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The dilemma of hyperoxia following positive pressure mechanical ventilation: role of iron and the benefit of iron chelation with deferasirox

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Abstract. – *Background and Objective:* **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, which is thought among different mediators, transition metal ion, iron, by generation of very reactive free radicals which play an important role. Disruption of normal iron homeostasis has been reported in hyperoxic conditions. We hypothesized that chelation of iron can reduce hyperoxia-induced lung injury.**

Methods: **Mechanically ventilated patients, who received oxygen with FiO_2 >0.5 for at least 3 days, underwent bronchoscopy before and 72 hours after receiving "Deferasirox". Oxidative injury index and iron homeostasis markers were measured in lavage fluid and plasma.**

Results: **In 12 patients, the concentrations of 8-isoprostane (p=0.005), 8-oxoguanine (p=0.04), carbonyl proteins (p=0.04) – as markers of oxidative stress – decreased significantly in lavage fluid after intervention. Levels of iron-related proteins, ferritin (p=0.04) and transferrin (p=0.005) also decreased significantly in lavage fluid.**

Conclusion: **Deferasirox – as an iron chelator – decrease oxidative injury index in hyperoxic condition and it could be consider safe and beneficial agent, along with other supportive measures in hyperoxia-induced lung injury for better toleration of oxygen therapy.**

Key Words:

Hyperoxia, Lung injury, Iron, Iron chelator, Deferasirox.

Introduction

High fraction of inspired oxygen (FiO_2) is necessary for managing of hypoxic respiratory failure in critically ill patients, but high concentration of oxygen in presence of systemic inflammatory responses promote production of oxygen derived free radical species[1-3] which can overwhelm the antioxidant defenses of the lung and result in damage to major macromolecules within pulmonary cells such as DNA, protein, lipid and finally lead to damage to airway and pulmonary parenchyma[4-7]. Toxicity of oxygen radicals are increased in the presence of transition metal ion especially the most abundant, iron, which results in the generation of powerful hydroxyl radicals that easily react with major macromolecules within cells[8-10]. Some studies demonstrate the disruption of iron homeostasis in lung oxidative injury and hyperoxic condition, increases in the level of ferritin and lactoferrin and changes in transferrin and transferrin receptors have been reported in both human and animal studies[11-16]. High levels of hemoxygenase-1-enzyme responsible for iron metabolism-has been shown following hyperoxic insult[17,18]. Generally lung epithelial cells, through uptake and storage of iron within ferritin and lactoferrin[19-21] and export of intracellular iron by either ferritin and transferrin release[22], provide mechanisms to diminish iron stress. These defenses may become overwhelmed. Thus, pharmaceutical intervention for sequestration of iron could be acceptable in this

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situation. We hypothesized that iron chelation through inhibition of generation of free radicals could be useful for decreasing of hyperoxia-induced lung injury.

Desferrioxamine (DFO) has a very high affinity for Fe^{3+}, and is very efficient in preventing its reduction to Fe^{2+} and the participation of iron in oxidative stress process. Chelating of free iron by deferoxamine had been demonstrated to provide protective effect against oxidative stress in some investigations including lung injury[23-28]. Deferasirox is a new oral iron chelator that recently approved for treatment of iron overload in beta-thalassaemic patients[29]. This drug has more favourable side effect profile in comparison with deferoxamine[30] and it seems because of its small size and lipophilic structure could chelate intracellular iron better than deferoxamine[29,31,32].

Our aim is to assess the effect of Deferasirox on hyperoxia-induced lung injury and possible effect of this drug on better toleration of oxygen therapy.

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 and Health Services. Our clinical trial had been registered in Iranian Registry of Clinical Trials (IRCT) with a Registration ID in IRCT of "IRCT138709181497N1".

Study Population

Between March 2009 and April 2010, all critically ill patients under mechanical ventilation (due to different reasons) were reviewed for inclusion and those who received oxygen with FiO_2 more than 50% for at least 3 days and Positive End Expiratory Pressure (PEEP) at least 5 cm H_2O, and had the ability to take the 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 >2 mg/dl or U/O <0.5 cc/kg/hr, or dialysis), shock state (pH ≤7.2, MAP <60, PaO_2/FiO_2 ≤100), patients who received other anti-inflammatory drugs especially corticosteroids or N-acetyl cysteine, history of leukemia, bone marrow transplantation, thalassemia, iron deficiency anemia, patients who received iron chelator drugs within past 3 months, history of chemotherapy or immunosuppression within past 3 months, ferritin level less than 30 mcg/L, grade 2 or 3 of heart block and prolonged QT interval, positive viral markers for HIV, HBV and HCV, WBC <3000, ANC <1500, platelets <50.000 and cardiac problems such as EF <55%.

All patients with a stable clinical status underwent bronchoscopy alveolar lavage (BAL) before and 72 hours after receiving Deferasirox and followed up for 1 week. BAL was not performed if patients had oxygen saturation <90%, clinically unstable with hypotension (systolic blood pressure <90 mmHg), myocardial ischemia or cardiac dysrhythmia or if risk of bleeding was high. Blood samples were collected by central catheter at the time of lavage.

BAL fluid and blood samples were spun at 1500 microg 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.

All patients received 1500 mg deferasirox (Osveral, Osve Company, Ghazvin, Iran) in order to deal with a mean weight of 70 kg and the recommended dose 20 mg/kg for thalasemic patients.

Patient's clinical and paraclinical characteristics were recorded as the following: consciousness according to the Glasgow coma scale (GCS), saturation oxygen tension (SPO_2), blood urea nitrogen (BUN), creatinine (Cr), white blood cells (WBC) count, platelet count, hemoglobin, arterial blood gas (ABG), body temperature (using a rectal probe),blood pressure,pulse rate and respiratory rate. All measurements were done after drug administration for 1 week. Patient's mortality was recorded during this period of time. Admitting diagnosis was selected from a list of diagnostic categories; all that applied were noted.

Patients also underwent the Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Assessment (SOFA) scoring systems for assessing severity of disease and organ dysfunction from day 1 and 7 days after dosing.

The objective of study was to determine the differences in oxidative injury index before and after intervention with the deferasirox and effect of this drug on oxygen therapy.

Bronchoalveolar Lavage (BAL)

All mechanically ventilated patients were sedated with midazolam/morphine or fentanyl. A

SrCr = Serum Creatinine; ULN = Upper Limit of Normal

flexible fibroptic bronchoscope (Olympus, type 20D, New Hyde Park, NY, USA) was passed through endothracheal tube of the ventilated patients after preoxygenation (FiO_2=1). After wedging into the right middle lobe, 4 successive 20 ml liquates of 0.9% saline were instilled and immediately aspirated. The recovered BAL fluid was pooled and immediately centrifuged (1500 microg, 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, CA, USA).

Concentration 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, USA).

Oxidative Injury Index

Based on the injury to major macromolecules within cells, 8-oxoguanine as a marker of DNA damage, 8-isoprostane and carbonyl protein as an end product of lipid peroxidation and protein oxidation were chosen to analyze.

8-oxoguanine and 8-isoprotane concentrations in lavage supernatant and plasma were analyzed using commercially available enzyme-linked immunosorbent assay kit (Cayman Chemical, Ann Arbor, MI, USA).

Determination of Protein-Bound Carbonyl Groups

For assessment of protein carbonyls spectrophotometric 2,4-dinitrophenylhydrazine (DNPH) method was used. In summary sample proteins were precipitated with trichloroacetic acid (TCA, 20% final concentrations) and then collected by centrifugation for 10 min. A solution of 10 mM DNPH in 2 N HCl is added to each sample, with 2 N HCl only added to corresponding sample aliquot reagent blanks. Samples are kept in 37°C for 50 min with vortexing every 15 min; they were then precipitated with 20% TCA (final concentration) and centrifuged for 10 min. The supernatants were discarded; the protein pellets washed 3-5 times with 1 ml portions of ethanol/ethylacetate (1:1, v/v) to remove any free DNPH. Samples are then suspended in 6 M guanidine hydrochloride (GdmCl, dissolved in 20 mM phosphate buffer, pH 2.3) at 37°C for 30 min and centrifuged again for 10 min. Carbonyl contents were determined from the absorbance at 380 nm (Biotek Gen 5, Bio Tek Instruments, Winooski, VT, USA) using a molar absorption coefficient of 22,000 M^{-1} cm^{-1}[33]. Protein was determined with Bradford Protein Assay[34].

Statistical Analysis

All data were expressed as mean ± standard error of mean. For parametric data Student's paired *t*-test was used to compare data before and after intervention, for nonparametric data Wilcoxon test was used. Significance was defined as a p value of <0.05.

Results

Twelve patients (6 male, 6 female) with a mean age of 52.5 ± 6.7 were enrolled in the study. Cerebrovascular accidents, intracranial hemorrhage, multiple traumas, neurological etiology such as multiple sclerosis and surgical procedures were the main underlying causes of Intensive Care Unit (ICU) admission of our cases. The changes in the levels of oxidative injury index and iron homeostasis biomarkers in BAL fluid and plasma were studied before and after deferasirox challenge. The comparisons of measured variables are shown in Table I and II. 8-isoprostane, as a marker of lipid peroxidation, were significantly decreased in BAL fluid after treatment (p=0.005) (Figure 1). However, reduced plasma levels of this biomarker were not significant (p=0.5). 8-oxoguanine, as a marker of DNA damage, had shown the same pattern, significant decrease in BAL fluid (p=0.04) (Figure 2), but not significant in plasma (p=0.8). The amount of oxidized protein, expressed as nmol carbonyl/ml BAL fluid was markedly decreased after intervention (p=0.04) (Figure 3).

To determine the potential influence of Deferasirox on iron homeostasis concentrations of total iron, ferritin and transferrin were measured in lavage fluid and blood samples.

By binding iron, transferrin act as an antioxidant in the lower respiratory tract[35], chelation of iron by deferasirox, decrease significantly concentrations of this glycoprotein in the BAL fluid of patients (p=0.005) (Figure 4).

Table I. Comparison of measured variables before and after intervention in BAL fluid.

Variable	Before drug	After drug	*p*
Total iron (mcg/dl)	170.5 ± 19.5	120 ± 15.7	0.07
Ferritin (ng/ml)	385.2 ± 105	206.5 ± 68.2	0.005
Transferrin (mg/dl)	20.9 ± 6.4	10.64 ± 3.2	0.04
8-isoprostane (pg/ml)	92.5 ± 44.8	9.2 ± 4	0.005
8-oxoguanine (pg/ml)	9877 ± 1984	6234 ± 1734	0.04
Carbonyl protein (nmol/ml)	0.61 ± 0.12	0.31 ± 0.04	0.04

p: Level of significance (<0.05).

Table II. Comparison of measured variables before and after intervention in plasma.

Variable	Before drug	After drug	*p*
Total iron (mcg/dl)	789 ± 194.5	364 ± 68.4	0.1
Ferritin (ng/ml)	677.3 ± 158	547.5 ± 86.5	0.6
Transferrin (mg/dl)	73.1 ± 8.07	66 ± 11.06	0.2
8-isoprostane (mcg/ml)	0.81 ± 0.51	0.21 ± 0.12	0.5
8-oxoguanine (mcg/ml)	911.1 ± 175.8	711 ± 75.4	0.8
Carbonyl protein (nmol/ml)	0.16 ± 0.04	0.12 ± 0.02	0.8

p: Level of significance (<0.05).

Ferritin levels, which stores iron in a non-catalytically reactive form, were also decreased significantly in lavage fluid (p=0.04) (Figure 5) but not in plasma. Total iron concentrations were decreased in plasma and lavage fluid after intervention but the values were not significant (Figure 6).

No death neither major side effects were detected during intervention and after one week follow up of patients. Organ function especially liver (level of transaminase and bilirubin) and renal function (Blood Urea Nitrogen, serum creatinine) didn't change before and after inter-

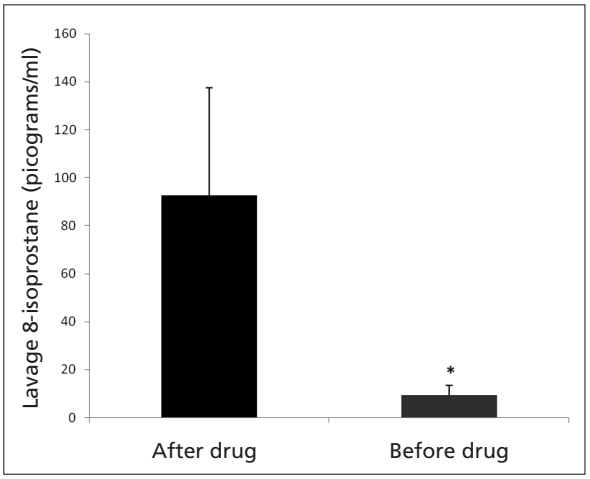

Figure 1. 8-isoprostane concentrations in the bronchoalveolar lavage fluid of patients before and after intervention. Levels of 8-isiprostane were decreased significantly after Deferasirox administration. *Significant decrease after intervention.

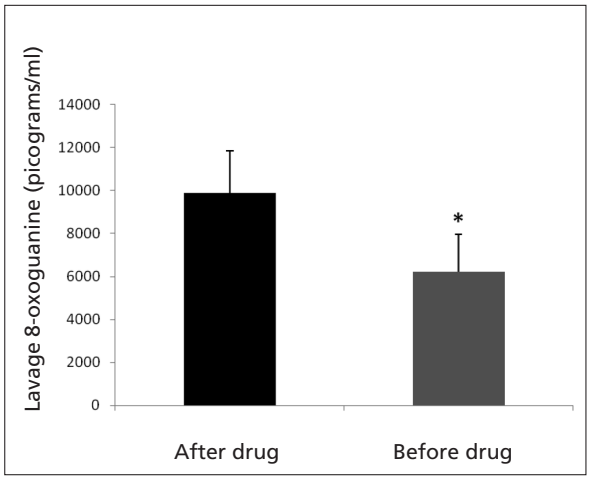

Figure 2. 8-oxoguanine concentrations in the bronchoalveolar lavage fluid of patients before and after intervention. Levels of 8-oxoguanine were decreased significantly after Deferasirox administration. *Significant decrease after intervention.

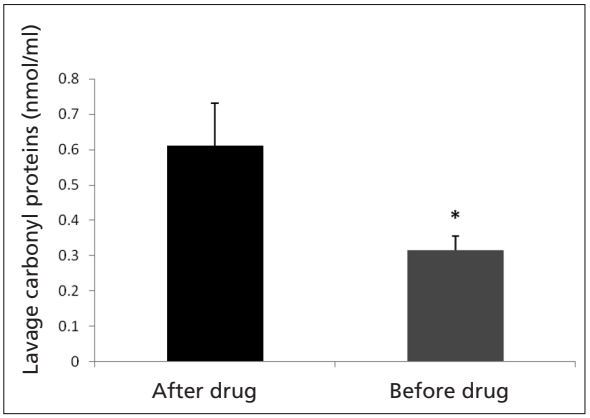

Figure 3. Carbonyl proteins concentrations in the bronchoalveolar lavage fluid of patients before and after intervention. Levels of oxidized proteins were decreased significantly after Deferasirox administration. *Significant decrease after intervention.

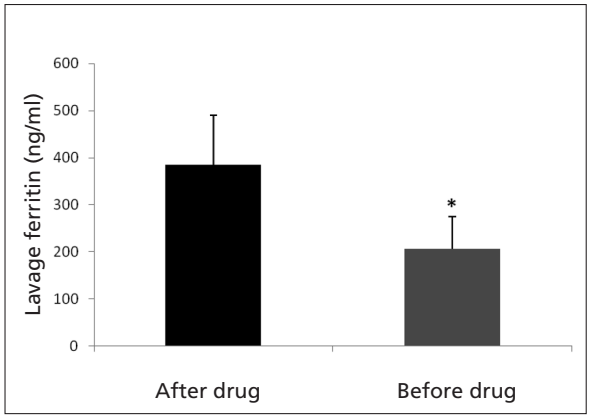

Figure 5. Ferritin concentrations in the bronchoalveolar lavage fluid of patients before and after intervention. Levels of ferritin were decreased significantly after Deferasirox administration. *Significant decrease after intervention.

vention. SOFA score didn't change significantly (from 5.8±0.53 to 5±0.28 after 1 week).

Discussion

The current study has shown that deferasirox – as an iron chelator – resulted in decline of oxidative injury markers in lavage fluid of patients who were under treatment with supraphysiological concentrations of oxygen. Thus, metal-catalyzed oxidation plays a key role in oxidative-induced lung injury and iron chelation may be effective as an antioxidant in this situation.

Normally iron remains tightly bound to storage and transport proteins including ferritin, transferrin and lactoferrin and these proteins act as antioxidants to preserve iron in less catalytically reactive form[6,8,35]. Levels of these haemostatic proteins have been reported to vary in hyperoxic conditions. Supplementation of iron free transferrin, for increase of plasma iron binding capacity result in decrease of iron-catalyzed oxidative stress in premature rabbits that were under hyperoxic condition[36]. Yang et al[37] 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) com-

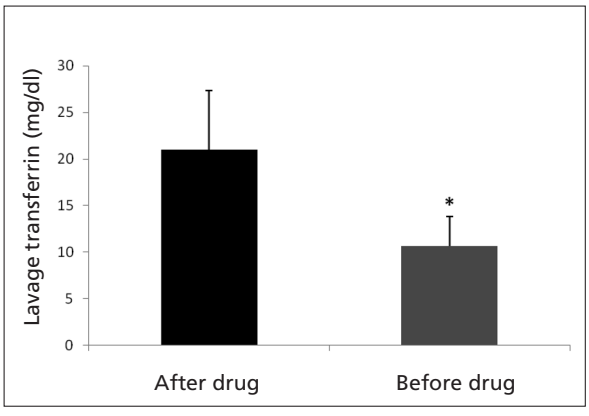

Figure 4. Transferrin concentrations in the bronchoalveolar lavage fluid of patients before and after intervention. Levels of this glycoprotein were decreased significantly after Deferasirox administration. *Significant decrease after intervention.

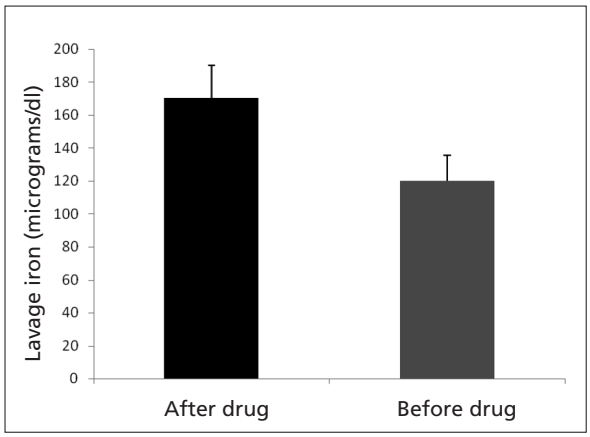

Figure 6. Total iron concentrations in the bronchoalveolar lavage fluid of patients before and after intervention. Levels of iron were decreased after Deferasirox administration, but it wasn't significant.

pared 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. In a study by Upton et al[12] ferritin protein levels were significantly down regulated in lungs of rodents treated with Lipo Poly Saccharide(LPS). However, nonheme iron levels were increased, and transferrin receptors are up regulated in rodent lung. Decreasing levels of ferritin and transferring in lavage fluid after our intervention may indicate that iron chelation could be useful in restoration of oxidant/antioxidant imbalance in lung injury.

Besides ferritin and transferrin, levels of oxidative injury index including 8-isoprostane (end product of lipid peroxidation), 8-oxoguanine (end product of DNA damage) and carbonyl protein (end product of protein oxidation) were decreased by iron chelation, increased levels of these end products have been reported in oxidative conditions including both pulmonary and nonpulmonary[38-40]. Therefore, based on our hypothesis, decreasing of iron through inhibition of generation of reactive oxygen intermediates could diminish lung oxidative injury.

Iron chelation has been recently used for conditions without iron overload such as neurodegenerative, infectious, reperfusion injury, cardioprotection[32,41] these unable indication for iron chelation, implies the role of iron as an oxidative-induced injury and possible role of chelation therapy as adjuvant, alternative or main therapy in many non-iron loading conditions. Some studies have been done with deferoxamine.

In one of the human studies using deferoxamine (DFO) on patients candidate for coronary artery bypass grafting (CABG), DFO was infused immediately after induction of anesthesia for 8 hours, the oxidative injury markers and hemodynamic parameters were measured and patients followed up for 1 year. They concluded that DFO infusion ameliorates oxygen free radical production, protects the myocardium against reperfusion injury and improve cardiac recovery and function[28]. In other studies addition of DFO to cardioplegic solution to prevent the generation of free radicals, improved cardiac index and decreased the lung injury which is frequently seen after surgery[24,25,42]. In this regard some research have been performed in order to evaluate role of iron chelators in lung injury. Ritter et al[23] showed that combination of N-acetyl cysteine plus DFO decreased lavage fluid thiobarbituric acid reactive species, carbonyl proteins, superoxide production, nonheme iron in rat after induction of acute lung injury by instillation of lipopolysaccharide (LPS), and also superoxide dismutase and catalase activity, inflammatory infiltration, proinflammatory cytokines were inhibited after intervention. They concluded that this combination significantly attenuated lung oxidative damage, mitochondrial superoxide production and histopathological alterations in injured lung.

In another animal experimental study, intratracheal administration of N-acetyl cyctein (20 mg/kg/day) alone or in combination with DFO (20 mg/kg/day) decreased the inflammatory response and the oxidative stress parameters in rats exposed to coal dust[43].

According to these studies DFO was effective in reducing or prevention of lung injury, but DFO not only has poor bioavailability and short half life but also because of its structure penetrates poorly to cells seems to be less effective than new generation of iron chelators[44,45]. The majority of injury by iron is induced by its free form within cells, the new generation of iron chelators such as deferiperone and deferasirox have a more lipophilic structure and better penetration within cells[29,31,46,47]. Therefore, significant reduction of oxidative injury biomarkers in our intervention may be related to these characteristics of deferasirox, also it is an oral agent with long half life, so its application is more convenient than DFO.

Deferasirox had well tolerated in our study, however our follow up duration was short, therefore just as an pilot study deferasirox might be suggested to enhance defenses against oxygen radicals and minimize or prevent lung damage.

Conclusions

Based on these data, deferasirox could be consider at least as safe agent in the setting of ICU, along with other supportive measures for better toleration of oxygen therapy in patients under hyperoxic conditions. However, we recommend further studies for investigation of key pharmacokinetics parameter of this drug following positive ventilation and also proper dosing schedule for establishment of dose-response relationship.

The major limitation in this study is the cross-sectional design. The ideal approach would be a longitudinal study with multiple samples from each individual but this is impractical because

the risk to the patients exceeds that of any benefit. Our research was conducted in an unicenter as a pilot study, so the number of patients compatible with our criteria is low, besides repeat of bronchoscopy procedure in a short interval (72 hour) not only has some adverse effect for patients but also increase risk of infections especially pneumonia.

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