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The effects of increased inspired oxygen with and without dopamine on lung and diaphragm hydrogen peroxide and apoptosis following hemorrhagic shock

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Abstract: During resuscitation of hemorrhagic shock (HS), clinicians employ high fractions of inspired oxygen (FIO_2) to restore maximal oxygen (O_2) saturations. Studies indicate that increased FIO_2 can be detrimental to cellular function. Our purpose was to determine the FIO_2 with and without dopamine (DA) that minimizes hydrogen peroxide (H_2O_2) production and apoptosis in lung and diaphragm following HS. Sprague-Dawley rats were randomized to FIO_2 groups: 0.21, 0.40, 0.60 and 1.0. Controlled HS was elicited by reducing mean arterial pressure to approx. 40 mm Hg. The rats were treated with various FIO_2s, with and without DA infusion (10 mcg/kg/min). Hydrogen peroxide was measured using dihydrofluorescein diacetate. Apoptosis was determined based on nuclear differential dye up-take. Compared to 0.21, lung and diaphragm H_2O_2 and apoptosis were significantly reduced in the 0.40 and 0.60 groups. At an FIO_2 of 1.0, H_2O_2 and apoptosis were greater than at 0.21. With the exception of an FIO_2 of 0.40, infusing DA with various FIO_2s resulted in H_2O_2 and apoptosis being significantly decreased. These results indicate that lung and diaphragm H_2O_2 and apoptosis are affected by inspired O_2 and DA. Results indicate using an FIO_2 of 0.40, with or without DA, is most beneficial in attenuating tissue damage following HS.

Key words: reactive oxygen species, DNA damage, hemodynamics, acid-base balance, hyperoxia

INTRODUCTION

Hemorrhagic shock (HS) is the result of an acute loss of blood from the intravascular space, often a consequence of traumatic injury and the leading cause of death in civilian and military trauma patients [1]. During HS, there is decreased perfusion of vital organs which leads to inadequate delivery of oxygen (O_2) necessary for normal cell function [2]. In patients who experience HS, failure of compensatory mechanisms and hemodynamic instability, decreased oxygen delivery (DO_2), and O_2 utilization result in hypoxic injury [3]. This alteration in cellular metabolism generates an increase in reactive oxygen species (ROS) formation [4].

In order to restore and maintain adequate tissue oxygenation during HS, clinicians apply increased fractional inspired oxygen (FIO_2). This is an intervention adopted in Advanced Trauma Life Support (ATLS), American College of Surgeons course guidelines [5]. There are published data indicating that increased FIO_2 can be detrimental to tissues [6, 7]. Administering supplemental O_2 during HS increases tissue O_2, which may subsequently lead to increased amounts of ROS. This increased production of ROS can lead to lipid peroxidation, protein alterations and deoxyribonucleic acid (DNA) damage [8].

Dopamine (DA) is a pharmacological agent that is sometimes used in patients experiencing HS when isotonic crystalloid administration fails to enhance tissue perfusion. Administering DA can augment tissue oxygenation by increasing cardiac output and thus decreasing free radicals. In addition, DA has been shown to be a free radical scavenger [9, 10].

The objectives of this study were to determine the effects of different FIO_2s, without and with the administration of DA (10 mcg/kg/min), following HS on lung and diaphragm damage. Tissue damage was assessed by measuring H_2O_2, a precursor of the hydroxyl radical, and DNA damage, a component of apoptosis.

The Institutional Laboratory Animal Research Division at the University of Kansas Medical Center (KUMC) provided care of the animals. The animal care facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Animal care approval was obtained for this study (2008-1708).

MATERIALS AND METHODS

Experimental design and protocol. Male Sprague-Dawley rats (350-450 g) were used in these volume-controlled HS experiments. The animals were ordered in batches of 6 and allowed to acclimate for 48 h prior to experimentation. This study was an experimental design in which rats were randomized among 8 treatment groups.

The independent variables for these experiments were FIO_2 and DA. The dependent variables were lung and diaphragm H_2O_2 and apoptosis. Mean arterial blood pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), arterial blood gases (ABGs), hemoglobin (Hgb) and body temperature were monitored and recorded

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during the experiment. The rat's core body temperature was maintained at 36-37°C.

***In-vivo* experiments**. The rats were anesthetized with sodium pentobarbital (50 mg/kg body weight) administered intraperitoneally. Atropine (0.1 mg/100 g body weight) was administered intraperitoneally to reduce respiratory secretions. When a surgical plane of anesthesia was reached, the following procedures were performed. The trachea was exposed and cannulated using polyethylene (PE) 240 tubing. A PE 50 catheter was placed in the right carotid artery to monitor arterial pressures and HR. Blood pressures were continuously monitored and measured with an accuracy of $\pm$ 2 mm Hg. A second PE 50 catheter was inserted in the femoral artery for blood withdrawal eliciting HS. A third PE 50 catheter was inserted in the right external jugular vein for administration of DA (10 mcg/kg/min) following HS. The ABGs were measured using an I-STAT instrument which measured pH, partial pressure of carbon dioxide ($PaCO_2$), bicarbonate (HCO_3^-), base excess (Beecf), partial pressure of O_2 (PaO_2), O_2 Saturation (SaO_2) and Hgb.

Control arterial blood pressures (SBP, DBP and MAP), acid-base and Hgb measurements were obtained. HS was elicited by removing approximately 40% of the blood volume via the femoral artery over 30 min. During this time, all rats breathed ambient air (FIO_2 = 0.21). At the end of the HS period, hemodynamic and acid-base parameters were recorded and one of the 8 treatments (various FIO_2, without and with DA) was initiated. No fluids were administered as treatments prior to the use of O_2 and DA in this controlled HS experimental model. Sixty min later, the treatment was completed and the above parameters recorded.

The animal was euthanized with sodium pentobarbital (150 mg/kg body weight) and the lung and diaphragm were rapidly excised for H_2O_2 and apoptosis determination. The tissues were divided into two equal portions and immersed in a Krebs Ringer's (KR) solution. Both lungs and the entire diaphragm were used for H_2O_2 and apoptosis measurements.

Hydrogen peroxide measurements. After rinsing the lungs and diaphragm in KR solution, isolated lung and diaphragm strips were mounted in a paraffin dish and loaded with dihydrofluorescein diacetate (Hfluor-DA). This chemical is a probe that is oxidized to fluorescein (Fluor) by H_2O_2. After 30 min of loading with Hfluor-DA, the lung and diaphragm strips were rinsed in phosphated buffer solution for 10 min. Lung and diaphragm strips were stretched and mounted on slides for measurement of fluorescent intensity (expressed as $\times 10^6$) which is directly proportional to the amount of H_2O_2 in the tissue. Changes in fluorescence due to Fluor were measured using a laser scanning cytometer with a detection limit of 1 µM H_2O_2.

Apoptosis measurements. The lung and diaphragm were minced into small pieces and homogenized with a KR solution containing trypsin, collagenase and antioxidants. After 30 min homogenization at 37°C, the supernatant was removed and centrifuged for 30 min at 6,000 rpm. The pellet was resuspended in 2 mL of KR solution. A 250 µL aliquot was added to a tube containing 2 µL of ethidium bromide (EB) and 2 µL acridine orange (AO). After vortexing, 20 µL of the sample was placed on a slide and fluorescent microscopy performed. Differential nuclear dye uptake fluorescence microscopy was employed to measure deoxyribonucleic acid (DNA) damage, an index of apoptosis. The images were analyzed with Boyce Scientific Analysis® software. The software eliminates human error by assessing exact hue values for each nucleus, thus allowing for determination of relative amounts of each dye [11]. Approximately 300 lung and diaphragm nuclei were analyzed for apoptosis.

Statistical analysis. Data are presented as mean ± SEM. Differences within the FIO_2 only and FIO_2 + DA groups at control and shock were analyzed for identification of significant main and interaction effects by 2-way analysis of variance (ANOVA). Treatment differences within the FIO_2 only and FIO_2 + DA groups were analyzed by 1-way ANOVA followed by *post hoc* test (Fisher's LSD). Differences between FIO_2 only and FIO_2 + DA groups were analyzed similarly by 1-way ANOVA followed by *post hoc* test (Fisher's LSD). Significance was defined as $P < 0.05$. Statistical analyses were performed using SPSS software (version 17 for Windows®; Chicago, IL.).

RESULTS

Hemodynamics. The hemodynamic data are summarized in Tables 1 and 2. At control, there were no significant differences in SBP, DBP, MAP and HR among the 8 groups. While breathing room air, removal of 40% of the rat's blood volume resulted in significant decreases in all arterial blood pressures. The blood pressures at shock were not significantly different among all the groups. Consequently, the hemodynamic status of all rats was comparable at the onset of treatment. All statistical differences are with respect to FIO_2 at 0.21. In Table 1, increasing FIO_2 resulted in a significant increase in SBP at an FIO_2 of 0.60 ($P < 0.05$). Diastolic blood pressures and MAPs were significantly increased at all FIO_2s greater than 0.21. Varying FIO_2 had no significant effect on HR. In Table 2, administering DA and increasing FIO_2s resulted in a significant decrease in SBP at 0.60 ($P < 0.05$). Increasing FIO_2 had no significant effect on DBP and MAP. Again, HR was not significantly increased in any of the FIO_2 plus DA groups.

Table 1 Hemodynamics for the different fraction of inspired oxygen concentrations.

	0.21	0.40	0.60	1.0
SBP (mm Hg)				
Control	148 ± 4	162 ± 9	157 ± 7	167 ± 9
Shock	97 ± 4	83 ± 3	93 ± 5	94 ± 7
Treatment	93 ± 9	121 ± 14	133 ± 10*	126 ± 14
DBP (mm Hg)				
Control	107 ± 5	122 ± 7	115 ± 7	124 ± 7
Shock	43 ± 1	39 ± 2	38 ± 3	46 ± 3
Treatment	38 ± 9	71 ± 13*	76 ± 6*	76 ± 15*
MAP (mm Hg)				
Control	127 ± 5	141 ± 7	135 ± 7	143 ± 7
Shock	61 ± 2	54 ± 3	57 ± 3	63 ± 4
Treatment	56 ± 9	94 ± 15*	98 ± 7*	95 ± 16*
HR (b/min)				
Control	357 ± 24	358 ± 10	355 ± 15	334 ± 26
Shock	366 ± 9	326 ± 17	380 ± 11	311 ± 24
Treatment	392 ± 23	379 ± 18	403 ± 19	405 ± 17

Data are presented as mean ± SEM, n = 6. SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, HR: heart rate.
* $p < 0.05$ in comparison with $FIO_2 = 0.21$.

Table 2 Hemodynamics for the different fraction of inspired oxygen concentrations plus dopamine.

	0.21	0.40	0.60	1.0
SBP (mm Hg)				
Control	161 ± 3	153 ± 6	162 ± 9	155 ± 3
Shock	85 ± 4	86 ± 4	93 ± 6	78 ± 6
Treatment	157 ± 10	130 ± 12	122 ± 6*	133 ± 11
DBP (mm Hg)				
Control	118 ± 2	111 ± 4	108 ± 6	112 ± 3
Shock	38 ± 1	35 ± 2	36 ± 3	40 ± 2
Treatment	80 ± 9	65 ± 11	70 ± 9	90 ± 11
MAP (mm Hg)				
Control	138 ± 2	132 ± 4	135 ± 7	132 ± 3
Shock	55 ± 1	52 ± 2	55 ± 2	54 ± 2
Treatment	104 ± 8	89 ± 11	92 ± 9	109 ± 11
HR (b/min)				
Control	351 ± 11	357 ± 20	314 ± 24	372 ± 18
Shock	324 ± 20	332 ± 17	291 ± 29	311 ± 17
Treatment	402 ± 20	438 ± 31	361 ± 38	396 ± 31

Data are expressed as mean ± SEM, n = 6. SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, HR: heart rate.
* $p < 0.05$ in comparison with $FIO_2 = 0.21$.

Arterial blood gases and hemoglobin. Tables 3 and 4 contain ABG data at control, HS and treatment at different FIO_2s, without and with DA infusion. There were no significant differences among the variables at control and shock among all 8 groups ($P > 0.05$). Plasma HCO_3 in rats breathing FIO_2 greater than 0.21 was significantly greater than that at ambient air (0.21), and correspondingly, the Beecf was less in these rats (Table 3). As expected, increasing the FIO_2 resulted in significant increases in the PaO_2 ($P > 0.05$). In the treatment groups without and with DA, increasing FIO_2 had no significance effects on pH, PCO_2, O_2 saturation or Hgb.

Table 3 Arterial blood gases for each fraction of inspired oxygen concentration.

	0.21	0.40	0.60	1.0
pH				
Control	7.42 ± 0.02	7.41 ± 0.02	7.40 ± 0.02	7.39 ± 0.02
Shock	7.46 ± 0.03	7.44 ± 0.03	7.47 ± 0.04	7.47 ± 0.04
Treatment	7.24 ± 0.07	7.39 ± 0.04	7.38 ± 0.05	7.31 ± 0.05
$PaCO_2$ (mm Hg)				
Control	38 ± 2	41 ± 3	41 ± 1	44 ± 2
Shock	24 ± 3	29 ± 3	24 ± 2	27 ± 3
Treatment	20 ± 2	25 ± 4	27 ± 3	27 ± 5
HCO_3 (mEq/L)				
Control	25 ± 1	26 ± 1	26 ± 1	27 ± 1
Shock	17 ± 1	19 ± 1	17 ± 1	19 ± 1
Treatment	8 ± 1	16 ± 3*	16 ± 2*	15 ± 3*
Beecf				
Control	0.3 ± 0.8	-1.3 ± 1.6	0.8 ± 0.9	1.2 ± 1.0
Shock	-7.3 ± 1.4	-5.0 ± 1.0	-6.3 ± 1.8	-4.3 ± 1.5
Treatment	-19.8 ± 1.5	-9.3 ± 3.1*	-8.8 ± 2.5*	-11.7 ± 4.0
PaO_2 (mm Hg)				
Control	80 ± 2	81 ± 4	78 ± 5	77 ± 3
Shock	89 ± 8	86 ± 6	112 ± 8	108 ± 9
Treatment	88 ± 5	196 ± 8*	244 ± 22*	519 ± 21*
O_2 Saturation (%)				
Control	94 ± 1	93 ± 1	95 ± 1	95 ± 1
Shock	97 ± 1	97 ± 1	99 ± 1	99 ± 1
Treatment	97 ± 1	99 ± 1	99 ± 1	99 ± 1
Hgb (g/100 mL)				
Control	12.6 ± 0.6	13.9 ± 0.3	12.5 ± 1.0	14.0 ± 0.9
Shock	7.7 ± 1.0	7.7 ± 0.5	5.8 ± 0.6	8.6 ± 1.0
Treatment	6.0 ± 0.7	6.8 ± 0.5	6.5 ± 0.8	7.0 ± 0.5

Data are expressed as mean ± SEM, n = 6. $PaCO_2$: partial pressure carbon dioxide, HCO_3: bicarbonate, Beecf: base excess, PaO_2: partial pressure of oxygen, O_2 Saturation: % of oxyhemoglobin, Hgb: hemoglobin.
* $p < 0.05$ in comparison with $FIO_2 = 0.21$.

Table 4 Arterial blood gases for each fraction of inspired oxygen concentration plus dopamine.

	0.21	0.40	0.60	1.0
pH				
Control	7.40 ± 0.09	7.43 ± 0.03	7.43 ± 0.04	7.41 ± 0.02
Shock	7.44 ± 0.07	7.43 ± 0.02	7.43 ± 0.05	7.39 ± 0.02
Treatment	7.43 ± 0.02	7.35 ± 0.06	7.39 ± 0.04	7.32 ± 0.05
$PaCO_2$ (mm Hg)				
Control	45 ± 1	35 ± 3	43 ± 5	41 ± 2
Shock	32 ± 5	27 ± 2	31 ± 5	34 ± 3
Treatment	25 ± 3	19 ± 2	28 ± 5	36 ± 7
HCO_3 (mEq/L)				
Control	28 ± 1	24 ± 1	27 ± 1	26 ± 1
Shock	21 ± 1	19 ± 1	19 ± 2	21 ± 1
Treatment	16 ± 2	11 ± 2	16 ± 3	19 ± 3
Beecf				
Control	2.0 ± 0.6	-0.3 ± 0.6	2.3 ± 0.6	1.0 ± 0.7
Shock	-3.5 ± 1.7	-5.0 ± 0.7	-5.8 ± 1.3	-4.5 ± 0.4
Treatment	-7.7 ± 1.5	-14.0 ± 3.3	-8.0 ± 3.0	-6.8 ± 3.0
PaO_2 (mm Hg)				
Control	72 ± 3	78 ± 5	75 ± 5	72 ± 4
Shock	95 ± 10	84 ± 6	92 ± 4	89 ± 5
Treatment	87 ± 6	181 ± 15*	282 ± 9*	506 ± 27*
O_2 Saturation (%)				
Control	94 ± 1	93 ± 1	95 ± 2	95 ± 1
Shock	97 ± 1	97 ± 1	99 ± 1	99 ± 1
Treatment	97 ± 1	99 ± 1	99 ± 1	99 ± 1
Hgb (g/100 mL)				
Control	13.1 ± 0.4	10.8 ± 0.5	13.1 ± 0.2	13.2 ± 0.5
Shock	8.1 ± 1.0	6.2 ± 0.3	7.3 ± 0.4	7.1 ± 0.7
Treatment	7.1 ± 0.6	5.9 ± 0.3	6.9 ± 0.4	7.1 ± 0.6

Data are expressed as mean ± SEM, n = 6. $PaCO_2$: partial pressure carbon dioxide, HCO_3: bicarbonate, Beecf: base excess, PaO_2: partial pressure of oxygen, O_2 Saturation: % of oxyhemoglobin, Hgb: hemoglobin.
* $p < 0.05$ in comparison with $FIO_2 = 0.21$.

Lung hydrogen peroxide. The effects of increasing inspired O_2 without and with DA infusion on lung H_2O_2 are illustrated in Figure 1 (next page). Increasing FIO_2 only (open bars) resulted in significant decreases in Fluor intensity (H_2O_2) at FIO_2 of 0.40 and 0.60 ($P < 0.05$). However, increasing FIO_2 to 1.0 resulted in H_2O_2 not being significantly different from FIO_2 equal to 0.21. Increasing FIO_2 plus DA (striped bars) was accompanied by significant decreases at all FIO_2s ($P < 0.05$). DA significantly decreased ($P < 0.05$) H_2O_2 at FIO_2s of 0.21, 0.60 and 1.0 (striped bars versus open bars). However, DA did not significantly decrease H_2O_2 at FIO_2 of 0.40.

Lung apoptosis. Figure 2 summarizes percent apoptosis in lung tissue at various FIO_2s, without and with the administration of DA. With the exception of FIO_2 equal to 1.0, increasing FIO_2 was accompanied by significant decreases (open bars) in lung apoptosis ($P < 0.05$). Increasing FIO_2 while infusing DA (striped bars) resulted in the percent of lung apoptosis being significantly increased only at FIO_2 of 1.0 ($P < 0.05$). Comparing FIO_2 plus DA to FIO_2 only groups

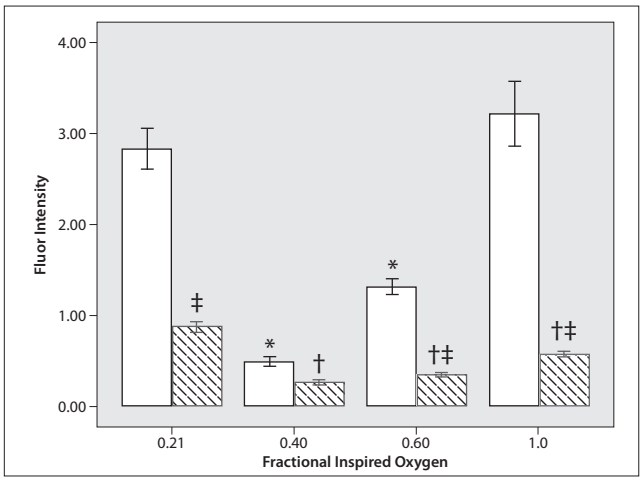

Figure 1 Fluor intensity of lung hydrogen peroxide for FIO_2 and FIO_2 plus DA.
Open bars = FIO_2 groups; Stripped bars = FIO_2 plus DA groups.
* significantly different from FIO_2 at 0.21 within the FIO_2 only group – □ ($p < 0.05$).
+ significantly different from FIO_2 at 0.21 within the FIO_2 plus DA group – ▨ ($p < 0.05$).
‡ significantly different between the FIO_2 only and FIO_2 plus DA groups – □ *vs.* ▨ ($p < 0.05$).
Data are expressed as mean ± SEM, n = 6.

(striped bars versus open bars), exhibits a significant decrease in the percentage of lung apoptosis in all groups except FIO_2 equal to 0.40.

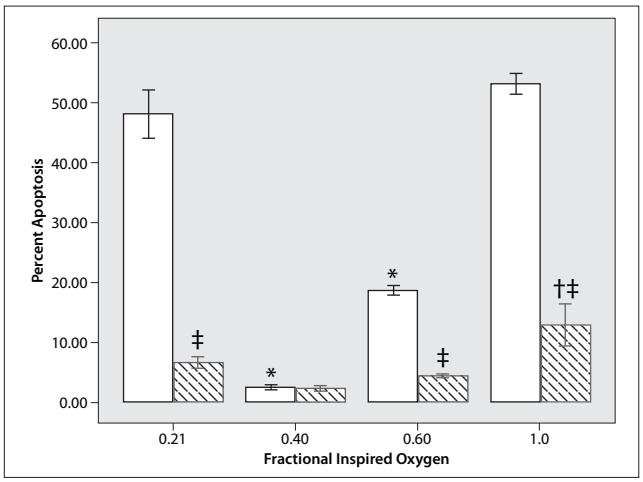

Figure 2 Percent lung apoptosis for FIO_2 and FIO_2 plus DA.
Open bars = FIO_2 groups; Stripped bars = FIO_2 plus DA groups.
* significantly different from FIO_2 at 0.21 within the FIO_2 only group – □ ($p < 0.05$).
+ significantly different from FIO_2 at 0.21 within the FIO_2 plus DA group – ▨ ($p < 0.05$).
‡ significantly different between the FIO_2 only and FIO_2 plus DA groups – □ *vs.* ▨ ($p < 0.05$).
Data are expressed as mean ± SEM, n = 8.

Diaphragm hydrogen peroxide. Figure 3 illustrates diaphragm H_2O_2 for FIO_2 only and FIO_2 plus DA groups. For the FIO_2 only groups (open bars), there were significant decreases in H_2O_2 at 0.40 and 0.60 with respect to 0.21 ($P < 0.05$). In contrast, when breathing 100% O_2 diaphragm H_2O_2 was significantly greater than breathing room air. In the FIO_2 with DA groups (striped bars), there were significant decreases in H_2O_2 in 0.40 and 0.60 FIO_2 groups ($P < 0.05$). However, at an FIO_2 of 1.0, diaphragm H_2O_2 was not significantly less than ambient air. Administering DA resulted in diaphragm H_2O_2 being significantly decreased at all FIO_2s except 0.40 (striped versus open bars) ($P < 0.05$).

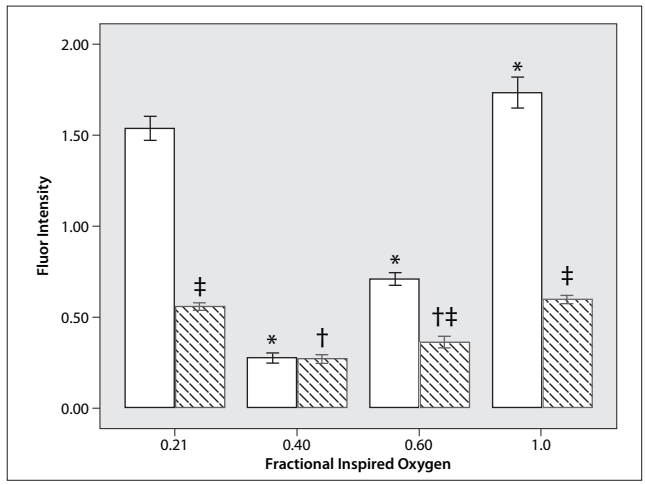

Figure 3 Fluor intensity of diaphragm hydrogen peroxide for FIO_2 and FIO_2 plus DA.
Open bars = FIO_2 groups; Stripped bars = FIO_2 plus DA groups.
* significantly different from FIO_2 at 0.21 within the FIO_2 only group – □ ($p < 0.05$).
+ significantly different from FIO_2 at 0.21 within the FIO_2 plus DA group – ▨ ($p < 0.05$).
‡ significantly different between the FIO_2 only and FIO_2 plus DA groups – □ *vs.* ▨ ($p < 0.05$).
Data are expressed as mean ± SEM, n = 6.

Diaphragm apoptosis. Percent diaphragm apoptosis results are summarized in Figure 4. Increasing FIO_2 to 0.40 and 0.60 resulted in significant decreases in apoptosis (open bars). In contrast, administering FIO_2 equal to 1.0 resulted in the apoptosis being significantly greater than 0.21 ($P < 0.05$). Infusing DA (striped bars) at FIO_2 0.40 and 0.60 did not result in significant decreases in apoptosis. In contrast, at an FIO_2 of 1.0 apoptosis was significantly greater than at 0.21 ($P < 0.05$). With the exception of FIO_2 at 0.40, infusing DA significantly decreased apoptosis at all FIO_2s (striped bars versus open bars).

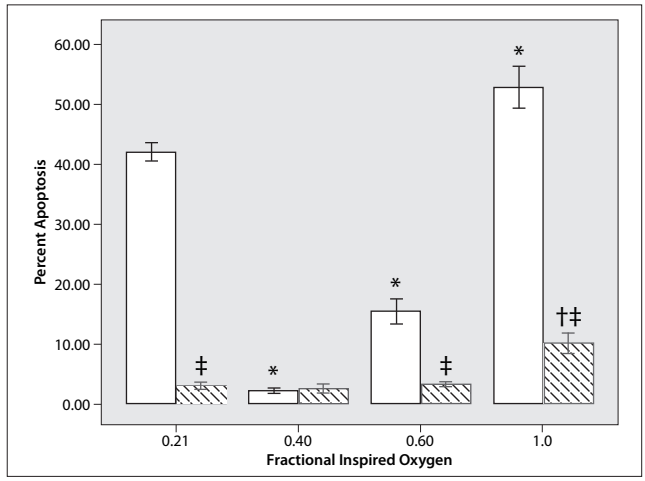

Figure 4 Percent diaphragm apoptosis for FIO_2 and FIO_2 plus DA.
Open bars = FIO_2 groups; Stripped bars = FIO_2 plus DA groups.
* significantly different from FIO_2 at 0.21 within the FIO_2 only group – □ ($p < 0.05$).
+ significantly different from FIO_2 at 0.21 within the FIO_2 plus DA group – ▨ ($p < 0.05$).
‡ significantly different between the FIO_2 only and FIO_2 plus DA groups – □ *vs.* ▨ ($p < 0.05$).
Data are expressed as mean ± SEM, n = 8.

DISCUSSION

In this study we investigated the effects of FIO_2 (0.21, 0.40, 0.60, 1.0) and DA (10 mcg/kg/min) on lung and diaphragm H_2O_2 and apoptosis after 30 min of HS. We observed that H_2O_2 and apoptosis in lung and diaphragm were minimized when rats breathed 40% O_2. The extent of H_2O_2 and apoptosis was comparable and greatest in animals that were administered 21% or 100% O_2. With the addition of DA with supplemental O_2, significant decreases in lung and diaphragm H_2O_2 and apoptosis in all FIO_2 groups were observed, except in the FIO_2 0.40 groups. At an FIO_2 of 0.21, arterial blood pressures remained decreased throughout the treatment period. Arterial blood gases and Hgb results were similar to other investigations studying HS.

Arterial blood pressures increased with supplemental O_2 administration (0.40, 0.60, 1.0) during the treatment period [12]. Infusing DA at all FIO_2s resulted in arterial blood pressures not being significantly different at the end of the treatment period.

Lung hydrogen peroxide. Hydrogen peroxide production contributes to lung injury during HS [13]. In HS, reduced DO_2 activates leukocytes resulting in increased free radical production [14]. In addition, cellular hypoxia results in increasing mitochondria free radical formation [15]. It has been reported that increasing DO_2 by increasing inspired O_2 may result in hyperoxic-induced lung damage. Turrens et al. observed increased lung mitochondrial H_2O_2 production at FIO_2 greater than 0.60 [16]. Other investigators reported lung injury accompanied by increased free radical formation during hyperoxia [17, 18]. Thus, our results related to lung H_2O_2 production being affected by the FIO_2 are consistent with these investigators. Our data indicate that an FIO_2 of 0.40 is most beneficial in minimizing lung H_2O_2 production following HS. In class IV HS when Hgb and cardiac output are decreased, negative effects on microcirculation leads to tissue and cellular hypoxia and acidosis impairing mitochondrial functioning. Oxygen administration of 0.40 improves DO_2 by supporting HR, MAP and increasing vascular resistance. The higher FIO_2 values had the same effect on HR and MAP. In 40% blood volume loss, vascular resistance is already very high, and increasing it without volume replacement may reduce cardiac output (pressure/resistance = flow). Oxygen delivery is proportional to cardiac output. Oxygen administration of 0.40 can also enhance antioxidant functions. Vento et al. observed that an FIO_2 of 0.30 enhanced glutathione free radical scavenging in prenatal neonates [19]. In addition, Lee et al. found that mice breathing 40% O_2 had increased tissue levels of vitamin E and C, known antioxidants [20]. Administering DA with varying FIO_2s resulted in significant decreases in lung H_2O_2 at all FIO_2 except 0.40. Gero et al. found that activating DA receptors was cytoprotective against H_2O_2 induced lung injury [21]. Our results suggest that DA scavenges ROS in lung tissue. Dopamine enhances tissue O_2 perfusion, increases cardiac contractility, and systemic pressure and HR in HS [22]. We have observed that DA increases diaphragm blood flow in rats following HS [23]. As a consequence, DO_2 to the tissues is increased with DA administration, which could in part account for the reduction in H_2O_2 production.

Lung apoptosis. Programmed cell death is associated with HS [24]. Shih et al. studied lung differential gene expression and found HS induced up-regulation of genes responsible for apoptosis [25]. High concentrations of FIO_2 cause ROS (H_2O_2) mediated apoptosis [26]. Hydrogen peroxide production results in the formation of hydroxyl radicals leading to caspase activation that trigger apoptotic events [27]. The increase in ROS causes a release of cytochrome c from the mitochondria, resulting in cell death [28]. Administering FIO_2 of 0.21 and 1.0 after inducing HS resulted in the greatest percent lung apoptosis. Similarly, H_2O_2 was also greatest at these two FIO_2. Hypoxia and hyperoxia were associated with apoptosis. In HS, an FIO_2 of 0.40 produced the least amount of lung H_2O_2 and apoptosis. When using an FIO_2 of 0.60, there was a slight increase in lung apoptosis and H_2O_2 compared to 0.40.

In HS, activated leukocytes are a source of free radicals [29]. Dopamine has been shown to reduce polymorphonuclear leukocyte superoxide production [30]. Similar to other antioxidants, in this study, DA decreased lung apoptosis at all FIO_2s (0.21, 0.40, 0.60, 1.0), presumably as of a result of decreasing H_2O_2. At FIO_2 of 0.40 lung apoptosis with DA was not significantly different from that in animals not administered DA. The extent of lung apoptosis at FIO_2 of 0.40 was similar to sham lung apoptosis (2%). Our results are similar to the Teramoto et al. study in which they concluded that H_2O_2 induced lung apoptosis was in part attributable to ROS production [31].

Diaphragm hydrogen peroxide. There is well established research of the effects of free radicals on diaphragm muscle function [32, 33]. Free radicals attenuate calcium release from diaphragm muscle cells resulting in decreased force generation that leads to respiratory distress [32]. Diaphragm ROS are generated during re-oxygenation following hypoxia [34]. Oxygen radical generation also occurs in the diaphragm during exposure to hyperoxia [35]. In the present study, administering FIO_2 of 0.40 resulted in the least amount of diaphragm H_2O_2. In contrast, FIO_2s of 0.21 and 1.0 were accompanied by the highest amount of diaphragm H_2O_2. With an exception of FIO_2 of 0.40, adding DA decreased H_2O_2. We have reported that DA increases diaphragm blood flow [36]. The resulting increase in DO_2 would reduce ROS formation.

Diaphragm apoptosis. The percent of diaphragm apoptosis at various FIO_2s paralleled the changes in diaphragm H_2O_2 and lung apoptosis. The percent diaphragm apoptosis was greatest at FIO_2s at 0.21 and 1.0 and lowest at 0.40. The addition of DA with various FIO_2 resulted in a marked reduction in the percent of apoptosis. Dopamine is a free radical scavenger that attenuates apoptosis in the diaphragm [37].

CONCLUSIONS

After bleeding is controlled, initial treatment of HS is directed at increasing DO_2 to cells. One hundred percent O_2 is routinely employed to achieve this objective. However, there are numerous studies indicating that 100% O_2 results in tissue injury which is attributed to increased free radical formation. We found that O_2 administered at an FIO_2 of 1.0 in HS increased H_2O_2 and apoptosis in both the lung and diaphragm. This study suggests that increased lung and diaphragm H_2O_2 and apoptosis resulting from the administration of 100% O_2 can be prevented by infusing DA (10 mcg/kg/min). Our results also indicate that while breathing 21% O_2 for 60

min following HS, increased lung and diaphragm H_2O_2 and apoptosis can be markedly decreased by administering DA. This effect of DA appears to be attributable to its free radical scavenging capabilities and an increase in DO_2 associated with an improved hemodynamic status. Administering 40% O_2 achieved the greatest reduction in H_2O_2-mediated apoptosis in lung and diaphragm accompanying HS.

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