

Therapeutic study of hyperbaric oxygen on Heme Oxygenase-1 (HO-1) in patients with acute carbon monoxide poisoning and myocardial injury

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ARTICLE INFO

Original paper

Article history:

Received: January 02, 2022

Accepted: April 06, 2022

Published: June 30, 2022

Keywords:

Hyperbaric Oxygen Therapy, CO Poisoning, Heme Oxygenase-1, Myocardial Injury.

ABSTRACT

Carbon monoxide (CO) poisoning causes myocardial injury, which is attenuated by hyperbaric oxygen therapy (HBOT). During CO poisoning, the body increases anti-inflammatory proteins, including heme oxygenase-1 (HO-1), in response to oxidative stress. Considering the myocardial injury resulting from CO poisoning and the lack of sufficient information about the effect of HBOT on HO-1, the present study evaluated the effect of hyperbaric oxygen therapy on heme oxygenase-1 (HO-1) in patients with acute carbon monoxide poisoning and myocardial injury. In this regard, in a before-after Quasi-Experimental study, 20 patients with carbon monoxide poisoning and myocardial injury were studied. All patients underwent 40 daily hyperbaric oxygen therapy sessions for 90 minutes at a pressure of 2.4 ATA. Also, 20 healthy individuals, as a control group, were participated. To evaluate and compare the mRNA level of the HO-1 gene, the Real-time PCR technique was used. Paired t-test was used to compare the two indices of 6min walking distance and pulmonary arterial pressure (PAP) before and after the intervention. The results showed that the difference during 12 weeks was 8.65 ± 4.91 for PAP, and this reduction in pressure was statistically significant ($P = 0.0092$). The distance traveled increased by 28 ± 10.88 m in 6 minutes at the end of the study ($P = 0.0084$). Regarding the expression level of HO-1, the results showed that the expression level in the intervention group before the test had a significant increase compared to the control group ($p = 0.0004$). However, after hyperbaric oxygen therapy, the expression of this gene decreased significantly, and there was no statistically significant difference with the control group ($p = 0.062$). Overall, the results showed that HBOT significantly decreased HO-1 gene expression in CO poisoning and myocardial injury patients. It indicates the importance of HBOT in the treatment and compensation of cardiac tissue damage caused by CO poisoning.

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Introduction

Carbon monoxide (CO) is an air pollutant and the by-product of the incomplete combustion of fossil fuels and hydrocarbons. CO poisoning is one of the most important causes of death due to poisoning (1). In some developed countries, such as the United States, CO is the leading cause of death from poisoning (2). It is rapidly absorbed from the lungs and then combined with hemoglobin and carboxyhemoglobin, disrupts cellular respiration, produces Reactive Oxygen Species, destroys cells, and apoptosis (3).

The significant effects of CO poisoning have been due to reduced oxygen delivery to the tissues, especially in the brain and heart. CO poisoning is usually accidental and due to disruption of systems related to fossil or hydrocarbon fuels (4). CO poisoning, in some cases, causes severe heart poisoning that can lead to the spread of cardiomyopathy, heart failure, and even death (2). The results of previous studies all indicate the destructive effects of CO poisoning on myocardial tissue (5-7). Meanwhile, some studies have shown that this poisoning leads to histological changes in heart tissue, such as vacuolation, heart cell fusion, and myocardial cell death (8, 9). There are several mechanisms

involved in CO poisoning. CO-induced cardiomyopathy has been reported to be strongly associated with increased cardiac oxidative stress (10). In addition, apoptosis and impaired intracellular calcium regulation are among the possible mechanisms related to CO poisoning (11).

When the heart is exposed to CO oxidative stress, it gradually develops hypertrophy (6). Cardiac hypertrophy, characterized by the growth of cardiomyocytes and remodeling of the intercellular matrix (such as fibrosis) in myocardial tissue, is a complex process involving growth factors and cytokines (9).

The body increases anti-inflammatory proteins, including heme oxygenase-1 (HO-1), in response to oxidative stress (12). HO-1 is one of the most sensitive antioxidant enzymes against cellular stress and has a variety of functions (13). Still, the most critical process of HO-1 is to defend against oxidative stress, which due to its significant expression and activity in the heart has received a lot of attention. Therefore, studying this enzyme in people with CO poisoning can provide valuable information (14).

One way to treat patients with acute carbon monoxide poisoning and myocardial injury is to use hyperbaric oxygen therapy (HBOT) (2). The mechanism of action in the treatment of HBOT is to create an environment with

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a pressure of more than one atmosphere and the ability to provide pure respiratory oxygen (15). This process allows a lot of dissolved oxygen to reach the bloodstream so that the cells' needs can be met by dissolved oxygen in the blood (16). In hyperbaric oxygen therapy, increasing the oxygen content of the blood (O_2 content) increases the transfer of oxygen from the blood to the tissues and facilitates the release of oxygen (17).

Considering the damages resulting from poisoning and death and the lack of sufficient information about the pattern of poisoning, the present study evaluated the effect of hyperbaric oxygen therapy on heme oxygenase-1 (HO-1) in patients with acute carbon monoxide poisoning and myocardial injury.

Materials and Methods

Studied patients

In a before-after Quasi-Experimental study, 20 patients with carbon monoxide poisoning and myocardial injury were studied. Inclusion criteria included:

- Carbon monoxide poisoning
- Minimum pulmonary arterial pressure (PAP) of 30 mm Hg based on the Bernoulli equation in Doppler echocardiography
- No history of heart failure
- No systemic disease

The Bernoulli equation was calculated based on the flow rate of tricuspid valve insufficiency and according to the following equation:

Pulmonary Arterial Pressure (PAP) = $4 \times V^2 + \text{Jagular Venus Pressure}$

People with a history of other lung, heart, and systemic diseases were excluded from the study. Consent was obtained from all patients studied. At the beginning of the study and after performing Doppler echocardiography and collecting demographic information to check the Exercise Capacity, a 6 min walking test (the distance that the patient can walk in 6 minutes) was performed on all samples.

Experimental evaluations

All patients underwent 40 daily hyperbaric oxygen therapy sessions for 90 minutes at a pressure of 2.4 ATA. Patients were kept unaware of the name and side effects of the drug until the end of the study. The patients' other medications continued during the study period, but the patients were not allowed to take the new medication. Patients were monitored weekly for drug side effects such as blurred vision, binoculars, hot flashes, and urine discoloration during the interview by a physician. Specialists visited them if they reported any significant side effects. Treatment was discontinued if necessary. After 12 weeks, patients underwent Doppler echocardiography, PAP calculation, and Six Min Walking test.

Heme oxygenase-1 (HO-1) gene expression

To evaluate and compare the mRNA level of the HO-1 gene, 5 ml of peripheral blood was obtained from all patients in the study, before and after the intervention. Also, 20 healthy individuals, as a control group, participated and 5ml of blood were prepared from them. RNA extraction and cDNA synthesis were evaluated by RNA extraction kit protocol (Qiagen, South Korea) and Vivantis cDNA synthesis kit (Malaysia). Specific primer pairs were designed to amplify the sequences of the HO-1 gene and β -actin gene (internal control). The primers were designed using Gene Runner 5 and Primer Express 1.0.3 software. Table 1 shows the sequence of primers for the real-time PCR technique.

The final volume for each reaction was 20 μ l, including 100ng of Power SYBR® Green PCR Master, 1 μ l of cDNA, 10 μ l of Master Mix (Applied Biosystems, USA), 10mmol/ μ l of primers, and 6 μ l of nuclease-free water. Temperature protocol was performed as initial denaturation at 94°C for 5 minutes. Subsequently, 35 cycles were performed as denaturation at 94°C for 10 seconds and annealing at 58°C for 40 seconds. Reproduction analysis and melting curve were performed using Applied Biosystems 7500. Then gene expression diagram was drawn using Prism 5 GraphPad software. Genetic data analysis was performed based on threshold cycle comparison (Ct). The Δ CT was calculated by the Ct difference obtained from the tested samples. Then, it was calculated using the formula $2^{-\Delta\Delta CT}$.

Statistical analysis

Data were analyzed using SPSS software version 15. Paired t-test was used to compare the two indices of distance and pulmonary artery pressure before and after the intervention. P-values less than 0.05 were considered significant, and all data were reported as mean \pm SD.

Results and discussion

The results of initial evaluations showed that the age of patients was between 16 and 48 years, with an average of 32.87 ± 3.91 years. Mean systolic blood pressure was 119 ± 20 mm Hg, and diastolic blood pressure was 69 ± 8 mmHg. The mean heart rate was 71 ± 7 , and the mean respiratory rate was 16 ± 3 . Of the 20 patients studied, 19 completed the course of treatment, and only one patient stopped treatment due to gastrointestinal side effects after six weeks. At the beginning of the study, the mean pulmonary arterial pressure (PAP) was 37.75 ± 8.21 , and the mean distance traveled over 6 minutes in patients was 263.35 ± 82.28 m. After 12 weeks, the average PAP was 29.10 ± 6.55 , and the average distance was 291.35 ± 82.39 . The difference during 12 weeks was 8.65 ± 4.91 for PAP, and this reduction in pressure was statistically signi-

Table 1. The Primer sequences of HO-1 and β -actin genes for the Real-time PCR technique.

Gene		Primer Sequence (5'-3')	Product size
HO-1	Forward	CCCAAACTGGCCTGTAAAA	104p
	Reverse	CGTGGTCAGTCAACATGGAT	
β -actin	Forward	AGCTGCGTTTTACACCCCTTT	165bp
	Reverse	AAGCCATGCCAATGTTGTCT	

ficant ($P = 0.0092$). The distance traveled increased by 28 ± 10.88 m in 6 minutes at the end of the study ($P = 0.0084$). Headache was reported in 2 patients (10%), and indigestion was reported in 3 patients (15%). No cases of vision loss and frequent urination were reported.

Regarding the expression level of HO-1, the results showed that the expression level in the intervention group before the test had a significant increase compared to the control group ($p = 0.0004$). However, after hyperbaric oxygen therapy, the expression of this gene decreased significantly, and there was no statistically significant difference with the control group ($p = 0.062$) (Figure 1).

Carbon monoxide (CO) poisoning is caused by breathing dangerous carbon monoxide gas. This poisoning, which is caused by various gas appliances such as heaters, water heaters, and even coal, is often seen in winter (18). If the chimney or ventilation of a place is blocked, the released carbon monoxide gas will not be able to escape from the mentioned devices; it will be released into the building environment and will cause poisoning and eventually death (19). Death from gas poisoning is called "silent death." Carbon monoxide is a colorless, odorless gas produced by the incomplete combustion of various fuels and can cause harm to people. The importance of the issue is that because it is colorless and odorless, it can quickly cause poisoning symptoms in people (20).

Carbon monoxide poisoning can include a wide range of symptoms that can be seen in various diseases (18). Unfortunately, many of these symptoms are similar to the common cold, and most people think they have the flu. Early signs of this type of poisoning include headache, weakness, dizziness and restlessness, nausea and vomiting, drowsiness, lethargy, and fatigue. CO poisoning, in some cases, causes severe heart poisoning that can lead to the spread of cardiomyopathy, heart failure, and even death (21). The results of previous studies all indicate the destructive effects of CO poisoning on myocardial tissue. Meanwhile, some studies have shown that this poisoning leads to histological changes in heart tissue, such as heart cell fusion and myocardial cell death (20).

CO-induced heart damage can have several mechanisms. Suggested mechanisms for CO-induced cardiac poisoning include the production of free radicals, mitochondrial metabolism degradation, calcium metabolism disorders, and direct DNA damage (22, 23). It has been reported that the most accepted mechanism for CO-induced cardiac poisoning is the production of free radicals that can damage heart cells through lipid peroxidation (24). Free radicals and CO-induced fat peroxidation, as well as the ability of CO to bind to mitochondrial fats, are the leading causes of CO poisoning in heart tissue. On the other hand, CO induces apoptosis through a mechanism dependent on reactive oxygen species (ROS). Apoptosis can be one of the most common mechanisms of cardiac cell death (25).

Hyperbaric oxygen therapy (HBOT) is one of the ways to treat patients with acute carbon monoxide poisoning and myocardial injury (15). In addition to treating respiratory failure, HBOT is used to repair damaged tissue, especially myocardial injury. In this case, oxygen reaches the damaged tissue through the lungs and blood circulation (16). By correcting the amount of tissue oxygen, the conditions are created to form new capillary vessels, which are a natural reaction of ischemic tissue, and neovascularization occurs, which leads to the elimination of (possibly perma-

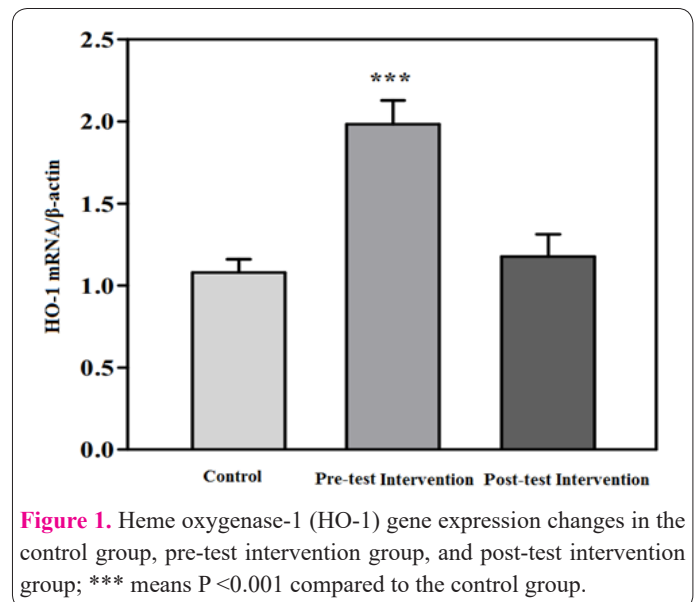


Figure 1. Heme oxygenase-1 (HO-1) gene expression changes in the control group, pre-test intervention group, and post-test intervention group; *** means $P < 0.001$ compared to the control group.

nent) tissue ischemia (26-28). Tissue hypoxia is followed by the destruction of anaerobic infections, which, when oxygen reaches the immune cells, modulates the activity of the immune system and reduces vasoconstriction due to hypoxia, leading to a decrease in edema in the ischemic region (15). The current study results showed that the expression level in the intervention group before the test had a significant increase compared to the control group ($p = 0.0004$). However, after hyperbaric oxygen therapy, the expression of this gene decreased significantly, and there was no statistically significant difference with the control group ($p = 0.062$).

Acknowledgments

The authors are thankful to the higher authorities for the facilities provided.

Authors' contribution

This study was done by the authors named in this article, and the authors accept all liabilities resulting from claims which relate to this article and its contents.

Interest conflict

The authors declare that they have no conflict of interest.

Funding

No funding was received for this study.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Statements and declarations

The author declares that no conflict of interest is associated with this study.

References

1. Ng PCY, Long B, Koyfman A. Clinical chameleons: an emergency medicine focused review of carbon monoxide poisoning. *Intern Emerg Med* 2018; 13(2): 223-229.
2. Lin C-H, Su W-H, Chen Y-C et al. Treatment with normobaric or hyperbaric oxygen and its effect on neuropsychometric dysfunction after carbon monoxide poisoning: A systematic review and

- meta-analysis of randomized controlled trials. *Medicine* 2018; 97(39):e12456.
3. Moon J, Chun B, Cho Y, Lee S. Diagnostic value of parameters related to white blood cell counts for troponin I elevation in CO poisoning. *Cardiovas Toxicol* 2019; 19(4): 334-343.
 4. Tekşam O, Sabuncuoğlu S, Girgin G, Özgüneş H. Evaluation of oxidative stress and antioxidant parameters in children with carbon monoxide poisoning. *Hum Exp Toxicol* 2019; 38(11): 1235-1243.
 5. Yesiloglu O, Gulen M, Satar S, Avci A, Acehan S, Akoglu H. Treatment of carbon monoxide poisoning: high-flow nasal cannula versus non-rebreather face mask. *Clin Toxicol* 2021; 59(5): 386-391.
 6. Owiredu S, Ranganathan A, Eckmann DM et al. Ex vivo use of cell-permeable succinate prodrug attenuates mitochondrial dysfunction in blood cells obtained from carbon monoxide-poisoned individuals. *Am J Physiol Cell Physiol* 2020; 319(1): C129-C135.
 7. Oliverio S, Varlet V. Carbon monoxide analysis method in human blood by airtight gas syringe–gas chromatography–mass spectrometry (AGS-GC-MS): relevance for postmortem poisoning diagnosis. *J Chromatogr B* 2018; 1090: 81-89.
 8. Lin M-S, Lin C-C, Yang C-C et al. Myocardial injury was associated with neurological sequelae of acute carbon monoxide poisoning in Taiwan. *J Chin Med Assoc* 2018; 81(8): 682-690.
 9. Cho DH, Ko SM, Son JW, Park EJ, Cha YS. Myocardial injury and fibrosis from acute carbon monoxide poisoning: a prospective observational study. *Cardiovasc Imaging* 2021; 14(9): 1758-1770.
 10. Kim JS, Ko BS, Sohn CH, Kim YJ, Kim WY. High-sensitivity troponin i and creatinine kinase-myocardial band in screening for myocardial injury in patients with carbon monoxide poisoning. *Diagnostics* 2020; 10(4): 242.
 11. Han Y, Wang Y, Zhao G, Yang J, Wang L, Wang W. Relationship between neutrophil-to-lymphocyte ratio and myocardial injury induced by acute carbon monoxide poisoning. *Chin J of Indust Hygiene Occupat Dis* 2018; 36(5): 362-364.
 12. Ryter SW. Therapeutic potential of heme oxygenase-1 and carbon monoxide in acute organ injury, critical illness, and inflammatory disorders. *Antioxidants* 2020; 9(11): 1153.
 13. Hopper CP, Meinel L, Steiger C, Otterbein LE. Where is the clinical breakthrough of heme oxygenase-1/carbon monoxide therapeutics? *Curr Pharma Design* 2018; 24(20): 2264-2282.
 14. Vijayan V, Wagener FA, Immenschuh S. The macrophage heme-heme oxygenase-1 system and its role in inflammation. *Biochem Pharmacol* 2018; 153: 159-167.
 15. Liao SC, Mao YC, Yang KJ, Wang KC, Wu LY, Yang CC. Targeting optimal time for hyperbaric oxygen therapy following carbon monoxide poisoning for prevention of delayed neuropsychiatric sequelae: A retrospective study. *J Neurol Sci* 2019; 396: 187-192.
 16. Huang CC, Ho CH, Chen YC et al. Impact of hyperbaric oxygen therapy on subsequent neurological sequelae following carbon monoxide poisoning. *J Clin Med* 2018; 7(10): 349.
 17. Liao SC, Shao SC, Yang KJ, Yang CC. Real-world effectiveness of hyperbaric oxygen therapy for delayed neuropsychiatric sequelae after carbon monoxide poisoning. *Sci Rep* 2021; 11(1): 1-10.
 18. Kinoshita H, Türkan H, Vucinic S et al. Carbon monoxide poisoning. *Toxicol Rep* 2020; 7: 169-173.
 19. Eichhorn L, Thudium M, Jüttner B. The diagnosis and treatment of carbon monoxide poisoning. *Dtsch Ärztebl Int* 2018; 115(51-52): 863.
 20. Jeon SB, Sohn CH, Seo DW et al. Acute brain lesions on magnetic resonance imaging and delayed neurological sequelae in carbon monoxide poisoning. *JAMA neurology* 2018; 75(4): 436-443.
 21. Rose JJ, Nouraie M, Gauthier MC et al. Clinical outcomes and mortality impact of hyperbaric oxygen therapy in patients with carbon monoxide poisoning. *Crit Care Med* 2018; 46(7): e649.
 22. Cha YS, Kim H, Lee Y et al. Evaluation of relationship between coronary artery status evaluated by coronary computed tomography angiography and development of cardiomyopathy in carbon monoxide poisoned patients with myocardial injury: a prospective observational study. *Clin Toxicol* 2018; 56(1): 30-36.
 23. Guo X, Yin B, Wang C, Huo H, Aziziaram Z. Risk assessment of gastric cancer in the presence of *Helicobacter pylori* cagA and hopQII genes. *Cell Mol Biol* 2021; 67(4): 299-305.
 24. Alvarez Vilella M, Wever-Pinzon O, Parikh M et al. Patterns of cardiac dysfunction after carbon monoxide poisoning. *Undersea Hyperb Med* 2020; 47(3): 477-485.
 25. Jang DH, Piel S, Greenwood JC et al. Alterations in cerebral and cardiac mitochondrial function in a porcine model of acute carbon monoxide poisoning. *Clin Toxicol* 2021; 59(9): 801-809.
 26. Eruygur N, Ucar E, Akpulat HA. et al. In vitro antioxidant assessment, screening of enzyme inhibitory activities of methanol and water extracts and gene expression in *Hypericum lydium*. *Mol Biol Rep* 2019;46: 2121–2129. <https://doi.org/10.1007/s11033-019-04664-3>.
 27. Akbari F, Arminian A, Kahrizi D, Fazeli A, Ghaheri M. Effect of nitrogen sources on gene expression of *Stevia rebaudiana* (Bertoni) under in vitro conditions. *Cell Mol Biol* 2018;64(2), 11–16. <https://doi.org/10.14715/cmb/2018.64.2.3>
 28. Nakajima M, Aso S, Matsui H, Fushimi K, Yasunaga H. Hyperbaric oxygen therapy and mortality from carbon monoxide poisoning: A nationwide observational study. *Am J Emerg Med* 2020; 38(2): 225-230.