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Effects of Hyperbaric Oxygen on the Total Blood Sulfhydryl Groups in Patients With Peripheral Vascular Disease

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Iyer EM, Bandopadhyay P, Baboo NS. Effects of hyperbaric oxygen on the total blood sulfhydryl groups in patients with peripheral vascular disease. J Hyperbaric Med 1992; 7(3):179–184.—Resting levels of total blood sulfhydryl (bl-SH) were assessed in 7 control subjects and 7 patients with peripheral vascular disease. Resting levels of total bl-SH groups were considerably lower in patients than in controls. The patients showed a significant rise in the resting levels of total bl-SH groups after 14 continuous sessions of hyperbaric oxygen (HBO) therapy, each consisting of breathing 100% O_2 in a recompression chamber at 2.5 atm abs for 90 min. The post-HBO utilization and regeneration of total bl-SH groups observed in patients before HBO therapy were significantly lower than in controls, but after HBO therapy post-HBO changes in the total bl-SH groups observed in patients were similar to those observed in control subjects. The rise in the resting levels of total bl-SH groups and the faster utilization and regeneration of these groups in patients after HBO therapy in the post-HBO session were associated with significant pain relief and therefore could be used to predict the recovery signs of patients undergoing HBO therapy.

hyperbaric oxygen therapy, total blood sulfhydryl groups

Introduction

Sulfhydryl (SH) groups form a vital component of many enzymes, coenzymes, and non-protein cellular substances. Among the important cellular constituents that contain non-protein SH groups are glutathione, cysteine, pantothenic acid, coenzyme A, and reduced lipoic acid. The role of glutathione in regulating the oxidation-reduction processes in the cell and the function of cysteine as a carrier of groups in a number of metabolic processes are two of the important, well-documented functions of non-protein SH groups. The SH groups of many enzymes, notably oxydoreductase, are essential for their enzymatic activity (1–3). Stress is likely to enhance the generation of hydroperoxide radical and is known to alter the blood/tissue levels of total SH groups as the reduced form of these groups are converted into their oxidized form (4–7). This study investigates whether the resting levels of whole blood SH (bl-SH) groups are different in patients than in controls and whether the resting levels of these groups are altered in patients by repeated exposure to hyperbaric oxygen (HBO). This study also investigates the utilization and

regeneration of whole bl-SH groups in patients before and after HBO therapy in the post-HBO session, and differentiates these changes from those seen in control subjects.

Materials and Methods

This study was done on 7 healthy subjects and 7 patients (5 cases of thromboangiitis obliterans, 1 case of diabetes mellitus, and 1 case of frost bite). The patients underwent HBO therapy because of painful vascular disease. Control subjects were exposed to 1 session of HBO therapy whereas the patients were exposed to 14 continuous session. Each session consisted of a 90-min exposure to HBO at 2.5 atm abs in a recompression chamber. Capillary blood samples were obtained before exposure to HBO and at 0, 30, and 60 min post-HBO sessions from the control subjects and from the patients before and after HBO therapy. Each capillary blood sample was analyzed for total bl-SH groups by the methods of Ellman (8) using 5,5′ dithiobis-2 (nitrobenzoic acid) as the coloring agent. Statistical comparisons were made by Student's t test.

Results

The resting levels of total bl-SH groups observed in control subjects (5.57 ± 0.65 mmol/liter) were significantly higher ($P < 0.001$) than the resting levels of total bl-SH groups observed in patients before HBO treatment (2.30 ± 0.68 mmol/liter). A significant rise in the resting level of total bl-SH groups to 4.97 ± 1.57 mmol/liter ($P < 0.001$) was observed in patients after HBO therapy. The differences in the resting levels of total bl-SH groups observed between the controls and patients after HBO therapy were small in contrast to the differences observed between controls and patients before beginning HBO therapy (Table 1).

The post-HBO utilization and regeneration of total bl-SH groups observed in patients before HBO therapy were significantly lower than in control, but after HBO therapy, post-HBO changes in the total bl-SH observed in patients were similar to those in control subjects. The differences at the 0, 30, and 60-min post-HBO values of total bl-SH observed in patients after HBO therapy compared to control subjects were lower than the differences observed between the patients before HBO therapy and the control subjects.

Discussion

Resting levels of whole bl-SH groups in normal healthy subjects have been reported to vary between 5.19 and 6.55 mmol/liter (6). In the present study the resting levels of whole bl-SH groups were 5.57 ± 0.65 mmol/liter and were within these limits, but in patients with peripheral vascular disease (PVD)

Table 1: Post-HBO Changes in the Levels of Total Blood Sulfhydryl (mmol/liter) in Control Subjects and in Patients Before and After HBO Therapy

Group	Pre-exposure	Post-exposure					
		0 min	*P*	30 min	*P*	60 min	*P*
Control	5.57 ± 0.65	1.30 ± 0.74	<0.001	3.30 ± 1.16	<0.01	5.16 ± 0.70	NS
Before HBO therapy	2.30 ± 0.68	0.64 ± 0.65	<0.001	1.66 ± 0.82	NS	2.30 ± 0.70	NS
After HBO therapy	4.97 ± 1.57	1.74 ± 0.80	<0.001	3.08 ± 1.51	<0.01	4.18 ± 1.48	<0.01

the levels of whole bl-SH group (2.30 ± 0.68 mmol/liter) were significantly lower.

Normal red blood cells are protected from lipid peroxidation by an existing antioxidant mechanism consisting of free GSH (reduced glutathione) of cell, SH components of the cell membrane, and vitamin E. The membrane SH compounds including GSH are destroyed by peroxidation. Secondary effects of lipid peroxides involve damage to red blood cell proteins, enzymes, and metabolic pathways as demonstrated by the inhibition of SH-bearing enzyme glyceraldehyde-3-PO_4 dehydrogenase in humans and decreased activity of erythrocyte acetylcholinesterase in dogs (9–13). Vitamin E plays a crucial role in providing protection against peroxidation (11, 14). The lower resting levels of whole bl-SH groups in patients with PVD suggest that in these subjects generation of hydroperoxide radicals may proceed faster and that these subjects are likely to be deficient in vitamin E as compared to the control subjects.

The activity of various scavenger enzymes, i.e., superoxide dismutase (SOD), glutathione peroxidase, and GSH transferase, has been reported to be significantly lower in both ischemicized and nonischemicized zones of the patients having transitory failure of the myocardium (15). The activity of SOD has been reported to be lower in multiple sclerosis patients as compared to that of controls (16, 17). It is likely that in our patients, the resting activity of various antioxidant enzymes may be considerably lower in view of their generalized state of stress, which might have affected the regeneration of the reduced form of SH groups from the oxidized form.

The lower resting levels of total bl-SH groups observed in patients with PVD can also result from increased secretion of catecholamines, cortisol, ACTH, endorphins as well as reduced levels of ascorbic acid (18–21), which is likely to increase the metabolic, enzymatic, and oxidation-reduced reaction leading to higher generation of reactive O_2 intermediates. The high generation of reactive O_2 intermediates is likely to enhance the conversion of reduced form of SH groups into the oxidized form (4–6).

It has been reported that after HBO therapy, multiple sclerosis patients show a significant increase in the activity of SOD and catalase, whereas patients with transitory failure of myocardium show a selective increase in the activity of SOD after reperfusion in both the ischemicized and nonischemicized zones (14, 16). Plasma levels of ACTH and endorphins have shown a significant reduction in patients with PVD after HBO therapy (20). This is likely to reduce the generation of reactive O_2 intermediates. At the same time, generated reactive O_2 intermediates are quickly utilized in view of the reported increase activity of SOD and catalase after HBO therapy. The higher resting levels of total bl-SH groups observed in patients after HBO therapy might be explained on this basis.

The utilization and regeneration of total bl-SH groups in patients before HBO therapy in the post-HBO session were much lower, but after HBO therapy they showed a significant increase and were similar to the control

subjects. This indicates that the patients after HBO therapy have developed a greater capacity to utilize the generated hydroperoxide radicals and resynthesize the reduced form of SH groups from its oxidized form in the post-HBO period. This also suggests that in patients after HBO therapy, activity of scavenger enzymes responsible for the conversion of oxidized SH groups into the reduced form show a significant increase.

The increase in the resting levels of total bl-SH groups and higher capacity to utilize and regenerate the SH groups in the post-HBO period in the patients after HBO therapy was significant and associated with pain relief and therefore could be used as an index to predict the recovery signs of patients undergoing HBO therapy.

References

1. Barron ESG, Singer TP. Enzyme systems containing active sulfhydryl groups. The role of glutathione. Science 1943; 97:356–358.
2. Ginsburg A, Stadtman ER. Multienzyme system. Ann Rev Biochem 1970; 39:429–472.
3. Glazer AN. Specific chemical modification of proteins. Ann Rev Biochem 1970; 39:101–124.
4. Bartlett RG, Register UD. Effect of cold and restraint on blood and liver non protein sulfhydryl compounds. Proc Soc Exp Biol Med 1953; 83:708–709.
5. Beck LV, Linkenheimer WH. Effect of shock and cold on mouse liver sulfhydryl. Proc Soc Exp Biol Med 1952; 81:291–294.
6. Iyer EM, Dikshit MB. Suryanarayan S. Effect of exposure to heat, hypoxia, cold, acceleration and vibration stress on the total blood sulfhydryl groups in human subjects. Aviat Space Environ Med 1985; 56:1097–1101.
7. Khuteta KP, Mathur KN, Mali SL. Effect of exposure to heat on total blood sulfhydryl groups in human being. Indian J Med Res 1969; 54:1767–1768.
8. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82:70–77.
9. Carolla RL, Mengel CE, Husney RM. Effect of in vivo hyperoxia on erythrocyte fatty acid composition. Aerosp Med 1968; 39:1290–1293.
10. Hochstein P. Antioxidant mechanism and hemolysis associated with lipid peroxidation. In: Brown IW, Cox BG, eds. Proceedings of third international conference on hyperbaric medicine. Washington DC: National Academy of Sciences—National Research Council. 1966:61–64.
11. Jacob HS, Lux SE IV. Degradation of membrane phospholipids and thiols in peroxide hemolysis: studies in vitamin E deficiency. Blood 1968; 32:549–567.
12. Kann HE Jr, Mengel CE. Mechanisms of erythrocyte damage during in vivo hyperoxia. In: Brown IW, Cox BG, eds. Proceedings of third international conference on hyperbaric medicine. Washington DC: National Academy of Sciences—National Research Council 1966:65–72.
13. Mengel CE, Kann HE Jr. Effects of in vivo hyperoxia on erythrocytes. III in vivo peroxidation of erythrocyte lipid. J Clin Invest 1960; 45:1150–1158.
14. Johnson WP, Jefferson D, Mengel CE. In vivo hemolysis due to hyperoxia: role of H_2O_2 accumulation. Aerosp Med 1972; 43:943–945.
15. Konovalova GG. Changes in the activity of "antioxidant" enzymes during ischemia and subsequent reperfusion of the myocardium. Bull Eksp Biol Med 1984; 98:271–273.
16. Ansari KA, Wilson M, Slater GE, Haglin JJ, Kaplan E. Hyperbaric oxygenation and erythrocyte antioxidant enzymes in multiple sclerosis patients. Acta Neurol Scand 1986; 74:156–60.
17. Hunter MI, Lao MS, Burtles SS, Davidson DL. Erythrocyte antioxidant enzymes in multiple sclerosis and effect of hyperbaric oxygen. Neurochem Res 1984; 9:507–516.
18. Golikov PP, Rogatsky GG, Nilolaeva N Yu. Glucocorticoid receptors in the lungs in the hyperbaric oxygenation. Patol Fiziol Eksp Ter 1986; 2:29–32.

19. Nakada T, Koike H, Katayama T, Watanabe H, Yamori Y. Increased adrenal epinephrine and norepinephrine in spontaneously hypertensive rats treated with hyperbaric oxygen. Hinyokika Kiyo 1984; 30:1357–1366.
20. Obrenovitch TP, Gillard JL. Decreased brain levels of ascorbic acid in rats exposed to high pressures. J Appl Physiol 1985; 58:839–843.
21. Tufano R, Del Gaudio A, De Cillis P. Hyperbaric oxygen effects on pain relief in patients with vascular disease. J Hyperbaric Med 1988; 3:29–33.