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 (FIo2) to restore maximal oxygen (O2) saturations. Studies indicate that increased FIo2 can be detrimental to cellular function. Our purpose was to determine the FIo2 with and without dopamine (DA) that minimizes hydrogen peroxide (H2O2) production and apoptosis in lung and diaphragm following HS. Sprague-Dawley rats were randomized to FIo2 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 FIo2s, 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 H2O2 and apoptosis were significantly reduced in the 0.40 and 0.60 groups. At an FIo2 of 1.0, H2O2 and apoptosis were greater than at 0.21. With the exception of an FIo2 of 0.40, infusing DA with various FIo2s resulted in H2O2 and apoptosis being significantly decreased. These results indicate that lung and diaphragm H2O2 and apoptosis are affected by inspired O2 and DA. Results indicate using an FIo2 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 (O2) necessary for normal cell function [2]. In patients who experience HS, failure of compensatory mechanisms and hemodynamic instability, decreased oxygen delivery (DO2), and O2 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 (FIo2). 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 FIo2 can be detrimental to tissues [6, 7]. Administering supplemental O2 during HS increases tissue O2, 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 FIo2s, without and with the administration of DA (10 mcg/kg/min), following HS on lung and diaphragm damage. Tissue damage was assessed by measuring H2O2, 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 FIo2 and DA. The dependent variables were lung and diaphragm H2O2 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.
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 ±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 (PaCO₂), bicarbonate (HCO₃⁻), base excess (Beecf), partial pressure of O₂ (PaO₂), O₂ Saturation (SaO₂) 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 (FIO₂ = 0.21). At the end of the HS period, hemodynamic and acid-base parameters were recorded and one of the 8 treatments (various FIO₂, without and with DA) was initiated. No fluids were administered as treatments prior to the use of O₂ 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 H₂O₂ 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 H₂O₂ 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 H₂O₂. 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 ×10⁸) which is directly proportional to the amount of H₂O₂ in the tissue. Changes in fluorescence due to Fluor were measured using a laser scanning cytometer with a detection limit of 1 μM H₂O₂.

**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 of 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 FIO₂ only and FIO₂ + 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 FIO₂ only and FIO₂ + DA groups were analyzed by 1-way ANOVA followed by post hoc test (Fisher's LSD). Differences between FIO₂ only and FIO₂ + 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 FIO₂, at 0.21. In Table 1, increasing FIO₂ resulted in a significant increase in SBP at an FIO₂ of 0.60 (P < 0.05). Diastolic blood pressures and MAPs were significantly increased at all FIO₂s greater than 0.21. Varying FIO₂ had no significant effect on HR. In Table 2, administering DA and increasing FIO₂s resulted in a significant decrease in SBP at 0.60 (P < 0.05). Increasing FIO₂ had no significant effect on DBP and MAP. Again, HR was not significantly increased in any of the FIO₂ plus DA groups.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Hemodynamics for the different fraction of inspired oxygen concentrations.</th>
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<tbody>
<tr>
<td></td>
<td>0.21</td>
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<tr>
<td><strong>SBP (mm Hg)</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>148 ± 4</td>
</tr>
<tr>
<td>Shock</td>
<td>97 ± 4</td>
</tr>
<tr>
<td>Treatment</td>
<td>93 ± 9</td>
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<tr>
<td><strong>DBP (mm Hg)</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>107 ± 5</td>
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<tr>
<td>Shock</td>
<td>43 ± 1</td>
</tr>
<tr>
<td>Treatment</td>
<td>38 ± 9</td>
</tr>
<tr>
<td><strong>MAP (mm Hg)</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>127 ± 5</td>
</tr>
<tr>
<td>Shock</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>Treatment</td>
<td>56 ± 9</td>
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<tr>
<td><strong>HR (b/min)</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>357 ± 24</td>
</tr>
<tr>
<td>Shock</td>
<td>366 ± 9</td>
</tr>
<tr>
<td>Treatment</td>
<td>392 ± 23</td>
</tr>
</tbody>
</table>

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 FIO₂ = 0.21.
Arterial blood gases and hemoglobin. Tables 3 and 4 contain ABG data at control, HS and treatment at different FIO2s, 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 HCO3 in rats breathing FIO2 greater than 0.21 was significantly greater than that at ambient air (0.21), and correspondingly, the Beef was less in these rats (Table 3). As expected, increasing the FIO2 resulted in significant increases in the PaO2 (P > 0.05). In the treatment groups without and with DA, increasing FIO2 had no significance effects on pH, PCO2, O2 saturation or Hgb.

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Shock</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.42 ± 0.02</td>
<td>8.44 ± 0.03</td>
<td>7.39 ± 0.02</td>
</tr>
<tr>
<td>PaCO2 (mm Hg)</td>
<td>7.24 ± 0.07</td>
<td>7.47 ± 0.04</td>
<td>7.31 ± 0.05</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>-7.7 ± 1.5</td>
<td>14.0 ± 3.3</td>
<td>-6.8 ± 3.0</td>
</tr>
<tr>
<td>HCO3 (mEq/L)</td>
<td>25 ± 1</td>
<td>26 ± 1</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>Beegf</td>
<td>0.3 ± 0.8</td>
<td>-1.3 ± 1.6</td>
<td>1.2 ± 1.0</td>
</tr>
<tr>
<td>Hgb (g/100 mL)</td>
<td>21 ± 1</td>
<td>19 ± 1</td>
<td>19 ± 1</td>
</tr>
</tbody>
</table>

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 FIO2 = 0.21.

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Shock</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.40 ± 0.09</td>
<td>7.43 ± 0.03</td>
<td>7.43 ± 0.04</td>
</tr>
<tr>
<td>PaCO2 (mm Hg)</td>
<td>7.43 ± 0.02</td>
<td>7.35 ± 0.06</td>
<td>7.39 ± 0.04</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>72 ± 3</td>
<td>78 ± 5</td>
<td>75 ± 5</td>
</tr>
<tr>
<td>HCO3 (mEq/L)</td>
<td>8 ± 1</td>
<td>16 ± 3</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>Beegf</td>
<td>2.0 ± 0.6</td>
<td>-0.3 ± 0.6</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>Hgb (g/100 mL)</td>
<td>94 ± 1</td>
<td>93 ± 1</td>
<td>95 ± 1</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM, n = 6. PaCO2: partial pressure carbon dioxide, HCO3: bicarbonate, Beegf: base excess, PaO2: partial pressure of oxygen, O2 Saturation: % of oxyhemoglobin, Hgb: hemoglobin. *p < 0.05 in comparison with FIO2 = 0.21.

**Lung hydrogen peroxide.** The effects of increasing inspired O2 without and with DA infusion on lung H2O2 are illustrated in Figure 1 (next page). Increasing FIO2 only (open bars) resulted in significant decreases in Fluor intensity (H2O2) at FIO2 of 0.40 and 0.60 (P < 0.05). However, increasing FIO2 to 1.0 resulted in H2O2 not being significantly different from FIO2 equal to 0.21. Increasing FIO2 plus DA (striped bars) was accompanied by significant decreases at all FIO2s (P < 0.05). DA significantly decreased (P < 0.05) H2O2 at FIO2s of 0.21, 0.40 and 0.60 (P < 0.05). However, increasing FIO2 to 1.0 resulted in H2O2 not being significantly different from FIO2 equal to 0.21. Increasing FIO2 plus DA (striped bars) was accompanied by significant decreases at all FIO2s (P < 0.05). DA significantly decreased (P < 0.05) H2O2 at FIO2s of 0.21, 0.60 and 1.0 (striped bars versus open bars). However, DA did not significantly decrease H2O2 at FIO2 of 0.40.

**Lung apoptosis.** Figure 2 summarizes percent apoptosis in lung tissue at various FIO2s, without and with the administration of DA. With the exception of FIO2 equal to 1.0, increasing FIO2 was accompanied by significant decreases (open bars) in lung apoptosis (P < 0.05). Increasing FIO2 while infusing DA (striped bars) resulted in the percent of lung apoptosis being significantly increased only at FIO2 of 1.0 (P < 0.05). Comparing FIO2 plus DA to FIO2 only groups
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being significantly decreased at all FIO2s except 0.40 (striped versus open bars) (P < 0.05).

Diaphragm apoptosis. Percent diaphragm apoptosis results are summarized in Figure 4. Increasing FIO2 to 0.40 and 0.60 resulted in significant decreases in apoptosis (open bars). In contrast, administering FIO2 equal to 1.0 resulted in the apoptosis being significantly greater than 0.21 (P < 0.05). Infusing DA (striped bars) at FIO2 0.40 and 0.60 did not result in significant decreases in apoptosis. In contrast, at an FIO2 of 1.0 apoptosis was significantly greater than at 0.21 (P < 0.05). With the exception of FIO2 at 0.40, infusing DA significantly decreased apoptosis at all FIO2s (striped bars versus open bars).

Diaphragm hydrogen peroxide. Figure 3 illustrates diaphragm H2O2 for FIO2 only and FIO2 plus DA groups. For the FIO2 only groups (open bars), there were significant decreases in H2O2 at 0.40 and 0.60 with respect to 0.21 (P < 0.05). In contrast, when breathing 100% O2 diaphragm H2O2 was significantly greater than breathing room air. In the FIO2 with DA groups (striped bars), there were significant decreases in H2O2 in 0.40 and 0.60 FIO2 groups (P < 0.05). However, at an FIO2 of 1.0, diaphragm H2O2 was not significantly less than ambient air. Administering DA resulted in diaphragm H2O2 (striped bars versus open bars), exhibits a significant decrease in the percentage of lung apoptosis in all groups except FIO2 equal to 0.40.

Figure 1  Fluor intensity of lung hydrogen peroxide for FIO2 and FIO2 plus DA. Open bars = FIO2 groups; Stripped bars = FIO2 plus DA groups.
* significantly different from FIO2 at 0.21 within the FIO2 only group – □ (p < 0.05).
+ significantly different from FIO2 at 0.21 within the FIO2 plus DA group – □ (p < 0.05).
‡ significantly different between the FIO2 only and FIO2 plus DA groups – □ vs. □ (p < 0.05).
Data are expressed as mean ± SEM, n = 6.

Figure 2  Percent lung apoptosis for FIO2 and FIO2 plus DA. Open bars = FIO2 groups; Stripped bars = FIO2 plus DA groups.
* significantly different from FIO2 at 0.21 within the FIO2 only group – □ (p < 0.05).
+ significantly different from FIO2 at 0.21 within the FIO2 plus DA group – □ (p < 0.05).
‡ significantly different between the FIO2 only and FIO2 plus DA groups – □ vs. □ (p < 0.05).
Data are expressed as mean ± SEM, n = 8.

Figure 3  Fluor intensity of diaphragm hydrogen peroxide for FIO2 and FIO2 plus DA. Open bars = FIO2 groups; Stripped bars = FIO2 plus DA groups.
* significantly different from FIO2 at 0.21 within the FIO2 only group – □ (p < 0.05).
+ significantly different from FIO2 at 0.21 within the FIO2 plus DA group – □ (p < 0.05).
‡ significantly different between the FIO2 only and FIO2 plus DA groups – □ vs. □ (p < 0.05).
Data are expressed as mean ± SEM, n = 6.

Figure 4  Percent diaphragm apoptosis for FIO2 and FIO2 plus DA. Open bars = FIO2 groups; Stripped bars = FIO2 plus DA groups.
* significantly different from FIO2 at 0.21 within the FIO2 only group – □ (p < 0.05).
+ significantly different from FIO2 at 0.21 within the FIO2 plus DA group – □ (p < 0.05).
‡ significantly different between the FIO2 only and FIO2 plus DA groups – □ vs. □ (p < 0.05).
Data are expressed as mean ± SEM, n = 8.
**DISCUSSION**

In this study we investigated the effects of FIO2 (0.21, 0.40, 0.60, 1.0) and DA (10 mcg/kg/min) on lung and diaphragm H2O2 and apoptosis after 30 min of HS. We observed that H2O2 and apoptosis in lung and diaphragm were minimized when rats breathed 40% O2. The extent of H2O2 and apoptosis was comparable and greatest in animals that were administered 21% or 100% O2. With the addition of DA with supplemental O2, significant decreases in lung and diaphragm H2O2 and apoptosis in all FIO2 groups were observed, except in the FIO2 0.40 groups. At an FIO2 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 O2 administration (0.40, 0.60, 1.0) during the treatment period [12]. Infusing DA at all FIO2s 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 DO2 activates leukocytes resulting in increased free radical production [14]. In addition, cellular hypoxia results in increasing mitochondrial free radical formation [15]. It has been reported that increasing DO2 by increasing inspired O2 may result in hyperoxic-induced lung damage. Turrens et al. observed increased lung mitochondrial H2O2 production at FIO2 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 H2O2 production being affected by the FIO2 are consistent with these investigators. Our data indicate that an FIO2 of 0.40 is most beneficial in minimizing lung H2O2 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 DO2 by supporting HR, MAP and increasing vascular resistance. The higher FIO2 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 FIO2 of 0.30 enhanced glutathione free radical scavenging in prenatal neonates [19]. In addition, Lee et al. found that mice breathing 40% O2 had increased tissue levels of vitamin E and C, known antioxidants [20]. Administering DA with varying FIO2s resulted in significant decreases in lung H2O2 at all FIO2 except 0.40. Gero et al. found that activating DA receptors was cytoprotective against H2O2 induced lung injury [21]. Our results suggest that DA scavenges ROS in lung tissue. Dopamine enhances tissue O2 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, DO2 to the tissues is increased with DA administration, which could in part account for the reduction in H2O2 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 FIO2 cause ROS (H2O2) 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 FIO2 of 0.21 and 1.0 after inducing HS resulted in the greatest percent lung apoptosis. Similarly, H2O2 was also greatest at these two FIO2. Hypoxia and hyperoxia were associated with apoptosis. In HS, an FIO2 of 0.40 produced the least amount of lung H2O2 and apoptosis. When using an FIO2 of 0.60, there was a slight increase in lung apoptosis and H2O2 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 FIO2s (0.21, 0.40, 0.60, 1.0), presumably as of a result of decreasing H2O2. At FIO2 of 0.40 lung apoptosis with DA was not significantly different from that in animals not administered DA. The extent of lung apoptosis at FIO2 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 H2O2 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 FIO2 of 0.40 resulted in the least amount of diaphragm H2O2. In contrast, FIO2s of 0.21 and 1.0 were accompanied by the highest amount of diaphragm H2O2. With an exception of FIO2 of 0.40, adding DA decreased H2O2. We have reported that DA increases diaphragm blood flow [36]. The resulting increase in DO2 would reduce ROS formation.

**Diaphragm apoptosis.** The percent of diaphragm apoptosis at various FIO2s paralleled the changes in diaphragm H2O2 and lung apoptosis. The percent diaphragm apoptosis was greatest at FIO2s at 0.21 and 1.0 and lowest at 0.40. The addition of DA with various FIO2 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 DO2 to cells. One hundred percent O2 is routinely employed to achieve this objective. However, there are numerous studies indicating that 100% O2 results in tissue injury which is attributed to increased free radical formation. We found that O2 administered at an FIO2 of 1.0 in HS increased H2O2 and apoptosis in both the lung and diaphragm. This study suggests that increased lung and diaphragm H2O2 and apoptosis resulting from the administration of 100% O2 can be prevented by infusing DA (10 mcg/kg/min). Our results also indicate that while breathing 21% O2 for 60
min following HS, increased lung and diaphragm H$_2$O$_2$ 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$_2$O$_2$-mediated apoptosis in lung and diaphragm accompanying HS.

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REFERENCES