO_2 preconditioning impacts inflammation through Syndecan-1, MDA, and IL-1a markers. **Method:** This study is a true experimental post-test design. Forty male 12- to 14-year-old Sprague Dawley rats were divided into four groups. HBO_2 and decompression diving were carried out in an animal hyperbaric chamber. All data were collected 12 and 24 hours after the decompression diving. **Result:** The incidence of decompression sickness was less frequent in the HBO_2 preconditioning treatment group as opposed to the control group (4 vs 9) but did not reach a significant level ($p > 0.05$). All parameters showed no difference between the control and treatment groups 12 hours after the dive ($p > 0.05$). Twenty-four hours after diving, the treatment group demonstrated substantially elevated IL-1a levels in comparison to the control group ($p = 0.030$), and the increase of IL-1a in the treatment group is significant ($p = 0.001$). Although MDA levels did not reach significant, the treatment group's increase in MDA levels 24 hours after diving was greater than that of the control group. Meanwhile, The treatment group had a smaller reduction in Syndecan-1 levels in comparison to the control group following diving 24 hours later. **Conclusion:** HBO_2 preconditioning prolongs the inflammation, as evidenced by increased levels of MDA, Syndecan-1, and IL-1a, even though it can prevent decompression sickness. Further research is needed to find the right time and dose of HBO_2 preconditioning to shorten the inflammation time.
Keywords: HBO_2 preconditioning, MDA, Syndecan-1, VCAM-1, IL-1a.

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BACKGROUND

Decompression sickness is an illness caused by nitrogen bubbles that form during the decompression phase of diving. The concept of acclimatization in diving has changed the paradigm of preventing decompression sickness.[1,2] One of the preventive methods is the use of hyperbaric oxygen (HBO_2) preconditioning.[3–5] HBO_2 preconditioning has been shown to decrease inflammatory markers, such as methane dicarboxylic aldehyde (MDA), monocyte chemoattractant protein-1 (MCP-1), and Interleukin 8 (IL-8).[5] Nevertheless, the precise mechanism of HBO_2 in inhibiting inflammation remains unclear.

The interaction of bubbles formed during the decompression phase of diving with the endothelial surface causes damage to the glycocalyx (shedding), facilitating complement activation and intravascular coagulation due to the loss of the gradient between blood and parenchyma.[6,7] As a component of the glycocalyx structure, Syndecan-1 holds the key to modulating flow-mediated endothelial cell phenotype and controlling shear stress-induced signaling, both in vitro and in vivo.[8] Hyperbaric oxygen preconditioning has been shown to reduce the volume and number of blood vessel micronuclei (de-nucleation), which is believed to be one of the mechanisms underlying the prophylactic effect of HBO_2 preconditioning.[9] However, the effect of HBO_2 preconditioning on changes in glycocalyx structure, particularly Syndecan-1, has not been studied.

Specifically, the impact of HBO_2 preconditioning on the glycocalyx component Syndecan-1 remains unknown despite numerous studies on endothelial injury caused by bubbles during diving. This study aims to observe how HBO_2 preconditioning impacts Syndecan-1, MDA, and IL-1α.

METHODS

This research employed a true experimental post-test design. The study involved 40 male 12- to 14-week-old Sprague Dawley rats aged 12–14 weeks, and they were split into four distinct groups: Group 1 (control) and Group 2 (treatment) were terminated 12 hours after the dive, while Group 3 (control) and Group 4 (treatment) were terminated 24 hours after the decompression dive. All animals were obtained from Animal Vet Laboratory Services.

Ethical approval for this study was granted by the Research Ethics Committee of Universitas Pembangunan Nasional Veteran, Jakarta (UPNVJ), Indonesia, with approval number 25/I/2023/KEPK. The guidelines for the care and use of animals for scientific and experimental purposes were followed at every step of the experiment at the experimental animal laboratory of the Medical Education and Research Centre (MERCe) of UPNVJ. The experimental animals were given unlimited access to rodent food in the form of pellets and water. The room lighting was controlled on a periodic cycle of 12 hours of light followed by 12 hours of darkness. The relative humidity varied between 50% and 56%, while the ambient temperature was regulated within the 23-25 °C range.

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HBO_2 treatment and dry diving were performed in the animal hyperbaric chamber at the Hyperbaric-Subaquatic Laboratory of UPNVJ. Tissue preparation and immunohistochemistry analysis were performed at the iRATco Laboratory, Bogor. A serological examination was conducted at the Pusat Studi Satwa Primata of Institute Pertanian Bogor.

Two groups were given HBO_2 18 hours prior to diving and were labeled as the treatment groups. HBO_2 was administered at a pressure of 2.4 ATA for 60 minutes using oxygen at a concentration of 94-98%. The descent and ascent speeds were 0.1 atm/minute, in accordance with clinical standards, and the time required for ascent or descent was 15 minutes each. No barotrauma or seizures were observed during the dive or up to 1 hour after the dive.

Eighteen hours after HBO_2 treatment, all four groups underwent diving (6 ATA using compressed air [O2 20%; N2 78%]) with a bottom time of 100 minutes. Diving was performed at a gradual speed to prevent barotrauma, starting from 0.5 atm/minute to 1.5 atm/minute. Decompression was conducted quickly (2 atm/minute), allowing the animals to reach the surface within 3-4 minutes. After surfacing, observations were made for decompression sickness. The experimental animals were terminated 12 and 24 hours after diving. If a rat died before the designated time, tissue was immediately collected.

Serum processing

Rats were anesthetized with 0.3 ml of ketamine intramuscularly until they became unconscious. Blood was drawn intracardially using a 3 cc syringe and placed in a plain vacutainer. The blood was left for 30 minutes at room temperature before being subjected to a 15-minute centrifugation at 3,000 rpm to isolate serum, followed by storing at -20 °C.

Serum testing was conducted at the Animal Research Laboratory of the Center for Primate Animal Studies (PSSP) of IPB University. Syndecan-1 and IL-1α were examined using immunoenzymatic ELISA following the instructions provided by the manufacturer. Colourimetric analysis of MDA was conducted using the TBA method.

All kits used were from Elabscience, Texas, USA: Rat Syndecan-1 (E-EL-R0996), IL-1α (E-EL-R0011), and MDA (E-BC-K023-M). The concentration was approximated by comparing the optical density with a standard curve, and the measurement of absorbance was conducted at a wavelength of 450 nm.

Data analysis

An assessment of normality was conducted on the acquired data using the Shapiro-Wilk test. Data following a normal distribution are reported as the mean ± SD, whereas data that do not follow a normal distribution are reported as the median (min-max). An independent t-test was employed to analyze the effect of HBO_2 preconditioning on all normally distributed variables, while the Kolmogorov-Smirnov test was employed for variables that were not normally distributed. The significance level for all statistical analyses was set at $p < 0.05$.

RESULTS

Decompression sickness (DCS)

The incidence of decompression sickness 12 hours after diving was the same in both the control (n = 2) and treatment (n = 2) groups. However, 24 hours after diving, the control group's incidence of decompression sickness (n = 7) was more than double that in the intervention group (n = 2). Overall, no substantial difference was identified in the incidence of decompression sickness between the control and treatment groups ($p > 0.05$).

MDA

Parameters	*Group*	*Control*	*Treatment*
MDA µmol/L	12h post dive	142.8 (95.04 - 2159.00)	149.90 (96.17 - 492.20)
	24h post dive	157.80 (142.50 - 262.90)	233.40 (170.70 - 2181.00)

Level of significance: $p < 0.05$

No substantial difference in MDA levels was observed between the control and treatment groups, either 12 hours or 24 hours after diving. While the observation did not reach statistical significance, the treatment group exhibited greater MDA levels 24 hours post-diving compared to the control group.

IL-1α

Parameters	*Group*	*Control*	*Treatment*
IL-1 (pg/mL)	12h post dive	36.73 (25.71 - 75.86)	35.15 (23.36 - 40.69)
	24h post dive	32.79 (27.28 - 38.31)**	53.40 (46.23 - 96.82)*

Level of significance: $p < 0.05$

** $p < 0.05$ between the 12 h and 24 h post-dive groups*

*** $p < 0.05$ between the control and treatment groups*

Our research results demonstrated that IL-1α levels in both the treatment and control groups 12 hours after diving were not meaningfully different ($p = 0.373$). However, following the diving activity, there was a notable disparity in the IL-1α levels of the treatment and control groups ($p = 0.030$) 24 hours later. Specifically, the treatment group exhibited greater IL-1α levels in comparison to the control group. Significant changes in IL-1α levels were not observed in the control group 24 hours after decompression diving ($p = 0.766$), whereas the treatment group showed a substantial increase in IL-1α levels ($p = 0.001$).

Syndecan-1

Parameters	*Group*	*Control*	*Treatment*
Syndecan-1 (ng/mL)	12h post dive	15.92 ± 5.30	16.88 ± 11.39
	24h post dive	11.32 ± 6.26	15.45 ± 3.98

Level of significance: $p < 0.05$

Our study demonstrated no substantial change in the levels of circulating Syndecan-1 between the treatment and control groups at 12 hours ($p = 0.823$) and 24 hours ($p = 0.234$) following the dive. Syndecan-1 levels decreased in both the control and treatment groups 24 hours after the dive, but this decrease was not substantial ($p = 0.238$ and 0.758 for the control and treatment groups, respectively).

DISCUSSION

This research demonstrates that HBO_2 preconditioning may prevent decompression sickness. However, the effects of HBO_2 preconditioning became apparent more than 12 hours after diving, as evidenced by the absence of significant differences in all parameters studied at that time. After 24 hours, IL-1α and MDA levels rose while Syndecan-1 levels declined. Moreover, the levels of all three parameters were lower in the control group compared to the treatment group. Of these parameters, only the difference in IL-1α levels reached statistical significance.

Adaptation is a process by which body functions adjust to achieve a new balance. Hans Selye divided this process into three stages—alarm, resistance, and exhaustion—known as General Adaptation Syndrome (GAS). The first stage, alarm, is further divided into two phases: the shock phase, marked by the onset of the stressor, and the counter-shock phase. The resistance stage represents the body's adaptive response in an effort to achieve cellular homeostasis. Finally, cells that fail to cope with the stressors enter the decompensation phase, followed by the exhaustion phase.[10]

The results of this study suggest that administering two stressors (HBO_2 and decompression diving) within an 18-hour interval causes cells to enter a more severe inflammatory phase compared to the administration of a single stressor (decompression diving alone). According to the GAS theory, this indicates two different adaptation responses. The diving stressor triggered a similar response in both the control and HBO_2 preconditioning groups 12 hours after the dive. However, there were differences in the parameter changes 24 hours after diving, suggesting a biphasic effect due to repeated stressors. Biphasic effects reflect a cell's response to a certain dose or intensity of an external stimulus. Hormesis, a concept describing a cell's adaptive dose, is illustrated by a U- or J-shaped dose-response curve. [11] The adaptive dose is a low dose that is sufficient to activate the cell's defense system in order to counteract the arising stress.[12] According to the hormesis theory, this research suggests that providing a stimulus in the form of HBO_2 preconditioning 18 hours before diving prolongs the inflammatory response after diving, keeping the cell in the alarm phase even 24 hours after the dive.

Syndecan-1 possesses both pro-inflammatory and anti-inflammatory properties, contingent upon its state—whether bound to the cell surface or released in soluble form—and it is crucial in controlling mechanical forces through adhesion molecules during different stages of the inflammatory process.[8] Early in the inflammatory process, Syndecan-1 appears to promote leukocyte movement to endothelial cells. However, later in the process, it prevents leukocyte adhesion and migration. The lack of Syndecan-1 in knockout mice is supported by the observed increase in the expression of pro-inflammatory cytokines (CCL-3, CCL-5, IL-6, and TNF-a) and adhesion molecules (ICAM-1 and VCAM-1) in models in models of oxazolone-induced hypersensitivity and dextran sodium sulfate-induced colitis.[13]

This study indicates that HBO_2 preconditioning maintains elevated levels of circulating Syndecan-1. The release of Syndecan-1 is critical in suppressing inflammation by eliminating CXC chemokines and reducing the production of pro-inflammatory cytokines.[14] Administering Syndecan-1-containing exosomes may potentially reduce the expression of other pro-inflammatory markers (IL-1b, IL-6, TNF-α). Increased expression of Syndecan-1 is required for leukocyte migration and the initiation of chemokine recruitment. However, this process must be tightly regulated. Syndecan-1, when released from the cell surface, reduces surface signaling and establishes a stable chemokine gradient by directing leukocyte migration specifically to inflammation sites, which is crucial for resolving inflammation at later stages.[13]

The primary stages of inflammation include the identification of inducers, transmission of signals, release of pro-inflammatory molecules, activation of inflammatory effectors, and resolution of inflammation.[15] Inducers are recognized by various receptors, including leucine-rich receptors, nucleotide-binding domain receptors, and toll-like receptors, which then activate and translocate NF-kB.[16] As a consequence, many pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), Interleukin-1β, IL-6, IL-8, and IL-12 are produced.[17] Cytokines, among the many molecules implicated in inflammation, provide dual functions as anti-inflammatory and pro-inflammatory mediators.[13]

Interleukin-1α is a cytokine that acts as a mediator in the immune system and inflammation. IL-1α is produced by various cells, particularly macrophages, monocytes, and dendritic cells, in response to infection or injury. IL-1α levels typically increase in response to infection, inflammation, or tissue damage.[18] Interestingly, the IL-1α levels of the control group continued to decrease 24 hours after diving, while the opposite occurred in the preconditioning group, where IL-1α levels increased. The increase in IL-1α levels in the preconditioning group was likely caused by exceeding the hormesis dose due to the two consecutive stressors, HBO_2 and decompression diving, causing the cells to re-enter the shock phase.

This study also revealed that decompression diving did not cause significant changes in MDA levels. Additionally, although HBO_2 preconditioning resulted in higher circulating MDA levels compared to the control group 24 hours after diving, no statistically significant difference was observed between the groups. This finding aligns with other studies that demonstrate that hyperoxic conditions induced by HBO_2 lead to an increase in ROS levels without causing severe oxidative stress.[19]

CONCLUSION

Clinically, HBO_2 preconditioning can prevent decompression sickness. However, at the biomolecular level, HBO_2 preconditioning acts as a stressor that may prolong oxidative stress and inflammation. These findings suggest that further research is required to establish the most effective hormetic dose of HBO_2 preconditioning to prevent inflammation during diving.

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ETHICAL CONSIDERATIONS

Ethical approval for this study was granted by the Research Ethics Committee of Universitas Pembangunan Nasional Veteran, Jakarta (UPNVJ), Indonesia (number 25/I/2023/KEPK).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

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REFERENCES

1. Zanchi J, Ljubkovic M, Denoble PJ, Dujic Z, Ranapurwala SI, Pollock NW. Influence of repeated daily diving on decompression stress. Diving Hyperb Med. 2014;44(3):166. DOI:10.1055/s-0033-1334968
2. Su C-L, Wu C-P, Chen S-Y, Kang B-H, Huang K-L. Acclimatization to neurological decompression sickness in rabbits. Am J Physiol - Regul Integr Comp Physiol. 2004;287(5). https://doi.org/10.1152/ajpregu.00260.2004
3. Katsenelson K, Arieli R, Arieli Y, Abramovich A, Feinsod M, Tal D. Hyperbaric oxygen pretreatment according to the gas micronuclei denucleation hypothesis reduces neurologic deficit in decompression sickness in rats. J Appl Physiol. 2009;107(2):558–63. doi: 10.1152/japplphysiol.91557.2008
4. Fan DF, Liu K, Xu WG, Zhang RJ, Liu Y, Kang ZM, et al. Hyperbaric oxygen preconditioning reduces the incidence of decompression sickness in rats via nitric oxide. Undersea Hyperb Med. 2010;37(3):173–80. PMID: 20568547
5. Qing L, Yi H jie, Wang Y wei, Zhou Q, Ariyadewa DK, Xu W gang. Benefits of hyperbaric oxygen pretreatment for decompression sickness in Bama pigs. J Exp Biol. 2018;221(5). https://doi.org/10.1242/jeb.171066
6. Nossum V. The effect of vascular bubbles on endothelial function. NTNU; 2003. https://ntnuopen.ntnu.no/ntnu-xmlui/handle/11250/264640
7. Klinger AL, Pichette B, Sobolewski P, Eckmann DM. Mechanotransductional basis of endothelial cell response to intravascular bubbles. Integr Biol. 2011;3(10):1033–42. DOI: 10.1039/c1ib00017a

8. Voyvodic P, Min D, Liu R, Williams E, Chitalia V, Dunn A., et al. Loss of syndecan-1 induces a pro-inflammatory phenotype in endothelial cells with a dysregulated response to atheroprotective flow. J Biol Chem. 2014;289(14):9547–59. DOI: 10.1074/jbc.M113.541573
9. Yount DE. On the evolution, generation, and regeneration of gas cavitation nuclei. J Acoust Soc Am. 1982;71(6):1473–81. https://doi.org/10.1121/1.387845
10. Cunanan AJ, DeWeese BH, Wagle JP, Carroll KM, Sausaman R, Hornsby WG, et al. The General Adaptation Syndrome: A Foundation for the Concept of Periodization. Sport Med [Internet]. 2018;48(4):787–97. https://doi.org/10.1007/s40279-017-0855-3
11. Calabrese EJ. Hormesis : principles and applications. 2015;(November 2014):69–82. DOI: 10.1016/j.homp.2015.02.007
12. Ji LL, Radak Z, Goto S. Hormesis and exercise: How the cell copes with oxidative stress. Am J Pharmacol Toxicol. 2008;3(1):41–55. DOI:10.3844/ajptsp.2008.44.58
13. Gopal S. Syndecans in Inflammation at a Glance. Front Immunol. 2020;11(February):1–8. doi: 10.3389/fimmu.2020.00227
14. Hayashida K, Parks WC, Pyong WP. Syndecan-1 shedding facilitates the resolution of neutrophilic inflammation by removing sequestered CXC chemokines. Blood [Internet]. 2009;114(14):3033–43. http://dx.doi.org/10.1182/blood-2009-02-204966
15. Hannoodee S, Nasuruddin D. Acute inflammatory response [Internet]. Vol. 206, Nature. StatPearls; 2024. p. 20. https://www.ncbi.nlm.nih.gov/books/NBK556083/
16. Kawai T, Akira S. Signaling to NF-κB by Toll-like receptors. Trends Mol Med. 2007;13(11):460–9. DOI: 10.1016/j.molmed.2007.09.002
17. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2. DOI: 10.1038/sigtrans.2017.23
18. Mantovani A, Dinarello CA, Molgora M, Garlanda C. Interleukin-1 and Related Cytokines in the Regulation of Inflammation and Immunity. Immunity [Internet]. 2019;50(4):778–95. https://doi.org/10.1016/j.immuni.2019.03.012
19. Jayatama A, Roestam A, Wardhani S, Suryokusumo G, Soemarko D, Suhadi. The Effects of Hyperbaric Oxygen Therapy on Carboxyhaemoglobin and Malondialdehyde Levels of Road Officers Exposed to Chronic Carbon Monoxide (Experimental Study of the Traffic Warden in Jakarta). University of Indonesia; 2021.

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