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Hyperbaric oxygenation improve red blood cell deformability in patients with acute or chronic inflammation

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ABSTRACT

Introduction: Red blood cells (RBC) are one of the key elements of the microcirculation. Their ability to pass through capillaries and to deliver oxygen to cells is due to their large degree of deformability linked to the characteristics of the RBC membrane. Alterations in RBC deformability as a result of membrane damage, linked in part to increased synthesis of reactive oxygen species (ROS), can be observed in several diseases, such as sepsis, and may contribute to the altered microcirculation observed in these pathologies. Hyperbaric oxygen therapy (HBOT), with inhalation of 100 % oxygen, has been proposed in several acute or chronic pathologies, including carbon monoxide poisoning.

Objective: We investigated the effects of HBOT on oxidative stress from ROS produced by myeloperoxidase (MPO) and on RBC deformability in patients with acute or chronic inflammation (n = 10), in patients with acute carbon monoxide poisoning (n = 10), and in healthy volunteers (n = 10).

Methods: RBC deformability was evaluated before and after HBOT in the various populations using the ektacytometry technique (Laser-assisted Optical Rotational Red Cell Analyzer – LORRCA). Deformability was determined by the elongation index (EI) in relation to the shear stress (SS) over a range of 0.3 to 50 Pa. Oxidative stress was estimated through changes in proteins (chlorotyrosine and homocitrulline) induced by MPO activity measured by liquid chromatography-tandem mass spectrometry analysis.

Results: Before HBOT, EI was significantly lower in patients with acute or chronic inflammation than in healthy volunteers and patients with acute carbon monoxide poisoning for the majority of SS values studied. After one session of HBOT, the EI was significantly higher than before HBOT for SS values of 1.93 Pa or higher in patients with acute or chronic inflammation. This effect remains constant after 10 sessions. There were no differences before and after HBOT in protein or amino acid oxidation due to ROS generation mediated by MPO in the three populations.

Conclusions: Our results confirm altered RBC deformability in patients with acute and chronic conditions associated with an underlying inflammatory process. HBOT improves deformability only after one session and therefore may improve microcirculation in this population. According to our results, this improvement does not seem mediated by the ROS pathway via MPO. These results need to be confirmed in a larger population.

1. Introduction

Red blood cells (RBC), as the only oxygen (O_2) carrying cells, are important elements of the microcirculation (Mohandas and Chasis, 1993). Alterations in RBC rheology (shape, aggregability and deformability) are observed in several diseases, both chronic (e.g., diabetes mellitus, coronary artery disease, heart failure or arterial hypertension), and acute (e.g., sepsis), and may contribute to the altered microcirculation observed in these conditions (Cho et al., 2008; Baskurt et al., 1998). We observed, in critically ill patients, a relationship between inflammation and altered RBC rheology (decreased deformability and increased aggregability) (Piagnerelli, 2009; De Backer et al., 2011),

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Table 1
Demographic and hemodynamic data of the study subjects at baseline.

	Healthy volunteers (n = 10)	CO poisoning (n = 10)	Acute or chronic inflammation (n = 10)	p-Value
Age (years)	31 {25–42}	52 {40–63}	53 {47–59}	0.016*ID
Men (%)	6 (60)	6 (60)	8 (80)	
BMI	22.7 {22.0–25.2}	24.1 {23.3–27.6}	24.6 {23.5–25.4}	0.25
HR (beat/min)	78 {70–84}	87 {68–102}	81 {71–100}	0.66
SBP (mm Hg)	128 {124–130}	131 {126–152}	134 {128–142}	0.16
DBP (mm Hg)	79 {64–84}	83 {75–95}	76 {62–86}	0.25
MBP (mm Hg)	89 {84–96}	98 {91–112}	96 {82–104}	0.10
SpO_2 (%)	99 {98–100}	99 {98–100}	99 {98–99}	0.66
$FiO_{2\ (\%)}$	21 {21–21}	100 (21–100}	21 {21–21}	0.006*CObis

Values are expressed in median (25-75th percentiles).
BMI = body mass index; HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure; SpO_2 = peripheral oxygen saturation; FiO_2 = fraction of inspired oxygen.
*ID = Patients with chronic and acute inflammatory disease versus healthy volunteers.
*CObis = Patients with CO poisoning versus healthy volunteers AND patients with acute and chronic disease.

Table 2
Biologic data at baseline.

	Healthy volunteers (n = 10)	CO poisoning (n = 10)	Acute and chronic inflammation (n = 10)	p-Value
Hb (g/dL)	14.4 {13.6–14.9}	14.1 {12.7–16.5}	14.9 {10.8–15.4}	0.91
Ht (%)	43.7 {41.4–46.3}	41.6 {39.8–49.7}	44.2 {32.9–47.2}	0.87
RDW (%)	12.6 {12.4–12.7}	13.3 {12.8–13.7}	12.9 {12.6–14.5}	0.1
MCV (fl)	90 {89–93}	90 {88–93}	90 {88–93}	0.92
RBC (10^6/mm^3)	4.77 {4.54–5.22}	5.11 {4.53–5.34}	4.85 {3.55–5.30}	0.81
ESR (mm/h)	4 {3–5}	14 {8–27}	5 {3–11}	0.02*CO
Platelets (10^3/mm^3)	280 {232–358}	237 {209–287}	227 {181–267}	0.31
WBC (10^3/mm^3)	6.98 {6.26–8.26}	10.39 {7.72–14.19}	9.64 {7.10–12.25}	0.14
SS ½ (Pa)	1.139 ± 0.336	1.137 ± 0.469	1.548 ± 0.660	0.07
EI_{Max}	0.670 ± 0.118	0.660 ± 0.015	0.641 ± 0.033	0.034*ID

Values were expressed in median (25-75th percentiles) or in mean ± standard deviation.
Hb = hemoglobin; Ht = hematocrit; RDW = red cell distribution width; MCV = mean corpuscular volume; RBC = red blood cell count; ESR = sedimentation rate, WBC = leucocyte count, SS ½ = shear stress required for half-maximal deformation, EI_{max} = elongation index-maximum.
*CO = CO poisoning versus healthy volunteers.
*ID = Acute and chronic inflammatory diseases versus healthy volunteers.

Table 2.A
Biological data before and after HBOT: Healthy volunteers.

	Healthy volunteers before HBOT (n = 10)	Healthy volunteers after HBOT (n = 10)	p-Value
Hb (g/dL)	14.4 {13.6–14.9}	13.6 {13.4–15}	0.06
Hct (%)	43.7 {41.4–46.3}	41.45 {40.5–45.9}	0.06
RDW (%)	12.6 {12.4–12.7}	12.6 {12.3–12.9}	0.12
MCV (fl)	90 {89–93}	91 {90–92}	0.92
RBC (10^6/mm^3)	4.77 {4.54–5.22}	4.66 {4.53–4.79}	0.039*
ESR (mm/h)	4 {3–5}	4 {3–5}	0.31
Platelets (10^3/mm^3)	280 {232–358}	299 {241–363}	0.28
WBC (10^3/mm^3)	6.98 {6.26–8.26}	8.82 {6.75–11.27}	0.06
SS ½ (Pa)	1.139 ± 0.336	1.183 ± 0.318	0.67
EI_{Max}	0.670 ± 0.118	0.667 ± 0.0181	0.69

Values were expressed in median (25-75th percentiles) or in mean ± standard deviation.
* = RBC in healthy volunteers before and after HBOT.
Hb = hemoglobin; Ht = hematocrit; RDW = red cell distribution width; MCV = mean corpuscular volume; RBC = red blood cell count; ESR = sedimentation rate, WBC = leucocyte count, SS ½ = shear stress required for half-maximal deformation, EI_{max} = elongation index-maximum.

explained in part by modifications of membrane carbohydrate content (Piagnerelli et al., 2009; Serroukh et al., 2012).

Several enzymes are involved in the destruction of the pathogen, including myeloperoxidase (MPO), an enzyme that produces reactive oxygen species (ROS) which is secreted into the extracellular space following the degranulation of neutrophils (Poret et al., n.d.; Khan et al., 2018). MPO binds to red blood cell membrane proteins, specifically band 3 and glycophorins A and B, resulting in altered red blood cell rheology with less deformable MPO-treated red blood cells (Poret et al., n.d.).

Hyperbaric oxygen therapy (HBOT) is proposed for the treatment of various pathological conditions, including carbon monoxide (CO) poisoning, open fractures with crush injury, decompression illness, gas embolism, sudden deafness, soft tissue radionecrosis (cystitis, proctitis), osteoradionecrosis, and in sepsis associated with anaerobic or mixed bacterial infections (Niinikoski et al., 2007). HBOT acts by delivering 100 % O_2 at a pressure >1 absolute atmosphere (ATA) in a hyperbaric chamber. As expected, because of this quantity of O_2, studies have shown that HBOT also increases the synthesis of ROS, which can cause cellular damage (Narkowicz et al., 1993; Benedetti et al., 2004).

We compared the effects of HBOT session on RBC deformability, as assessed using the laser-assisted optical rotational cell analyzer (Lorrca), and on oxidative stress mediated by MPO in patients with acute and chronic conditions associated with an underlying inflammatory process, in patients with CO poisoning, and in healthy volunteers. We hypothesized that RBC deformability was improved by HBOT session in patients with CO poisoning or with acute or chronic inflammatory process.

2. Material and methods

2.1. Study population

After agreement from the local and central institutional ethics committees (ISPPC OM 008, P 17/23_29/03), this prospective interventional study was performed over a 5-month period in the hyperbaric medicine center at CHU-Charleroi, André Vésale Hospital, Montigny-le-Tilleul, Belgium.

All adult (≥18 years) patients who required HBOT for CO poisoning or for acute or chronic inflammation, as assessed by the physician in charge, were considered for inclusion.

Pregnant women, epileptic patients, patients with chronic obstructive pulmonary disease (COPD) GOLD III or IV, patients with hematologic disease (leukemia, lymphoma and/or erythrocytic diseases), and patients being treated with erythropoietin were excluded. We also

Table 2.B
Biological data before and after HBOT: Carbon monoxide poisoning.

	Carbon monoxide poisoning before HBOT (n = 10)	Carbon monoxide poisoning after HBOT (n = 9)	p-Value
Hb (g/dL)	14.1 {12.7–16.5}	13.7 {13.2–17.2}	0.7
Hct (%)	41.6 {39.8–49.7}	42.6 {40.7–50.5}	0.8
RDW (%)	13.3 {12.8–13.7}	13.2 {12.7–13.9}	0.38
MCV (fl)	90 {88–93}	90 {82–93}	0.38
RBC (10^6/mm^3)	5.11 {4.53–5.34}	5.04 {4.59–5.64}	0.9
ESR (mm/h)	14 {8–27}	/[a]	
Platelets (10^3/mm^3)	237 {209–287}	240 {213–279}	0.3
WBC (10^3/mm^3)	10.39 {7.72–14.19}	8.53 {7.23–13.21}	0.47
HbCO (% total Hb)	13.2 {10.3–22.7}	4.6 {4.4–5.9}	0.004*
SS ½ (Pa)	1.137 ± 0.469	1.364 ± 0.477	0.8
EI_{Max}	0.660 ± 0.015	0.658 ± 0.015	0.59

Values were expressed in median (25-75th percentiles) or in mean ± standard deviation.
* = HbCO in patient with carbon monoxide poisoning before and after HBOT.
Hb = hemoglobin; Ht = hematocrit; RDW = red cell distribution width; MCV = mean corpuscular volume; RBC = red blood cell count; ESR = sedimentation rate, WBC = leucocyte count, SS ½ = shear stress required for half-maximal deformation, EI_{max} = elongation index-maximum.
[a] Missing data.

Table 2.C
Biological data before and after 10 sessions HBOT: Acute or chronic inflammation.

	Chronic disease before HBOT (n = 10)	Chronic disease after HBOT (n = 9)	p-Value
Hb (g/dL)	14.9 {10.8–15.4}	14.8 {10.6–15.4}	0.65
Hct (%)	44.2 {32.9–47.2}	43.4 {32.1–45.4}	0.43
RDW (%)	12.9 {12.6–14.5}	12.9 {12.6–14.5}	1
MCV (fl)	90 {88–93}	91 {88–92}	0.63
RBC (10^6/mm^3)	4.85 {3.55–5.3}	4.73 {3.48–5.21}	0.43
ESR (mm/h)	5 {3–11}	5 {3–25}	0.09
Platelets (10^3/mm^3)	227 {181–267}	228 {209–294}	0.85
WBC (10^3/mm^3)	9.64 {7.1–12.25}	9.75 {7.44–12.26}	0.9
SS ½ (Pa)	1.548 ± 0.660	1.563 ± 0.590	0.79
EI_{Max}	0.641 ± 0.033	0.652 ± 0.0239	0.78

Values were expressed in median (25-75th percentiles) or in mean ± standard deviation.
Hb = hemoglobin; Ht = hematocrit; RDW = red cell distribution width; MCV = mean corpuscular volume; RBC = red blood cell count; ESR = sedimentation rate, WBC = leucocyte count, SS ½ = shear stress required for half-maximal deformation, EI_{max} = elongation index-maximum.

enrolled 10 healthy volunteers as part of the paramedical staff accompanying the patients during the HBOT sessions. All subjects provided written consent before participating in the study.

2.2. Data collection

Demographic data (age, sex, body mass index) were collected on inclusion. Hemodynamic (heart rate, arterial blood pressure) and oxygenation (fraction of inspired oxygen (FiO_2) and peripheral oxygen saturation (SpO_2)) variables were recorded before and after the HBOT session. For the patients with acute or chronic inflammation who needed several sessions of HBOT, measurements were taken before and after the first session of HBOT and the 10th session.

The following biological values were recorded before and after the HBOT in all subjects: hemoglobin, hematocrit, RBC count, mean corpuscular volume, RBC distribution width (RDW), erythrocyte sedimentation rate (ESR), leukocyte count. For patients with acute CO poisoning, carboxy-hemoglobin (HbCO) measurements were measured and recorded before and after the HBOT.

2.3. RBC deformability

RBC deformability as assessed using ektacytometry (LORRCA; Mechatronics Instrumeents BV, AN Zwaag, Netherlands). We used the same analytical method as that used by Piagnerelli et al. (2022): a suspension of 5 microliters (μL) of whole blood was mixed with 1000 μL of polyvinylpyrrolidone 360 solution, an isotonic viscous medium (PVP, 4 %; MW 360 kDa; viscosity 30 ± 2 mPa·s), to obtain a final solution with a constant hematocrit of 0,2 %. Using a Couette system composed of glass cup and a precisely fitting bob, with a gap of 0.36 mm between the cylinders, the liquid solution was sheared and illuminated by a laser beam in order to obtain a diffraction pattern produced by the deformed cells. This diffraction was analyzed by a computer, which also controls the cup rotational speed and the predetermined shear stresses. The elongation index (EI) is calculated as: EI = (L − W) / (L + W), where L and W are the length and width of the diffraction pattern, respectively. The diffraction pattern is circular for cells at rest and elliptical for deforming RBC. We calculated EI for different values of shear stress (SS) up to 50 Pa.

From the SS-response curves of shape change, we calculated the maximal RBC elongation (EI max) and the shear stress required for one-half of this maximal deformation (SS ½). The curves are presented in logarithmic scales (Condon et al., 2003).

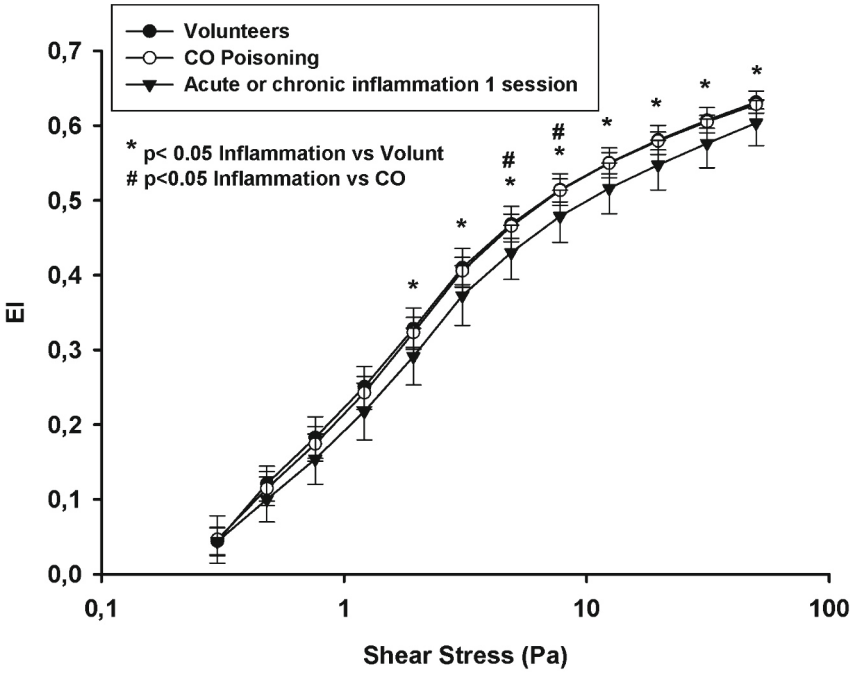
2.4. Hyperbaric oxygen therapy

The HBOT (Hyperbaric Oxygen Treatment Systems, IHC Hytech, type HYOT 2200/12/2/FD, Raamsdonksveer, the Netherlands) sessions were administered in a twelve place hyperbaric chamber located at the Department of Hyperbaric Medicine at CHU-Charleroi, André Vésale Hospital, Belgium. Each HBOT session lasted 120 min., as is routine in our center, and was conducted under medical supervision. For each session, there was a 15-min compression phase. The subjects then breathed 100 % O_2 at a pressure of 2.5 ATA (absolute atmosphere) for 90 min. Finally, there was a 15-min decompression phase.

2.5. Oxidative stress measurement

To evaluate oxidative stress in plasma before and after the first session of HBOT, we assessed specific and non-specific modification of proteins produced by MPO activity, notably chlorothyrosine (Cl-Tyr) and homocitrulline (Hcit). Cl-Tyr is a specific product from the oxidation of tyrosine (Tyr) by MPO (only MPO can induce Cl-Tyr production in humans) and Hcit is produced by the reaction of lysine (Lys) residues and cyanate resulting from urea decomposition and thiocyanate oxidation by MPO (Delporte et al., 2012).

We assessed protein-bound Cl-Tyr and Hcit using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The full method has been published elsewhere (Delporte et al., 2012) but, briefly, to perform the acid hydrolysis, 20 μL of plasma were placed in a 3 mL hydrolysed quartz vial. Ten μL of internal standard ([^{13}C, ^{15}N] lysine 3.4 μM and [^{13}C9] tyrosine 34 μM, final concentration) and 200 μL HCl 6 N supplemented with phenol 0.05 % were added to the vial. Acid hydrolysis was carried out by heating to 110 °C for 30 min. Free amino acids were then derivatized with butanolic-HCl to butyric esters. Rapid liquid chromatography separation and quantification of amino acids was then performed to determine the ratios of Hcit/Lys and Cl-tyr/Tyr, which indicate the enzymatic activity of MPO.



Shear stress (Pa)	Healthy volunteers (n=10)	CO poisoning (n=10)	Acute and Chronic diseases (n=10)	p-value
SS 0.3	0.044 ± 0.018	0.047 ± 0.032	0.044 ± 0.019	0.96
SS 0.48	0.121 ± 0.023	0.115 ± 0.023	0.100 ± 0.030	0.19
SS 0.76	0.183 ± 0.028	0.174 ± 0.023	0.203 ± 0.164	0.79
SS 1.21	0.251 ± 0.027	0.243 ± 0.022	0.218 ± 0.033 *	0.049
SS 1.93	0.329 ± 0.023	0.323 ± 0.020	0.291 ± 0.038 *#	0.02
SS 3.07	0.410 ± 0.026	0.406 ± 0.018	0.373 ± 0.040 *#	0.02
SS 4.89	0.468 ± 0.024	0.465 ± 0.016	0.431 ± 0.036 *#	0.006
SS 7.78	0.514 ± 0.021	0.513 ± 0.015	0.479 ± 0.035 *#	0.005
SS 12.39	0.550 ± 0.020	0.550 ± 0.014	0.516 ± 0.03 *#	0.005
SS 19.72	0.581 ± 0.019	0.579 ± 0.012	0.547 ± 0.033	0.005
SS 31.4	0.607 ± 0.017	0.605 ± 0.009	0.576 ± 0.033 *#	0.006
SS 50	0.631 ± 0.015	0.628 ± 0.006	0.603 ± 0.010 *#	0.007

Values were expressed in mean ± standard deviation

* = Patients with acute disease versus healthy volunteers
# = Patients with Acute disease versus healthy volunteers and CO poisoning

Fig. 1. RBC deformability, as assessed using the elongation index, before HBOT.

2.6. Statistical analysis

IBM SPSS (Statistical Package for Social Sciences) was used for the statistical analyses. Results are presented as mean ± SD or as median values with 25-75th interquartile range.

A Kruskal Wallis with Tukey test was used for comparison of data. A Wilcoxon Signed Rank test or *t*-test was used for pairwise comparisons. A value of $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Study population

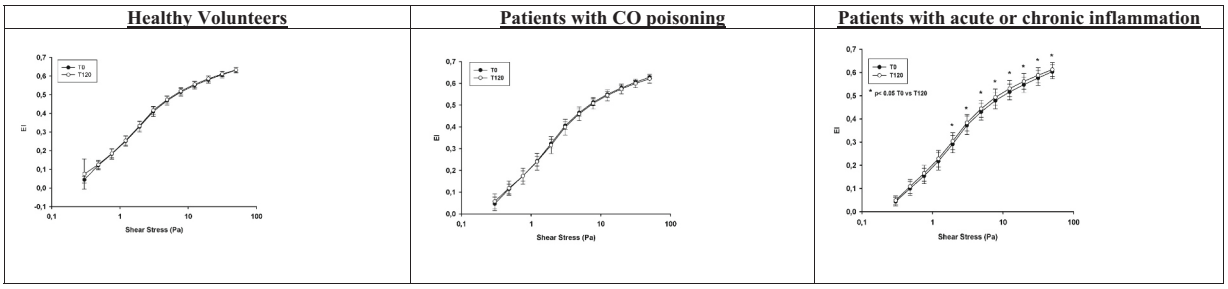
During the study period, data were collected from 10 healthy volunteers, 10 patients with CO poisoning, and 10 patients with an acute or chronic inflammatory disease (overall age range 25 to 88 years) for the first HBOT session. Nine patients were investigated in this group for 10 sessions. The patients with inflammatory conditions were prescribed HBOT for acute inflammatory diseases: necrotizing fasciitis (n = 2), gangrene (n = 1), and chronic inflammatory diseases: progressive or sudden deafness (n = 4), spinal cord decompression (n = 1), tinnitus (n = 1), and radiation proctitis (n = 1). In the group with acute CO poisoning, 1 patient with cognitive disorders did not receive the complete HBOT treatment because of anxiety. All patients survived until the end of treatment except 1 in the group with acute inflammatory disease (necrotizing fasciitis).

Demographic and hemodynamic data are shown in Table 1. FiO_2 before HBOT was significantly higher in the CO poisoned patients. Hemodynamic variables did not change during the HBOT in any of the patients.

3.2. Biological data

Laboratory data at baseline are shown in Table 2. ESR was significantly higher in patients with CO intoxication than in the other patients and the healthy subjects.

The RBC count decreased significantly after HBOT therapy in healthy volunteers, from 4.77 [4.54–5.22] to 4.66 [4.53–4.79] $\times 10^6/mm^3$, p = 0.039. There were no significant differences in any of the other variables before and after HBOT, HbCO in patients with CO poisoning decreased



Shear stress (Pa)	Before HBOT (n=10)	Ater HBOT (n=10)	p-value	Before HBOT (n=10)	After HBOT (n=9)	p-value	Before HBOT (n=10)	After HBOT (n=10)	p-value
SS 0.3	0.044 ± 0.018	0.075 ± 0.081	0.27	0.047 ± 0.032	0.058 ± 0.035	0.63	0.044 ± 0.019	0.049 ± 0.019	0.32
SS 0.48	0.121 ± 0.023	0.126 ± 0.023	0.18	0.115 ± 0.023	0.119 ± 0.032	0.91	0.100 ± 0.030	0.109 ± 0.030	0.07
SS 0.76	0.183 ± 0.028	0.185 ± 0.024	0.71	0.174 ± 0.023	0.174 ± 0.037	0.86	0.153 ± 0.034	0.165 ± 0.035	0.03
SS 1.21	0.251 ± 0.027	0.254 ± 0.025	0.55	0.243 ± 0.022	0.240 ± 0.039	0.76	0.218 ± 0.038	0.228 ± 0.036	0.06
SS 1.93	0.329 ± 0.028	0.333 ± 0.024	0.46	0.323 ± 0.020	0.316 ± 0.039	0.56	0.291 ± 0.038	0.304 ± 0.037	0.02
SS 3.07	0.410 ± 0.026	0.416 ± 0.022	0.31	0.406 ± 0.018	0.399 ± 0.036	0.60	0.373 ± 0.040	0.383 ± 0.036	0.05
SS 4.89	0.468 ± 0.024	0.474 ± 0.020	0.32	0.465 ± 0.016	0.460 ± 0.032	0.58	0.431 ± 0.036	0.444 ± 0.011	0.01*
SS 7.78	0.514 ± 0.021	0.520 ± 0.018	0.27	0.513 ± 0.015	0.508 ± 0.027	0.56	0.479 ± 0.035	0.493 ± 0.035	0.005*
SS 12.39	0.550 ± 0.020	0.556 ± 0.017	0.25	0.550 ± 0.014	0.545 ± 0.025	0.56	0.516 ± 0.034	0.530 ± 0.035	0.002*
SS 19.72	0.581 ± 0.019	0.586 ± 0.015	0.27	0.579 ± 0.012	0.574 ± 0.022	0.51	0.547 ± 0.033	0.562 ± 0.034	0.001*
SS 31.4	0.607 ± 0.017	0.611 ± 0.014	0.30	0.605 ± 0.008	0.599 ± 0.020	0.44	0.576 ± 0.033	0.588 ± 0.032	0.001*
SS 50	0.631 ± 0.015	0.633 ± 0.014	070	0.628 ± 0.006	0.621 ± 0.020	0.41	0.603 ± 0.030	0.612 ± 0.031	0.01*

Values were expressed in mean ± standard deviation

Fig. 2. RBC deformability, as assessed using the elongation index, before and after one session of hyperbaric oxygen therapy (HBOT).

from 13.2 [10.3–22.7] to 4.6 [4.4–5.9] % of total Hb; p = 0.004 (Table 2.A.; Table 2.B; Table 2.C)

3.3. LORRCA measurements

At baseline, EI was significantly lower in patients with acute or chronic inflammatory conditions compared to healthy volunteers and patients with acute CO poisoning for the majority of SS values studied. EI was significantly lower in patients with acute CO poisoning than in healthy volunteers at SS values of 4.89 and 7.78 Pa (Fig. 1).

For healthy volunteers and patients with acute CO poisoning, the EI did not change significantly after one session of HBOT (Fig. 2).

For patients with acute or chronic conditions, the EI had increased for the majority of SS values ≥ 1.93 Pa after one session of HBOT (Fig. 2). No significant modifications compare to baseline were observed after 10 sessions (Fig. 3).

3.4. Oxidative stress measurements

Oxidative stress measurements were comparable at baseline across the three groups and did not change significantly after the first session of HBOT (Table 3).

4. Discussion

HBOT has been approved for various pathologies like air or gas embolism, CO poisoning, gangrene and necrotizing soft tissue infections, crush injury, decompression sickness, delayed radiation injury, problem wounds or idiopathic sudden hearing loss (Mathieu, 2006; Löndahl, 2012; Shandley et al., 2012; Parra et al., 2015; Chen et al., 2014; Ortega et al., 2021). It is a therapy in which patients breathe 100 % O_2 at a pressure >1 ATA. However, the effect of these high O_2 concentrations on hemorheological parameters has been poorly studied.

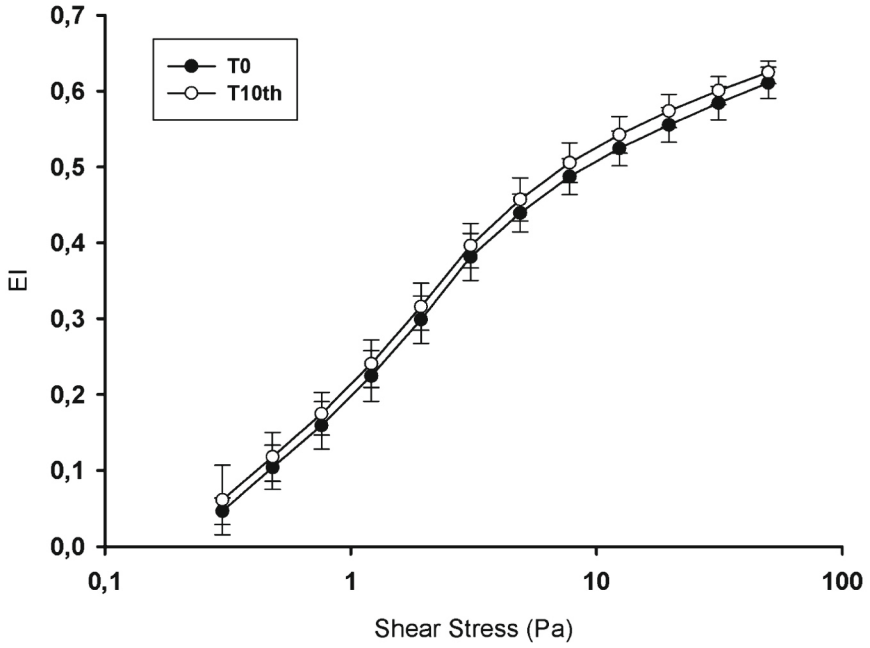
In our study, the EI, which reflects the deformability of the RBC, significantly lower at baseline from patients with acute or chronic inflammation than in those with CO poisoning or in healthy volunteers, already from low SS (≥1.93 Pa) values. Impaired RBC deformability has already been observed in patients with coronary artery disease, heart failure, hypertension, diabetes mellitus, and sepsis (Cho et al., 2008; Baskurt et al., 1998).

HBOT significantly increased RBC deformability for the majority of SS values ≥ 1.93 Pa in patients with acute or chronic conditions after one session but not with the 10th session which took place, on average, after 10 days after the first one. These observations seem congruent with those from a study by Sinan et al. (2016), which showed no changes in hemorheological parameters after 20 sessions of HBOT taking place in at least 26 days after the first one in 33 patients with various disorders such as diabetic foot ulcers (n = 11), osteomyelitis (n = 8), avascular necrosis (n = 4), acute lost hearing (n = 2), unhealed wounds (n = 2) or others causes (n = 6). The reason for these differences is unclear but we find that the delay between the first and the last session of HBOT is higher in the study of Sinan et al. (2016). Perhaps, no improvements were observed in this study due to more severe alterations of the RBC membrane. We also have included more acute patients (gangrene, fasciitis) and improvement of the RBC deformability could be related to the improvement of the inflammation in these particular patients (Sinan et al., 2016).

In a study in trauma patients, Langenfeld et al. (1991) showed that alterations in deformability, as assessed using a RBC deformability index, were present on admission. In patients who later developed an infection, the deformability index decreased further, an average of 4 days before the first signs of infection. A decrease in RBC deformability may therefore be an early predictor of worse prognosis. This same effect is possibly also seen in our study where the inflammatory marker like white blood cell count was not elevated in patients with inflammatory disease at baseline before HBOT while the deformability index was lowered.

Contrary to Gunes and Aktas (2017) who showed no modification on complete blood count following HBOT, our study reports a reduction in RBC count in healthy volunteers after 1 session of HBOT. The reason of our results remains unclear. The contrasting results are probably explained by the number of participants and the number of HBOT sessions in the study of Gunes and Aktas (2017): 140 patients who received >60 chamber sessions.

Acute CO exposure did not alter erythrocyte deformability. This result is in agreement with that of Neslihan and Nurten (2010) who observed that chronic low-level CO exposure did not alter erythrocyte deformability in humans. By contrast, Ozturk et al. reported decreased



Shear stress (Pa)	Before HBOT (n=10)	After 10 sessions HBOT (n=9)	p-value
SS 0.3	0.044 ± 0.019	0.061 + (0.066	0.45
SS 0.48	0.100 ± 0.030	0.118 + (0.032	0.59
SS 0.76	0.153 ± 0.034	0.175 ± 0.028	0.65
SS 1.21	0.218 ± 0.038	0.240 ± 0.031	0.58
SS 1.93	0.291 ± 0.038	0.316 ± 0.031	0.65
SS 3.07	0.373 ± 0.040	0.396 ± 0.029	0.65
SS 4.89	0.431 ± 0.036	0.457 ± 0.028	0.65
SS 7.78	0.479 ± 0.035	0.506 ± 0.026	0.65
SS 12.39	0.516 ± 0.034	0.542 ± 0.024	0.66
SS 19.72	0.547 ± 0.033	0.574 ± 0.022	0.63
SS 31.4	0.576 ± 0.033	0.600 ± 0.018	0.60
SS 50	0.603 ± 0.030	0.625 ± 0.015	0.54

Values are expressed in mean ± Standard deviation

Fig. 3. RBC deformability, as assessed using the elongation index, before and after ten session of hyperbaric oxygen therapy (HBOT) in patients with acute or chronic inflammation.

Table 3
Oxidative stress measurements before and after hyperbaric oxygen therapy (HBOT).

	Healthy volunteers - before HBOT (n = 10)	Healthy volunteers - after HBOT (n = 10)	CO poisoning - before HBOT (n = 10)	CO poisoning - after HBOT (n = 9)	Acute or chronic inflammation -before HBOT (n = 10)	Acute or chronic inflammation -after 10sessions HBOT (n = 9)
Hcit/ Lys	56.3 {54.3–57.8} × 10^5	56.6 {54.4–57.8} × 10^5	55.6 {53.2–65.6} × 10^5	55 {54–61.3} × 10^5	55.5 {51–60.7} × 10^5	53.9{52.4–60.8} × 10^5
Cl-tyr/ Tyr	6.43 {6.21–6.77} × 10^5	6.55 {6.22–6.98} × 10^5	7.18 {6.45–8.65} × 10^5	6.97 {6.35–7.72} × 10^5	7.28 {6.9–8.25} × 10^5	7.07 {6.34–9.28} × 10^5

Values are expressed as median (25-75th percentiles).
Hcit = homocitrulline; Lys = lysine; Cl-thyr = chlorotyrosine; Tyr = tyrosine.

deformability after acute exposure to CO in humans (Ozturk et al., 2016). The contrasting findings may be explained by dose and/or time dependency. In our study, the mean HbCO level at the time of arrival in the hospital was 13.2 % (10.3 %–22.7 %), but the duration of acute CO exposure was not recorded. In the study by Ozturk et al. (2016), with a larger population, the mean CO level at admission was 25 ± 10 % with a mean exposure duration of 4 h 7 min ± 2 h 29 min (1–8 h). In animal models, Shperling et al. (2008) also reported a decrease in RBC deformability in rats, which was most evident at the end of the first day after a single exposure to CO, whereas Bor-Kucukatay et al. (2010) observed higher deformability indices at SS levels ≤ 5.33 Pa immediately after a single toxic dose of CO. Rapid improvement in RBC deformability may be interpreted as a defense mechanism in order to maintain oxygenation, despite the CO-linked erythrocytes with limited O_2 transport.

The mechanical properties of RBCs and their membrane are different in different species, which explains these different ektacytometer results between humans and animals. At the lowest SS value employed (0.5 Pa), rat RBCs had a higher EI index than human RBCs and maximal deformation was observed at lower SS for RBCs rat than humans (Baskurt et al., 1997).

HBOT is used to increase local oxygenation, which facilitates the

activation of phagocytes. Several studies have focused on the oxidative effects of HBOT, most showing that HBOT leads to an increase in ROS in the blood (Matsunami et al., 2010; Oter et al., 2005; Deby-Dupont et al., 2002). We only studied the damage caused by ROS linked to systemic MPO activity. We did not explore the different pathways of ROS production (Forrester et al., 2018; Huet and Duranteau, 2008). MPO, a lysosomal enzyme produced mainly from polynuclear neutrophils, is released into the extracellular fluid after oxidative stress and various inflammatory responses. An increased level of systemic MPO activity is one of the diagnostic biomarkers for inflammatory and oxidative stress (Poret et al., n.d.; Khan et al., 2018).

Unexpectedly, in the group with acute or chronic inflammation, oxidation mediated by the MPO pathway was comparable with that in healthy volunteers before HBOT. However, in this group, the EI was significantly lower for the majority of SS values studied compared with both the other groups. This mismatch between RBC deformability and MPO activity suggests that RBC deformability is likely dependent on another ROS pathway or another mechanism. Other explanations are that we measured the systemic MPO activity and not the MPO binding on RBCs. Indeed, Gorudko et al. (2016) showed that MPO could bind to skeletal membrane proteins (especially band 3, glycophorins A and B) and alter RBC deformability. This is even more altered when the sialic acid membrane content is reduced. What we have already demonstrated on the RBCs of patients with acute inflammation such as in sepsis (Piagnerelli et al., 2009; Klebanoff, 2005). Other possible explanation is that we have only studied one pathway of the complex physiopathology of ROS. Here, linked to MPO activity and not all of the pathways and the oxidant-antioxidant status of the patient, nor these effects on RBC deformability (Harabin et al., 1990). To confirm these hypotheses, it might be interesting in the future to measure other rheological parameters such as RBC aggregation, the activity of RBC anti-oxidant defenses and/or RBC biochemistry as other compounds of the microcirculation like glycocalyx (Piagnerelli et al., 2009; Reggiori et al., 2009; Mohanty et al., 2014; Lipowsky, 2019).

After a two-hour HBOT treatment period with compression and decompression phases each of 15 min, MPO activity did not change significantly in any of our groups. The difference in the results of the present study compared to those of previous studies on HBOT and MPO (Matsunami et al., 2010; Oter et al., 2005) may be due to the continuous exposure to HBOT without compression and decompression phases during the 2 hour-session in those studies. In this context, ROS levels may decrease to baseline levels within 10 min of stopping HBO exposure (Narkowicz et al., 1993). MPO can be released outside the phagocytic cells, increasing the potential for damage of an extracellular target. A major obstacle to the production of distant toxicity by ROS is the presence of scavengers, proteins such as albumin as well as a number of reducing agents, in the intermediate fluid. These react quickly with the highly reactive products of the MPO system and prevent them from reaching a sensitive target of biological importance (Piagnerelli et al., 2003). In addition, discontinuous HBOT sessions (with air inhalation sequences) have been shown to stimulate the activity of anti-oxidant enzymes (Bateman et al., 2017).

RBC rheology can be influenced by many other factors that were not studied in the present study, including alterations in intracellular calcium and adenosine triphosphate (ATP) concentrations, effects of nitric oxide, effects of temperature variation, decrease in some RBC membrane components, such as sialic acid, and an increase in others, such as 2,3 diphosphoglycerate (Klebanoff, 2005).

. This non-interventional, prospective study has several limitations. First, the number of patients included is small, but despite this, several parameters approach significance. Increasing the number of patients would probably confirm these results. Second, this was a single center study and the decision to treat with HBOT was left to the attending physician. Third, the healthy volunteers in the control group were younger than the patients with acute CO poisoning or with acute or chronic inflammatory conditions. Four, we measured only systemic MPO activity and not MPO binding on RBCs. This measurement could perhaps explain the evolution of RBC deformability. Five, we investigated only RBC deformability and not really the roles of the cycle deoxygenation-oxygenation on RBC deformability. This cycle are known to affect RBC deformability mostly due to conformational state of the hemoglobin molecule which determines the interactions with the cytoskeletal proteins (Ugurel et al., 2020). New technique like ektacytometry with oxygen gradient could improve the understanding of our study.

5. Conclusion

Our results confirmed the altered deformability of RBCs in patients with acute and chronic conditions associated with an underlying inflammatory process. HBOT improves, at least temporarily RBC deformability and may therefore improve the microcirculation in this population. This potential link needs to be studied in a larger population. The results suggest that HBOT does not increase systemic oxidative stress mediated by the MPO pathway.

CRediT authorship contribution statement

Françoise Steenebruggen: Investigation, Data curation, Writing – original draft, Visualization. **Daniel Jacobs:** Resources. **Cédric Delporte:** Validation, Resources. **Pierre Van Antwerpen:** Validation, Resources. **Karim Zouaoui Boudjeltia:** Software, Validation, Resources. **Patrick Biston:** Project administration. **Michael Piagnerelli:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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