# Is ionized oxygen $O_2^{\bullet -}$ or $O_2^{\bullet +}$ more effective for carboxyhemoglobin reduction compare to medical oxygen at atmospheric pressure? The pilot study in vitro and in humans

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**Is ionized oxygen effective for carboxyhemoglobin reduction?**

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**Summary**

Carbon monoxide (CO) reversibly binds to hemoglobin forming carboxyhemoglobin (COHb). CO competes with $O_2$ 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 $O_2$ 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 dm3/min of either medicinal oxygen $O_2$, $O_2\bullet^-$ or $O_2\bullet^+$ for 15 minutes, then the COHb content was checked. In vivo study, 15 smokers inhaled of either medicinal oxygen $O_2$ or $O_2\bullet^-$, than we compared CO levels in expired air before and after inhalation. In both studies we found the highest elimination of CO when we used $O_2\bullet^-$. These results confirmed the benefit of short inhalation of $O_2\bullet^-$, in frame of Ionized Oxygen Therapy ($IO_2Th$/Engler) which could be used in smokers for decreasing of COHb in blood.

**Key words:** ionized oxygen, $O_2\bullet^-$, $O_2\bullet^+$, Ionized Oxygen Therapy ($IO_2Th$/Engler), CO, COHb, chronic hypoxia, smokers

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 haemoglobin (Hb), than oxygen, even low levels of CO in air can create COHb. CO competes with $O_2$ for binding place in Hb leading to tissue hypoxia and death (Mayes 1993, Von Berg 1999).

CO from Hb can be removed by lungs ventilation, but the half-life for COHb is 4-5 hours at a normal atmospheric pressure. Already 30% saturation of COHb can be deadly therefore there is no time to wait 4-5 hours for CO removal by respiration. Therefore inhalation of hyberbaric oxygene $O_2$ 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 (Perecinsky *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 $O_2•^-$ in Pico doses increasing trans membrane resting potentials of cells (TMRP), decreasing sludge of erythrocytes, has anti-inflammatory effect (Engler et al 2009, Engler 2004).

Present study evaluates effect of partially ionized oxygen (Pico doses of $O_2•^-$ or $O_2•^+$) 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.

**A. In vitro experiment removal of CO from COHb in human blood using ionized forms of oxygen** ($O_2•^-$ or $O_2•^+$) **or oxygen ($O_2$) without ionization.**

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 $dm^3$/min during simultaneous shaking (100 $min^{-1}$) for 15 minutes = 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., (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 (% 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 negative ionized oxygen $O_2•^-$ (120 000 ions of $O_2•^-/cm^3$ of $O_2$) or partially positive ionized oxygen $O_2•^+$ (135 000 ions of $O_2•^+/cm^3$ of $O_2$) respectively. For ionization of O2 was used device Oxygen Ion 3000 by Dr. Engler (CS Tronik, Austria). All samples were simultaneously shaking during 15 minutes 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 $O_2•^-$ or $O_2•^+$ ions, respectively.

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 negative ionized oxygen $O_2•^-$ ($P < 0.01$) compare to medical oxygen $O_2$.

***Table 1*** *should be placed here*

From the Table 1 is obvious the strongest effect of negative ionized oxygen ($O_2•^-$) 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 ($O_2•^-$) increases with decreasing COHb level.

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

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 negative ionized oxygen ($O_2•^-$), phase 3 – control without therapy (not any form of oxygen were used - subjects breathing room air resting at room temperature). For preparation of $O_2•^-$ the same device Oxygen Ion 3000 (generated 200.000 ions of $O_2•^-$ in 1 $cm^3$ of $O_2$ at a flow 8 L/minute) was used. The effect of oxygen therapy with ionized $O_2•^-$ 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 sec and then slowly to expire into the CO meter (the first measurement). Subsequently, the subject inhaled molecular $O_2$ or ionized oxygen $O_2•^-$ during 20 minutes (because of the beneficial effects of $O_2•^-$ in vitro study, subjects inhaled only $O_2•^-$ and never $O_2•^+$). Immediately after 20 minutes of oxygen forms inhalation the repeated measurement of CO in exhaled air was performed using the same technique (second measurement).

Statistic 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 $O_2•^-$ or $O_2$.

The best elimination of CO from COHb in smokers was achieved by inhalation of $O_2•^-$. 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 $O_2•^-$ inhalation. There was statistically significant difference in the increase of CO value in the second measurement between groups with inhalation of $O_2•^-$ or $O_2$ and control group. Also we observed difference between the inhalation of $O_2•^-$ and $O_2$ (P=0,016) (fig. 1)

***Figure 1*** *should be placed here*

In both studies (A and B) partially ionized oxygen $O_2•^-$ 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 $O_2•^-$ 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 $O_2•^-$ may have a beneficial effect in COHb elimination in vitro experiments or in vivo in smokers. The oxygen radicals O2•- in Pico doses in medical oxygen (20 minutes of inhalation at 8L/min flow) has beneficial biological effect as a signal molecule. For example partially negatively ionized oxygen ($O_2•^-$) 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 ($O_2•$-), 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 Treshold 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 dosis ($O_2$•- or $O_2$•$^+$) 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 ($O_2$•-). On the other hand, when inhaled oxygen was partially positively charged ($O_2$•$^+$) changes were lowered or completely eliminated. In this study a 36 hour of continuous inhalation of ionized oxygen O2 in guinea pigs (250 gram) was used, which was much longer exposition compared with 20 minutes in our study with smokers (70 kg).

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

These results are interesting and may have the important clinical implications. Short 20 minutes inhalation of partially ionized oxygen ($O_2$•$^-$) 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 $O_2$•$^-$ could be better option than only medical oxygen inhalation.

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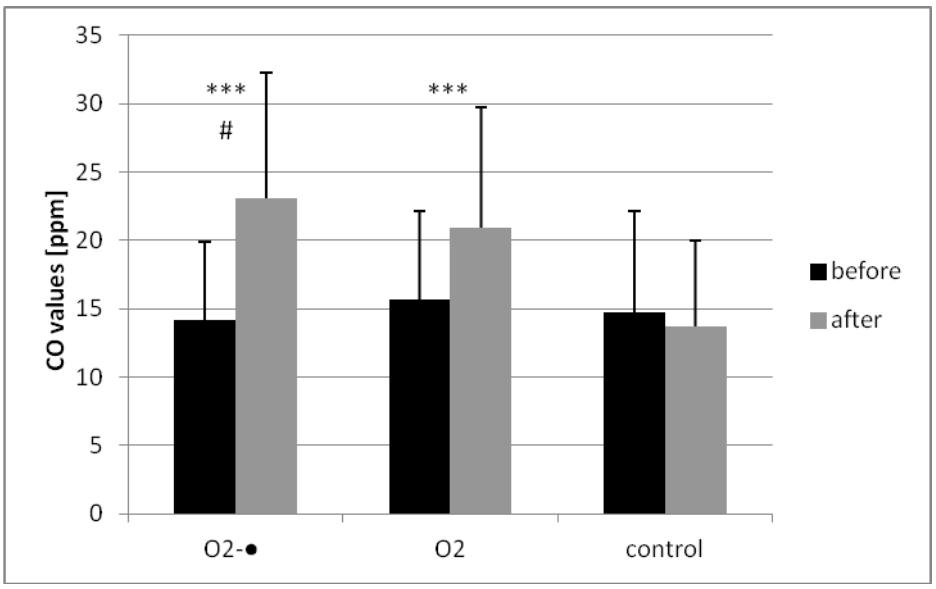
**Tab. 1 The efficiency (in %) of various oxygen species in elimination of CO from COHb in the heparinized human blood.**

| % 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** |

* $P < 0.01$




*****P<0.001 ($O_2$•$^-$ and $O_2$ vs. control), # P=0.016 ($O_2$•$^-$ vs. $O_2$).**

**Fig. 1 Differences in expired CO mean levels (in ppm) before and after inhalation of oxygen** $O_2$•$^-$ or $O_2$ or in **control group in 15 smokers.**