Role of Iron in Hyperoxia-Induced Lung Injury: One Step Forward in Iron Chelation

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ABSTRACT

Background: An increased oxidative stress in patients under treatment with high concentrations of oxygen (hyperoxia) is considered to be one of the major mechanisms of lung injury. Between different mediators, transition metal ions especially iron, by generation of very reactive free radicals play an important role in oxidative stress process. Disruption of normal iron hemostasis has been reported in hyperoxic conditions. So we hypothesized that chelation of iron can reduce hyperoxia-induced lung injury.

Methods: Mechanically ventilated patients, who received oxygen with FiO2 > 0.5 for at least 3 days, underwent bronchoscopy at baseline and 72 hours thereafter. Data from external control cases were collected prospectively to provide a comparative reference group. Iron and Iron-related proteins were measured in lavage fluid and plasma.

Results: In 24 patients and in comparison with the results of previous study, Iron concentration decreased significantly in lavage fluid (P<0.001). Reduction of ferritin was not significant in lavage fluid (P: 0.7). Transferrin decreased significantly in plasma (P: 0.01). Acute Physiology And Chronic Health Evaluation (APACHE) II (P: 0.006) score decreased significantly after 7 days of follow-up.

Conclusion: Deferasirox did not change Iron and Iron-related protein in hyperoxic condition and it just only could be considered along with other supportive measures for better toleration of oxygen therapy.

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Introduction

Oxygen therapy is necessary and life-saving component of the treatment of acute respiratory failure, however supraphysiological concentration of oxygen (hyperoxic condition) has long been recognized as a potential cause of lung injury (1-3). Hyperoxia through direct oxygen toxicity and accumulation of inflammatory mediators within lungs result in a process that initiate by damaging to pulmonary epithelium and endothelium and finally lead to pulmonary interstitial fibrosis (4-6). It is assumed that lung damage caused by hyperoxia is mediated by excess production of Reactive Oxygen Species (ROS) (7). Metal ions especially iron catalyze ROS production such as superoxide anion, hydrogen peroxide and hydroxyl radical that could damage biological macromolecules within cells (8,9). In physiologic condition, lung epithelium cells through sequestration of free iron in
ferritin and transportation limit the levels of catalytically active iron(8;10-12), but some disease condition and toxic exposures disrupt iron hemostasis which can contribute to the process of oxidative stress in the lungs(10).

Iron chelation is capable of preventing the generation of ROS. Several studies with deferoxamine have indicated that the use of deferoxamine could limit the effect of oxidative stress directly and alter inflammatory process (13-17). New long acting oral iron chelator, deferasirox, recently been approved for iron overload in beta thalassemic patients. It seems that this drug has better side effect profile in comparison with deferoxamine and because of its small size and lipophilic structure could chelate intracellular iron better than deferoxamin(18;19). Therefore we here describe the effects of deferasirox on iron and iron-related protein in hyperoxic condition. To determine the efficacy of iron chelation when randomization was not possible, we designed an external control group.

Patients and Methods

In all cases, informed consent was obtained from patients or their closest relatives. The study procedure and protocol were approved by the ethical committee of Tehran University of Medical Sciences.

Between March 2009 and April 2010, mechanically ventilated patients who received oxygen with Fio2 more than 0.50 for at least 3 days and Positive End Expiratory Pressure (PEEP) at least 5mmHg, and had the ability to take drug by oral route were enrolled in the study. Exclusion criteria for all patients were age less than 18, severe liver failure (AST, ALT > 5 times ULN), moderate to severe renal failure (SrCr > 2mg/dl or u/o<0.5cc/kg/hr, or dialysis), shock state (pH<7.2, MAP<60, Pao2/Fio2<100), patients who received other drugs which affect iron and patients who received iron chelator drugs within past 3 months. The term external indicates that these patients were not randomized as a prespecified control group in the original study plan and not parallel with treatment group.

All patients (in treatment and control group) that their clinical status was stable enough to allow underwent bronchoscopy alveolar lavage (BAL) at baseline and 72 hours thereafter. Blood samples were collected by central catheter at the time of lavage.

BAL fluid and Blood samples were spun at 1500×g for 15 minutes to remove cells and cellular debris. The cell-free supernatant and plasma were stored at – 80°C until the time of analysis.

Patient’s clinical and paraclinical characteristics were recorded as the following: consciousness according to the Glasgow Coma Scale (GCS), saturation oxygen tension (SPO2), Blood Urea Nitrogen (BUN), Creatinine (Cr), WBC count, Platelet count, Hemoglobin, Arterial Blood Gas (ABG), body temperature (using a rectal probe) and blood pressure, pulse rate, respiratory rate. All measurements were done after drug administration for 1 week. Admitting diagnosis was selected from patient’s chart.

Patients also underwent the Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Assessment (SOFA) scoring systems for assessing mortality rate and organ function at baseline and 7 days thereafter.

Bronchoalveolar lavage

All mechanically ventilated patients were sedated with midazolam/morphine or fentanyl. A flexible fibroptic bronchoscope (Olympus, type 20D, New Hyde Park, NY, USA) was passed through endotracheal tube of the ventilated patients after preoxygenation (Fio2=1). After wedging into the right middle lobe, 4 successive 20ml irrigations of 0.9% saline were instilled and immediately aspirated. The recovered BAL fluid was pooled and immediately centrifuged (1500×g, 15 min) and then stored at -80°C.

Iron Concentration

Concentrations of total iron in the lavage supernatant and plasma were measured with a standard colorimetric assay (Bioassay systems, Hayward, USA).

Concentrations of Transferrin and Ferritin

Transferrin protein concentrations in lavage supernatants...
and plasma were analyzed by an immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany). Ferritin was measured by a chemiluminescence assay (DiaSorin-Liaison, Stillwater, MN).

**Statistical analysis**

All data were expressed as mean ± standard error of mean. To assess differences between the treatment groups demographic characteristic, the unpaired t test and the Mann-Whitney test were used for parametric and nonparametric variables, respectively. To assess differences between the time points in each treatment group, repeated-measure two-way analysis of variance was used to analyze changes in levels of markers. All tests reported a 2-sided P value with the level of significance set at 0.05.

**Results**

Between March 2009 and April 2010, 307 consecutive patients admitted to the ICU were screened and 24 met the criteria for enrollment. Twelve patients were enrolled in each group. The patients in each group were similar at baseline regarding to age admission APACHE II and SOFA score (Table 1). Intracranial Hemorrhage, Multiple traumas, Neurological etiology such as Multiple sclerosis were the main underlying causes of ICU admission of our cases. The changes in the levels of Iron, ferritin and transferrin in BAL fluid and plasma were studied at baseline and 72 hours thereafter. The comparisons of measured variables are shown in table 2 and 3. Iron levels were decreased significantly in BAL fluid after intervention and in comparison with control groups (P<0.001). Changes in ferritin levels were not significant in BAL fluid (P: 0.73). Unfortunately due to analytical problems, transferrin levels in lavage fluid were not reported but levels of this glycoprotein were decreased in plasma significantly (P: 0.018). Concentrations of Iron and ferritin were decreased in plasma but not significantly (P: 0.18 and P:0.056 respectively).

In comparison with deferasirox group, APACHE II scores were decreased after 1 week of follow up in comparison to control group ,the values were significant(P:0.006). SOFA scores did not change significantly between groups (P:0.08) (Table 4).

**Discussion**

The results of this study shows that Iron and Iron-related proteins didn’t change significantly after 72 hours of follow up in hyperoxic condition and deferasirox might be considered -along with other supportive measures and antioxidant - for prevention of hyperoxia-induced lung injury.

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**Table 1.** Demographic characteristics of patients.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=12)</th>
<th>Deferasirox (n=12)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>53±17</td>
<td>52.5±6.7</td>
<td>0.31</td>
</tr>
<tr>
<td>Male, No.</td>
<td>1</td>
<td>6</td>
<td>0.027</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>22.5±0.76</td>
<td>16.5±0.76</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SOFA score</td>
<td>7±0.36</td>
<td>5.8±0.53</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Reason of admission</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Multiple trauma</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Intracranial hemorrhage</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Neurological etiology</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

P values <0.05 considered as significant.

Data shown are mean ± SE

APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sepsis-related Organ Failure Assessment.

**Table 2.** Comparison of measured variables at baseline and 72 hours thereafter in lavage fluid.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=12)</th>
<th>Deferasirox (n=12)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Iron (mcg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>44±7.2</td>
<td>29.8±2.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>72</td>
<td>170±19.55</td>
<td>120±15.7</td>
<td></td>
</tr>
<tr>
<td><strong>Ferritin (ng/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>385±105.53</td>
<td>206.53±68.29</td>
<td>0.73</td>
</tr>
</tbody>
</table>

The values were reported as mean ± SE.

$t_{72}$: 72 hours after drug administration, P values <0.05 considered as significant.

Data shown are mean ± SE.
Iron is the most abundant metal ion in the body. Although it is required for essential function including biosynthesis and cell proliferation, if it is not appropriately balanced in biological tissues, it can form harmful free radicals such as hydroxyl radicals which subsequently could have deleterious effect on cell function and result in injury and death(20). Disruption of iron hemostasis in the lower respiratory tract have been reported in numerous pulmonary diseases such as pneumonia, Acute Respiratory Distress Syndrome (ARDS) and hyperoxic condition(21;22). Ghio and coworkers studied 14 patients with ARDS and 21 healthy volunteers the concentrations of total and nonheme iron were increased in the lavage fluid of patients. Concentration of hemoglobin, haptoglobin, transferrin, transferrin receptor, lactoferrin and ferritin in BAL fluid were all significantly increased in ARDS patients (23).

Several studies demonstrate role of iron in lung oxidative injury and hyperoxia(22;24-27). Yang et al in a study proved that there was no increase in the levels of intracellular antioxidants, inflammatory cytokines, and hemeoxygenase-1 in the hypotransferrinemic mouse lung exposed to hyperoxia (95% O$_2$) compared with those in wild-type mice, however there were elevated expressions of ferritin and lactoferrin in the lung of hypotransferrinemic mice, especially in the alveolar macrophages(28). Based on these studies it seems that iron was increased in lungs in hyperoxic condition and catalyzed oxidative stress process and may be limitation of iron availability prevent deleterious ROS generation and lung injury.

Iron chelation has been recently used for conditions without iron overload such as neurodegenerative, infectious, reperfusion injury, cardioprotection and others suggesting the role of iron as an oxidative-induced injury (29; 30). Deferoxamin has been studied in some in vitro and in vivo setting of lung injury (31-33) and significantly attenuate lung oxidative damage. In our previous study(34), using of deferasirox for hyperoxia-induced lung injury resulted in reduction levels of iron, ferritin, transferrin and marker of oxidative damage (8-Isoprostan, 8-Oxoguanin and Carbonyl protein) in lavage fluid but this was an uncontrolled study and in comparison with externally controlled patients, deferasirox was not effective in reduction of iron and iron-related protein, completed study with better design and larger number of patients is necessary to prove antioxidant effect of deferasirox.

In summary, we can conclude that Iron chelation could be considered in the setting of hyperoxic conditions along with other supportive measures for better toleration of oxygen therapy and patient care. However further clinical studies are required for definite recommendation.

### References


### Table 3. Comparison of measured variables at baseline and 72 hours thereafter in plasma

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=12)</th>
<th>Deferasirox (n=12)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (mcg/dl)</td>
<td>817.75±116.8</td>
<td>681±112.18</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>786.5±194</td>
<td>367.83±68.46</td>
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</tr>
<tr>
<td>Ferritin(ng/dl)</td>
<td>1077.08±211.48</td>
<td>1028.68±190.3</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>677.3 ± 158</td>
<td>547.5 ± 86.5</td>
<td></td>
</tr>
<tr>
<td>Trans(mg/dl)</td>
<td>106.67±9.2</td>
<td>90.58±6.6</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>73.17±8</td>
<td>66.08±11.06</td>
<td></td>
</tr>
</tbody>
</table>

$t_{72}$: 72 hours after drug administration, P values <0.05 considered as significant.
Data shown are mean ± SE.

### Table 4. Comparison of APACHE II and SOFA scores between groups.

<table>
<thead>
<tr>
<th>Score</th>
<th>Control (n=12)</th>
<th>Deferasirox (n=12)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>APACHE II</td>
<td>22.5±0.76</td>
<td>21.83±0.86</td>
<td>0.006</td>
</tr>
<tr>
<td>SOFA</td>
<td>7±0.36</td>
<td>7.1±0.47</td>
<td>0.08</td>
</tr>
</tbody>
</table>

P values <0.05 considered as significant.
Data shown are mean ± SE.
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