SHORT COMMUNICATION

Is Ionized Oxygen Negatively or Positively Charged More Effective for Carboxyhemoglobin Reduction Compare to Medical Oxygen at Atmospheric Pressure?

S. PEREČINSKÝ¹, I. KRON², I. ENGLER³, L. MURÍNOVÁ¹, V. DONIČ³, M. VARGA¹,
A. MAROSSY³, L. LEGÁTH¹

¹Department of Occupational Medicine and Clinical Toxicology, Faculty of Medicine, P. J. Šafárik University and the L. Pasteur University Hospital, Košice, Slovakia, ²Institute of Medical and Clinical Biochemistry and Labmed, Faculty of Medicine, P. J. Šafárik University, Košice, Slovakia, ³Department of Human Physiology, Faculty of Medicine, P. J. Šafárik University, Košice, Slovakia

Received August 21, 2014

Accepted March 10, 2015

On-line June 5, 2015

Summary

Carbon monoxide (CO) reversibly binds to hemoglobin forming carboxyhemoglobin (COHb). CO competes with O₂ for binding place in hemoglobin leading to tissue hypoxia. Already 30 % saturation of COHb can be deadly. Medical oxygen at atmospheric pressure as a therapy is not enough effective. Therefore hyperbaric oxygen O₂ inhalation is recommended. There was a question if partially ionized oxygen can be a better treatment at atmospheric pressure. In present study we evaluated effect of partially ionized oxygen produced by device Oxygen Ion 3000 by Dr. Engler in elimination of COHb *in vitro* experiments and in smokers. Diluted blood with different content of CO was purged with 5 l/min of either medicinal oxygen O₂, negatively ionized O₂ or positively ionized O₂ for 15 min, then the COHb content was checked. *In vivo* study, 15 smokers inhaled of either medicinal oxygen O₂ or negatively ionized O₂, then we compared CO levels in expired air before and after inhalation. In both studies we found the highest elimination of CO when we used negatively ionized O₂. These results confirmed the benefit of short inhalation of negatively ionized O₂, in frame of Ionized Oxygen Therapy (IO₂Th/Engler) which could be used in smokers for decreasing of COHb in blood.

Key words

Ionized oxygen • Negatively ionized O₂ • Positively ionized O₂ • Ionized Oxygen Therapy (IO₂Th/Engler) • CO • COHb • Chronic hypoxia • Smokers

Corresponding author

S. Perečinský, Department of Occupational Medicine and Clinical Toxicology, Faculty of Medicine, Pavol Jozef Safarik University and the L. Pasteur University Hospital, Rastislavova 43, 04910, Košice, Slovakia. Fax: +421-55-615-2699. E-mail: slavomir.perecinsky@upjs.sk

Carbon monoxide (CO) is a colorless, odorless, tasteless gaseous poison formed during imperfect combustion of any fuel containing carbon. It is also found in cigarette smoke. It reversibly binds to hemoglobin forming carboxyhemoglobin (COHb). Usually this level is less than 1 % saturation, but patients with hemolytic anemia and smokers may have concentrations greater than 5 %. Because carbon monoxide binds about 200 times more strongly to hemoglobin (Hb), than oxygen, even low levels of CO in air can create COHb. CO competes with O₂ for binding place in Hb leading to tissue hypoxia and death (Mayes 1993, von Burg 1999).

CO from Hb can be removed by lungs ventilation, but the half-life for COHb is 4-5 h at a normal atmospheric pressure. Already 30 % saturation of COHb can be deadly therefore there is no time to wait 4-5 h for CO removal by respiration. Therefore inhalation of hyperbaric oxygen O₂ at 2.5 at is recommended. This can reduce COHb half-life to 22 min (Mayes 1993, Weaver *et al.* 2000, Prockop and Chichkova 2007).

However, the ideal dose of O_2 during such therapy is unknown so far (Gorman *et al.* 2003). There are still many controversies on using of oxygen in therapy of carbon monoxide intoxication (Raphael *et al.* 1989, Juurlink *et al.* 2005). Despite that, the hyperbaric oxygen therapy (HO_2Th) represents a golden standard in CO intoxication today (Prockop and Chichkova 2007). However, usually if any limited numbers of hyperbaric chambers are available in the case of CO intoxication. The patients need the transport to the facility and the time is crucial. Using of medical oxygen O_2 at atmospheric pressure is not effective enough.

On the other side, because of permanent increasing of COHb, smokers are at risk of chronic hypoxia. High level of COHb is also associated with coagulopathies, dyslipidemia, atherosclerosis and ischemic heart disease. In the case of Raynaud syndrome $O_2^{\bullet-}$ induces periphery vasodilatation (Perečinský *et al.* 2014). There was a question if partially ionized oxygen ($O_2^{\bullet-}$ or $O_2^{\bullet+}$) can be a better treatment even at atmospheric pressure. Inhalation of O_2 enriched partially with $O_2^{\bullet-}$ or $O_2^{\bullet+}$ ions produced by commercially available medical device Oxygen Ion 3000 was introduced in 1980 by Dr. Engler in Salzburg in frame of Ionized Oxygen Therapy ($IO_2Th/Engler$). In experiment, $O_2^{\bullet-}$ improves the oxygenation of tissues, increases the mobility of respiratory cilia. Inhalation of $O_2^{\bullet-}$ during bicycle ergometry showed an increased body performance measured in watt/kg, which was not achieved by medical O_2 inhalation (Engler 2004). Presence of $O_2^{\bullet-}$ in Pico doses increasing trans membrane resting potentials of cells (TMRP), decreasing sludge of erythrocytes, has anti-inflammatory effect (Engler 2004, Engler *et al.* 2009).

Present pilot study evaluates effect of partially ionized oxygen (Pico doses of $O_2^{\bullet-}$ or $O_2^{\bullet+}$) produced by device Oxygen Ion 3000 for elimination of COHb *in vitro* experiments and in smokers. According to our knowledge the similar study has not been published yet.

In vitro experiment removal of CO from COHb in human blood using ionized forms of oxygen ($O_2^{\bullet-}$ or $O_2^{\bullet+}$) or oxygen (O_2) without ionization (Study A)

Human heparinized blood provided by the Blood transfusion unit at the University hospital of L. Pasteur in Košice, Slovakia, blood group 0, Rh+ was used. Two samples of 400 ml of blood in a 2-liter round-bottom flask was purged either with CO (Tatragas Messer-Slovakia, purity 99.9 %) with the flow rate 5 l/min during

simultaneous shaking (100 min^{-1}) for 15 min = 100 % COHb or with medicinal oxygen (Tatragas Messer-Slovakia, purity 99.9 %) under the same conditions = 0 % COHb. The COHb content in blood was determined according to Dijkhuizen *et al.* (1977) by UV-VIS Diode array spectrophotometer Multispec-1501 (Shimadzu, Japan). To 2000 μl of distilled water (Millipore-Simplicity, France) was added 2 μl of blood and vigorously mixed in the 1 cm quartz cell. The measurements were performed in triplicate within 5 min from the sample withdrawal to avoid the losses of CO content on standing (Beutler and West 1984). The COHb content was calculated by an experimentally determined equation ($\% \text{ COHb} = (383.58 * A_{562}/A_{540}) - 233.33$). 100 % CO saturated blood was diluted with untreated blood to desired concentrations 50 %, 25 %, 12.5 %, 6.25 %, and 3.1 % of the original carbon monoxide concentration, which were checked by the spectrophotometer. Diluted blood with different content of CO (100 ml in a volume of 1-liter round-bottom flask) was purged with 4 l/min flow of either medicinal oxygen O_2 or partially negatively ionized oxygen $O_2^{\bullet-}$ (120 000 ions of $O_2^{\bullet-}/\text{cm}^3$ of O_2) or partially positively ionized oxygen $O_2^{\bullet+}$ (135 000 ions of $O_2^{\bullet+}/\text{cm}^3$ of O_2), respectively. For ionization of O_2 was used device Oxygen Ion 3000 by Dr. Engler (CS Tronik, Austria). All samples were simultaneously shaking during 15 min in a fume cupboard and then the COHb content was checked by spectrophotometer. All experiments were performed at least 3 times at 22 °C room temperature. The results were evaluated statistically using t-test.

Table 1 shows results of elimination of CO from blood with different initial COHb concentrations in percentage (the first column) by using medicinal oxygen O_2 and oxygen enriched with $O_2^{\bullet-}$ or $O_2^{\bullet+}$ ions, respectively.

Table 1. The efficiency (in %) of various oxygen species in elimination of CO from COHb in the heparinized human blood.

% COHb	O_2	$O_2^{\bullet-}$	$O_2^{\bullet+}$
91.2	-19.6	-18.2	-18.6
46.7	-28.1	-20.6	-19.5
24.4	-28.3	-33.2	-31.1
17.6	-39.8	-43.8	-32.2
11.9	-30.3	-35.3	-28.7
5.9	-69.5	-83.1*	-58.5

* $P < 0.01$.

The percentage was calculated as a difference (initial % COHb – final % COHb) divided by initial % COHb and multiplied by 100. The most effective in elimination of CO from blood seems to be the negatively ionized oxygen $O_2^{\bullet-}$ ($P < 0.01$) compare to medical oxygen O_2 .

From the Table 1 is obvious the strongest effect of negatively ionized oxygen ($O_2^{\bullet-}$) on decrease of COHb levels below 25 % of COHb (numbers in bold), which is significant ($P < 0.01$) especially for the level of 5.9 % COHb – a typical level of COHb for smokers (Lawther and Commins 1970, Beutler and West 1984, Kambam *et al.* 1986, Gabriel da Costa *et al.* 1998). The effectiveness of partially negatively ionized oxygen ($O_2^{\bullet-}$) increases with decreasing COHb level.

Study in vivo represents a group of 15 smoking subjects, inhaled partially negatively ionized oxygen ($O_2^{\bullet-}$) or medical oxygen (O_2) or room air without ionization, respectively (Study B)

This study was realized in 3 phases during 3 days in the same 15 smoking subjects. Phase 1 – therapy with molecular oxygen O_2 , phase 2 – therapy with partially negatively ionized oxygen ($O_2^{\bullet-}$), phase 3 – control without therapy (not any form of oxygen were used – subjects breathing room air resting at room temperature). For preparation of $O_2^{\bullet-}$ the same device Oxygen Ion 3000 (generated 200 000 ions of $O_2^{\bullet-}$ in 1 cm³ of O_2 at a flow 8 l/min) was used. The effect of oxygen therapy with ionized $O_2^{\bullet-}$ or molecular O_2 on the CO level in exhale air was monitored by a CO meter (GCO 100 Greisinger Electronic, Germany) and expressed in ppm. Determination of CO level in expired air was chosen due to its simplicity, non-invasiveness and low cost. Moreover, the CO level in expired air correlates very well with COHb level in blood (Wald *et al.* 1981, Andersson and Moller 2010). The measurement of CO was performed within 1 min after smoking a cigarette by a person. The person was asked to held breath for 20 s and then slowly to expire into the CO meter (the first measurement). Subsequently, the subject inhaled molecular O_2 or ionized oxygen $O_2^{\bullet-}$ during 20 min (because of the beneficial effects of $O_2^{\bullet-}$ *in vitro* study, subjects inhaled only $O_2^{\bullet-}$ and never O_2^+). Immediately after 20 min of oxygen forms inhalation the repeated measurement of CO in exhaled air was performed using the same technique (second measurement).

Statistical analysis was carried out with the programs Arcus QuickStat (Biomedical). The effect of

various oxygen species inhalation was evaluated by percentage of CO level after inhalation versus CO level before inhalation. Using analysis of variance and conversion by Tukey-Kramer test were compared differences in the values of exhaled CO (expressed as percentages) between groups. Difference between the first and second measurement represents the amount of CO removed from COHb by the treatment – inhalation of $O_2^{\bullet-}$ or O_2 .

The best elimination of CO from COHb in smokers was achieved by inhalation of $O_2^{\bullet-}$. There were no differences in average values of CO in the first measurement between all groups. However, in the second measurement the highest average CO value (in ppm) in exhaled air was seen in group with $O_2^{\bullet-}$ inhalation. There was statistically significant difference in the increase of CO value in the second measurement between groups with inhalation of $O_2^{\bullet-}$ or O_2 and control group. Also we observed difference between the inhalation of $O_2^{\bullet-}$ and O_2 ($P = 0.016$) (Fig. 1).

Fig. 1. Differences in expired CO mean levels (in ppm) before and after inhalation of oxygen $O_2^{\bullet-}$ or O_2 or in control group in 15 smokers. *** $P < 0.001$ ($O_2^{\bullet-}$ and O_2 vs. control), # $P = 0.016$ ($O_2^{\bullet-}$ vs. O_2).

In both studies (A and B) partially ionized oxygen $O_2^{\bullet-}$ showed the best effect in CO elimination from binding with Hb. The best effect was seen when COHb concentration in human blood was less than 25 % and CO in smokers was under 15 ppm in exhaled air. Inhalation of $O_2^{\bullet-}$ in Pico doses may improve oxygenation, mitochondrial functions especially production of ATP as an energetic molecule which is decreasing during hypoxia for example caused by acute or chronic exposition to CO (Engler 2004). This may explain that even very small concentration of $O_2^{\bullet-}$ may have a beneficial effect in COHb elimination *in vitro*

experiments or *in vivo* in smokers. The oxygen radicals $O_2^{\bullet-}$ in Pico doses in medical oxygen (20 min of inhalation at 8 l/min flow) has beneficial biological effect as a signal molecule. For example partially negatively ionized oxygen ($O_2^{\bullet-}$) inhalation was effective in treatment of vibration white finger syndrome in patients (Perecinsky *et al.* 2014).

Very low doses and short time inhalation of partially ionized oxygen ($O_2^{\bullet-}$), according to our opinion cannot increase oxidative stress. It is in accordance with theory of Hormesis (Calabrese and Baldwin 2003).

The similarly as Hormesis theory also Linear-No Threshold Theory of Radiation (Cohen 1999) explain that a small dosis of Radon radiation (Rn) prevent cancer incidence, but high dose of Rn cause lung cancer. In our experiments with lung fibroblast we find that partially ionized oxygen in Pico doses ($O_2^{\bullet-}$ or $O_2^{\bullet+}$) improve cells damage caused by Rn (Engler *et al.* 2009).

In the experimental work (Kaplan *et al.* 2009) free radical-induced oxidative damage and enzyme inhibition was even more pronounced when inhaled oxygen was partially negatively charged ($O_2^{\bullet-}$). On the other hand, when inhaled oxygen was partially positively charged ($O_2^{\bullet+}$) changes were lowered or completely eliminated. In this study a 36 h of continuous inhalation of ionized oxygen O_2 in guinea pigs (250 g) was used, which was much longer exposition compared with 20 min

in our study with smokers (70 kg).

It could be helpful to test antioxidant status of the smokers before $O_2^{\bullet-}$ inhalation, because it can shows how decreasing of COHb can change oxidative status after inhalation of partially ionized oxygen $O_2^{\bullet-}$.

These results are interesting and may have the important clinical implications. Short 20 min inhalation of partially ionized oxygen ($O_2^{\bullet-}$) can be used in smokers for decreasing of COHb in their blood as a prevention of consequences of chronic hypoxia and CO effect (face skin changes, decreased of physical and psychical performances, arteriosclerosis, ischemic heart disease, etc.). In acute intoxication with CO, inhalation of $O_2^{\bullet-}$ could be better option than only medical oxygen inhalation.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The authors would like to acknowledge The Scientific Grant Agency at the Ministry of Education, Slovak Republic and The Agency of the Slovak Ministry of Education for the Structural Funds of the EU, under project ITMS: 26220120067 for the financial support of the research.

References

- ANDERSSON MF, MÖLLER AM: Assessment of carbon monoxide values in smokers: a comparison of carbon monoxide in expired air and carboxyhaemoglobin in arterial blood. *Eur J Anaesthesiol* **27**: 812-818, 2010.
- BEUTLER E, WEST C: Simplified determination of carboxyhaemoglobin. *Clin Chem* **30**: 871-874, 1984.
- CALABRESE EJ, BALDWIN LA: Hormesis: the dose-response revolution. *Annu Rev Pharmacol Toxicol* **43**: 175-197, 2003.
- COHEN BL: Validity of the linear-no threshold theory of radiation, carcinogenesis in the low dose region. In: *Radon and Health*. DETJEEN P, FALKENBACH A (eds), Peter Lang Verlag, Vienna, 1999, pp 13-37.
- DIJKHUIZEN P, BUURSMA A, GERDING AM, VAN KAMPEN EJ, ZIJLSTRA WG: Carboxyhaemoglobin, spectrophotometric determination tested and calibrated using a new reference method for measuring carbon monoxide in blood. *Clin Chim Acta* **80**: 95-104, 1977.
- ENGLER I: *Handbuch Ionisierter Sauerstoff Therapie im Spiegel der Ganzheitsmedizin*. Spurbuch Verlag, D-Baunach, 2004, p. 264.
- ENGLER I, ATZMÜLLER C, DONIC V, STEINHÄUSLER F: Reactive oxygen species, especially $O_2^{\bullet+}$ in cancer mechanisms. *J Exp Ther Oncol* **8**: 157-165, 2009.
- GABRIEL DA COSTA MALHEIROS ACC, PEREIRA BASTOS DE SIQUEIRA ME, ALVAREZ-LEITE EM: Studies on spectrophotometric method for carboxyhaemoglobin determination. *Acta Toxicol Argentina* **6**: 4-7, 1998.
- GORMAN D, DREWRY A, HUANG YL, SAMES C: The clinical toxicology of carbon monoxide. *Toxicology* **187**: 25-38, 2003.

-
- JUURLINK DN, BUCKLEY NA, STANBROOK MB, ISBISTER GK, BENNETT M, MCGUIGAN MA: Hyperbaric oxygen for carbon monoxide poisoning. *Cochrane Database Syst Rev* **21**: CD002041, 2005.
- KAMBAM JR, CHEN LH, HYMAN SA: Effect of short-term smoking halt on carboxyhaemoglobin levels and P50 values. *Anesthesiol Analgesiol* **65**: 1186-1188, 1986.
- KAPLAN P, TATARKOVA Z, ENGLER I, CALKOVSKA A, MOKRA D, DRGOVA A, KOVALSKA M, LEHOTSKY J, DOBROTA D: Effects of long-term oxygen treatment on alpha-ketoglutarate dehydrogenase activity and oxidative modifications in mitochondria of the guinea pig heart. *Eur J Med Res* **14** (Suppl 4): 116-120, 2009.
- LAWTHER PJ, COMMINS BT: Cigarette smoking and exposure to carbon monoxide. *Ann NY Acad Sci* **174**: 135-174, 1970.
- MAYES RW: Measurement of carbon monoxide and cyanide in blood. *J Clin Pathol* **46**: 982-988, 1993.
- PERECINSKY S, MURINOVA L, ENGLER I, DONIC V, MURIN P, VARGA M, LEGATH L: Effect of partially ionized medical oxygen, especially O₂•⁻ in vibration white finger patients. *Int J Environ Res Public Health* **11**: 5698-5707, 2014.
- PROCKOP LD, CHICHKOVA RI: Carbon monoxide intoxication: an updated review. *J Neurol Sci* **262**: 122-130, 2007.
- RAPHAEL JC, ELKHARRAT D, JARS-GUINCESTRE MC, CHASTANG C, CHASLES V, VERCKEN JB, GAJDOS P: Trial of normobaric and hyperbaric oxygen for acute carbon monoxide intoxication. *Lancet* **2**: 414-419, 1989.
- VON BURG R: Toxicology update. Carbon monoxide. *J Appl Toxicol* **19**: 379-386, 1999.
- WALD NJ, IDLE M, BOREHAM J, BAILEY A: Carbon monoxide in breath in relation to smoking and carboxyhaemoglobin levels. *Thorax* **36**: 366-369, 1981.
- WEAVER LK, HOWE S, HOPKINS R, CHAN KJ: Carboxyhemoglobin half life in carbon monoxide-poisoned patients treated with 100 % oxygen at atmospheric pressure. *Chest* **117**: 801-808, 2000.
-