

THE OXIDANT EFFECTS OF HYPERBARIC OXYGENATION AND AIR POLLUTION IN ERYTHROCYTE MEMBRANES (HYPERBARIC OXYGENATION IN AIR POLLUTION)

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Aim: The aim was to investigate the interactions of hyperbaric oxygenation (HBO) and sulphur dioxide (SO₂) inhalation (at 10 ppm, one hour daily for 45 days) on lipoperoxidation and fragility of RBC membranes.

Methods: Malonyldialdehyde (MDA) levels, osmotic fragility ratios and hematologic parameters of a total of 24 rats were compared in four different groups controls, HBO, SO₂ and (HBO+SO₂). HBO was applied at 3 Atm abs (303.9 kPa), 1 hour daily, for 45 days in a specifically designed exposure chamber.

Results: MDA levels and osmotic fragility ratios were significantly higher in groups-B, C and D than in control group ($p < .05$ for all). Marked decrease in MDA levels and osmotic fragility ratios were observed in group-D (HBO+SO₂) compared to groups-B and C ($p < 0.05$). However, MDA levels and osmotic fragility ratios in group-D were also significantly higher than in group-A ($p < 0.05$ for all).

Conclusion: HBO-treatment or SO₂ inhalation alone resulted in structural and functional oxidant damage, indicated by higher MDA levels and osmotic fragility ratios, but when they were applied together, decreases in oxidant damage in RBC membranes were observed indicating HBO may also be useful in the cities with a serious air pollution problem, at least it does not cause additional oxidant stress.

Key words: HBO, SO₂, RBC, lipoperoxidation, fragility

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INTRODUCTION

The increased O₂ content of the blood caused by hyperbaric oxygenation (HBO) is very helpful in treating several diseases arising from or complicated by decreased tissue oxygen availability (1,2). However, many researchers have reported that O₂ in excess may have harmful effects. Lipid peroxidation due to free oxygen radicals (FOR) is known to occur during HBO (3-10). Peroxidation of membrane phospholipid PUFAs (Polyunsaturated fatty acids) which is an important complication of HBO treatment (HBOT) was reported to lead to the disruption of the structural integrity of the membrane lipid bilayer and/or alter the boundary lipid microenvironment of membrane proteins

especially those that maintain selective transmembrane ionic and potential gradients (11,12). Lipid peroxidation products accumulate in various tissues following hyperbaric hyperoxia and also depress the activity of Na-K ATPase (13-15). Lipid peroxidation, an autocatalytic free radical process, has been reported to have a very important effect in many diseases including various hemolytic anemias and hemoglobin disorders (16-18).

Air pollution is a very important problem which continues to threaten human health for a long time all over the world. Some of the major air pollutants are sulphur dioxide (SO₂), carbon monoxide, nitrogen dioxide (NO₂) and ozone (O₃). SO₂ is reported to cause an

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Table 1. Comparison of MDA levels and hematologic parameters (Mean±SEM)

Parameters	Group-A (control)	Group-B (HBO)	Group-C (SO ₂)	Group-D (HBO+ SO ₂)	
MDA, n.mol/grHb	30.5±1.79	58.03±8.68*	54.23±6.79*	45.58±2.94	*†
WBC, 10 ³ /mm ³	5.63±1.06	7.65±0.90*	7.66±0.90*	9.78±1.1	*†
RBC, 10 ⁶ /mm ³	6.23±0.42	7.82±0.20*	7.62±0.40*	7.38±0.8	*
Hematocrit, %	38.70±2.33	49.43±1.10*	47.09±2.20*	46.23±2.4	*
Hemoglobin, gr/dl	12.03±0.70	15.30±0.90*	14.40±0.50*	14.28±1.2	*
Mean cor.vol, fl	62.10±0.59	63.28±0.50*	61.64±2.20	62.80±0.7	*
Mean cor.Hb, pgr	19.26±0.30	19.55±0.10	18.93±0.70	19.47±0.7	
Mean cor Hb con, gr/dl	31.36±0.40	30.93±1.00	30.66±0.70	30.98±0.9	
Sulfhemoglobin, %	0.07±0.02	0.06±0.03	2.24±0.07	2.18±0.06	

* Statistically significant when compared with control group ($p < .05$),

† Statistically significant when compared with HBO and SO₂ groups ($p < .05$)

increase in the daily number of deaths due to respiratory and circulatory system diseases. SO₂ is absorbed readily (99%) through the respiratory tract and shows a wide tissue distribution. The amount of SO₂ in blood and other tissues increases proportionally with its concentration in the inhalation air. Radioactive sulphur (S³⁵) can be detected in the protein structures of tissues at least for one week (19-21).

Haider et al. reported an increase in the lipid peroxidation of the lungs and heart of the rats due to SO₂ inhalation at 10 ppm 1 hour daily for 30 days (22). Significant increases in sulfhemoglobin ratios and RBC activities of superoxide dismutase and glutathione peroxidase during an heavy air pollution were determined in an epidemiological study (23). Higher sulfhemoglobin and osmotic fragility ratios, WBC and RBC counts, hematocrit and hemoglobin values and lower erythrocyte deformabilities induced by SO₂ inhalation were detected in various studies (24-28). Moreover, the inhalations of various air pollutants (NO₂, O₃, cigarette smoke) were reported to result in higher MDA levels and osmotic fragility ratios (29-40).

The aims of the present study firstly, was to investigate if HBOT caused an increase in the formation of free O₂ radicals in RBC membranes. Secondly, since HBOT is administered all over the world including many cities with a serious air pollution problem, it was investigated if there were any adverse effects on RBC membranes in HBO treatments during heavy air pollutions.

MATERIALS AND METHODS

Animals and exposures. A total of 24 rats (Swiss-Albino, male, weighing 214-222 gr.) were divided into four groups by "Simple

random sampling method" as follows: Group-A (n:6): control, group-B (n:6): HBO, group-C (n:6): SO₂ and group-D (n:6): HBO+SO₂. Groups B and D exposed to HBO in a cylindrical pressure chamber under 3 Atm abs 303.9) pressure, 1 hour daily for 45 days. A dish with sodalime and sicca-gell spheres were placed in the chamber to prevent CO₂ accumulation and an increase in humidity. Pressurisation was supplied with compressed O₂ from commercial cylinders. The pressure was increased beginning with a slow initial phase of 1 ATA/30 s. and then doubled every 30 s. according to a previously validated schedule (41).

SO₂ were applied in groups C and D in a specifically designed exposure chamber at 10 ppm (MAC: Maximum acceptable dose), 1 hour daily, for 45 days. The flow rate of air containing SO₂ was 30 L/min. SO₂ concentration of the chamber air was measured by H₂O₂-acid titration method. Ambient air was applied at a similiar flow rate under the same conditions (1 hour daily, for 45 days) in groups-A (control) and B (HBO) (22).

Heparinized blood specimens were obtained by cardiac puncture under ketalar (20-40 mg/kg) + xylocaine (4-8 mg/kg) anesthesia at the end of the inhalation period. All the studies were completed in 4 hours.

Animals were supplied with standart rat chow and tap water. Minimal numbers of animals were sacrificed for ethical purposes.

Hematological measurements. RBC and WBC counts, hemoglobin, hematocrit, mean cell volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration values were determined using an electronic hematology analyzer (Coulter counter model S plus VI).

The extent of lipid peroxidation was determined by measurement of MDA levels

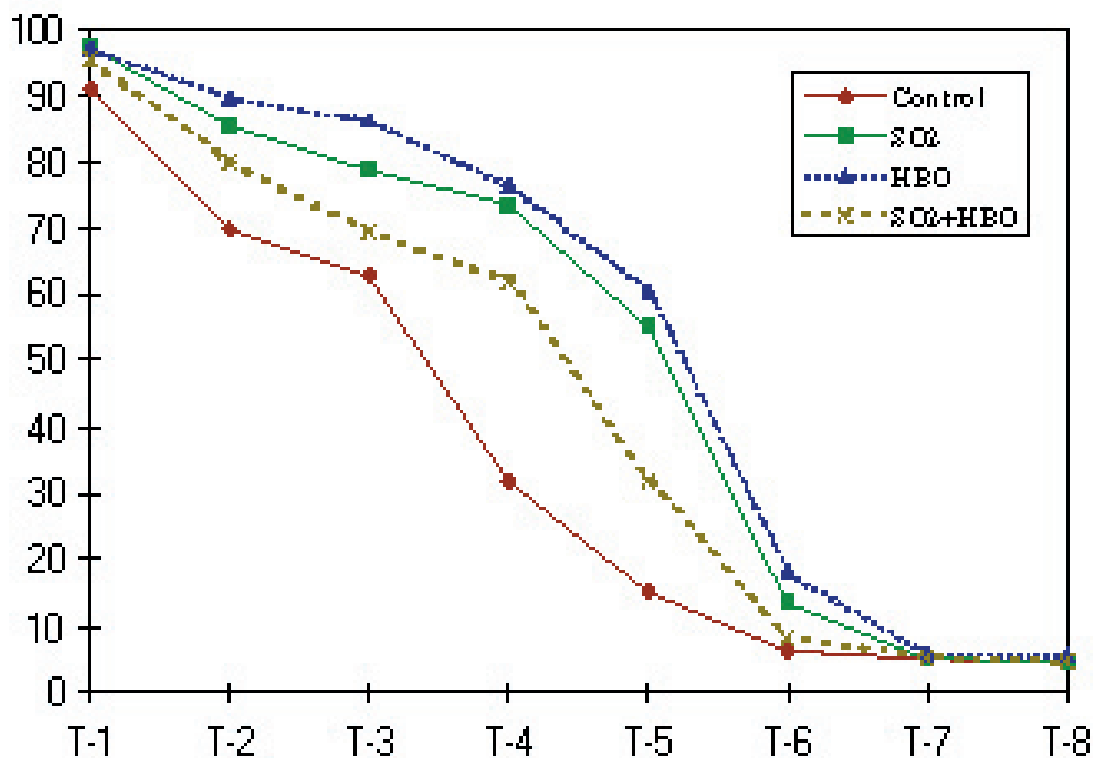


Figure 1. Comparison of the osmotic fragility curves

(T-1=20%, T-2=30%, T-3=40%, T-4=45%, T-5=50%, T-6=55%, T-7=60%, T-7=70%, T-8=80% concentrations of NaCl)

according to the method of Stocks and Dormandy (42,43). Reagents. (1) Buffered saline: 17.6 ml 0.5 M KH₂PO₄ + 60.8 ml 0.5 M K₂HPO₄ were made up to 1 liter with deionized water. An aliquot of this solution (100 ml) was added to 1 liter 0.15 M NaCl, pH 7.4 (2) TCA-Arsenite solution: 280 g trichloroacetic acid (TCA) were added to 500 ml deionized water followed by 13 g Na-arsenite. The mixture was dissolved by heating, then cooled. Finally, it was made up to 1 liter with deionized water and filtered. (3) TBA solution: 5 g thiobarbituric acid (TBA) was dissolved by heating in 200 ml deionized water together with 25 ml, 1 N NaOH. The solution was cooled and made up to 500 ml with deionized water and filtered.

Procedure: Fresh blood obtained by cardiac puncture was centrifuged. The plasma and buffy coat (the layer composed of leukocytes and thrombocytes) were aspirated. Erythrocytes were washed with 50 volume of P-tampone and centrifuged twice. Hemoglobin concentration was measured by adding 0.02 ml of blood to 5 ml Drapkin solution, shaking well, waiting and reading the absorbance value at 540 nm spectrophotometrically (Shimadzu UV-120-01). Afterwards, Hb

concentration was brought to 3 gr/100 ml by adding P-tampone. 3 ml of the cell suspension was added to 2 ml TCA-arsenite solution. The mixture was centrifuged, 3 ml of the supernatant was transferred to a 15 ml centrifuge tube and 1 ml of TBA solution was added. An air condenser was fitted to the tube and the mixture was incubated for exactly 15 min in a boiling-water bath. The tube was cooled with tap water immediately. The absorption spectrum of the mixture between 500 and 600 nm was plotted by using a spectrophotometer. The formula $(OD_{532-600}) \times 900$ gave the MDA concentration in n-mol/g Hb. Osmotic fragility measurements were done spectrophotometrically by using heparinized blood (44). Results were expressed as mean \pm standard error and the groups were compared by Mann/Whitney U-Wilcoxon Rank Sum W test.

RESULTS

Lipid peroxidation (Table 1). The extent of lipid peroxidation of RBC membranes in groups B, C and D were significantly higher than in control group ($p < .01$). Marked decreases in MDA levels were observed in group D compared with groups B and C

($p < 0.05$). Osmotic fragility ratios (Figure 1).

The results obtained in group B and C at concentrations of 0.30%, 0.40%, 0.45%, 0.50%, 0.55%, 0.60% were significantly higher than in group-A ($p < 0.05$). The values of group D were significantly lower than in group B at concentrations of (0.30%, 0.40%, 0.45%, 0.50%, 0.55%, 0.60%, 0.70%) ($p < 0.05$) and lower than in group C at concentrations of (0.45%, 0.50%, 0.55%, 0.60%, 0.70%) ($p < 0.05$). However, results of group D were also significantly higher than in group-A at concentrations of (0.40%, 0.45%, 0.50%, 0.55%, 0.60%) ($p < 0.05$).

Hematologic parameters (Table 1). WBC counts in all of the groups were significantly higher than in control group ($p < .05$). The results of group D were significantly higher than in groups B and C ($p < 0.05$).

RBC counts, hematocrit and hemoglobin values in all of the groups were significantly higher than in control group ($p < 0.05$) and no statistically significant differences were determined between the other groups ($p > 0.05$). No significant differences in MCV, MCH and MCHC values were detected between the groups also ($p > 0.05$).

DISCUSSION

Five important features of erythrocytes have caused them to be preferred extensively as a model for investigating oxidant stress: 1) RBCs are always in contact with molecular O₂ directly. 2) They contain metal ions catalysing oxidative reactions. 3) RBC membranes have got plenty of PUFA (polyunsaturated fatty acids) which is susceptible to lipid peroxidation. 4) They do not have ability to repair membrane damages and regenerate. 5) They circulate in the plasma which is poor in antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase (45). Therefore, erythrocytes may be accepted as an useful and convenient cell system in which to evaluate the effects of HBO administered in therapeutic situations or in alternate environments (e.g. deep sea diving) (46).

In recent years, oxidant stress related damages of biological systems have been attracting a growing interest of researchers. FOR cause oxidant-induced changes in the structure and function of erythrocyte membranes. Major changes reported by different researchers are lipid peroxidation, membrane protein-crosslinkings and oxidation of heme proteins resulting in their crosslinkings to skeletal proteins, i.e spectrin,

actin, cytoplasmic component of band 3 (47-50). Free oxygen radicals induced changes result in severe deterioration of membrane structure and function causing increases in osmotic fragility ratios, accelerated cell aging and premature cell death ultimately. The susceptibility to auto-oxidation is greatly increased in many haemolytic diseases and hemoglobin disorders and the oxidation of lipids are known to lead to cell death inevitably.

In this study, increased RBC counts, hemoglobin and hematocrit values as in the previous studies were also determined in groups B, C and D (25,26,27,51,52,53,54). These changes may be due to one of the followings: decreased plasma volume related with increased vascular permeability induced by free oxygen radicals, erythropoetic effect of fragmentation products of highly fragile erythrocytes or splenic contraction due to anesthesia complication resulting in RBC release or functional anemia due to sulfhemoglobinemia (for groups C and D) or decreased plasma volume related with increased vascular permeability induced by the irritant effect of SO₂ (for groups C and D) (41,55,56,57,58,59,60). Since no differences in hemoglobin, hematocrit and RBC counts were determined in group D compared with group C, these findings indicate that increases in these hematological parameters are not directly related with oxidant stress. Probably, these increases, also determined in group D, are related with the other factors mentioned above. MCV values in group-B were found significantly higher than in the control group. Highest osmotic fragility ratios determined in this group may be evaluated as a result of increased MCV values.

Higher WBC counts in groups B, C and D than in control group were also determined as in the previous studies. Activated neutrophils are known to release reactive O₂ species causing oxidant damage in blood and the other tissues. This increase which is known to be a response to the oxidant damage may have contributed to lipid peroxidation and structural and functional changes in erythrocyte membranes (63-63). But, while MDA levels in group D is lower than the groups B and C, WBC levels is significantly higher than the groups B and C, so the importance of the contribution of WBC to the lipid peroxidation must be very little or WBC increase must be related with another unknown factor.

We determined in this study that HBO and

SO₂ exposures resulted in an increase in MDA levels which is known to cause hemoglobin denaturation, membrane lipid peroxidation, cross-linkings between membrane skeletal proteins and between membrane and hemoglobin. Increases in osmotic fragility ratios due to structural and functional membrane damage were also observed in both HBO-treated group and SO₂-inhaled group. The changes following HBOT may be related with various free oxygen radicals and the other changes obtained following increased sulfhemoglobinemia induced by SO₂ inhalation may be due to sulfur dioxide anion radical (SO₂⁻) which was reported to form via one electron transfer resulting in the reduction of SO₂ to SO₂⁻ by cytochrome P-450 (64).

MDA levels were significantly lower in group D than in groups B and C. Moreover, significant decreases in osmotic fragility ratios were observed in group D compared with the same groups. This finding may be evaluated as a result of the presence of sulfur dioxide anion radical under the anaerobic conditions only. This radical can not be detected in the presence of air (65). The sulfur dioxide radical metabolite may be produced under aerobic conditions, but subsequently react with oxygen. The air oxidation of this free radical (to form superoxide and regenerate (bi)sulfite) occurs with a rate constant of $4.1 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$ (65). Moreover, since rat hepatic microsomes in the presence of NADPH will reduce molecular oxygen to superoxide, (bi)sulfite and oxygen may be reduced at the same site(s) (66,67). If oxygen is the better electron acceptor, it could prevent the reduction of (bi)sulfite by competitive inhibition (51). Therefore, as a conclusion, these findings indicate that the combination of SO₂ and HBO can reduce lipid peroxidation and oxidant damage in erythrocytes induced by free O₂ radicals or at least, this combination does not cause an additional radical damage.

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