Journal of Pharmaceutical Care
jpc.tums.ac.ir

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

Arezoo Ahmadi[1], Mojtaba Mojtahedzadeh[2], Farzaneh Javadi[3], Mohammad Abdollahi[4], Atabak Najafi[1], Sarah Mousavi[2*]

[1]Anesthesiology and Intensive Care Department, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[2] Clinical Pharmacy Department, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[3] Clinical Pharmacy Department, Faculty of Pharmacy, Islamic Azad University of Medical Sciences, Tehran, Iran.
[4] Faculty of Pharmacy, and Pharmaceutical Sciences Research Centre, Tehran University of Medical Sciences, Tehran, Iran.

ARTICLE INFO

Article type:
Original article

Keywords:
Iron
Hyperoxia
Oxidative Stress
Iron chelators
Deferasirox

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 $FiO_2 > 0.5$ 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.

J Pharm Care 2013; 1(3): 90-94.

► Please cite this paper as:
Ahmadi A, Mojtahedzadeh M, Javadi F, Abdollahi M, Najafi A, Mousavi S. Role of Iron in Hyperoxia-Induced Lung Injury: One Step Forward in Iron Chelation. J Pharm Care 2013; 1(3):90-94.

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

* *Corresponding Author*: Dr Sarah Mousavi
Address: 2nd floor, Old building, 16 Azar Ave., Keshavarz Blvd., Pharmacotherapy Department, Faculty of Pharmacy, Tehran14155-6451, Iran.
Tel/Fax: +982188814158
Email: Smousavi@razi.tums.ac.ir

ferritin and transportation by transferrin or lactoferrin and other mechanisms 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 deferoxamin 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 deferoxamin 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 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 30mcg/L, grade 2 or 3 of heart block and prolonged QT interval, positive viral markers for HIV,HBV and HCV,WBC<3 000,ANC<1500,Plt<50000, cardiac problems such as EF<55%.

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 20mg/kg for thallasemic patients. Osveral is a 500 mg dispersable tablet which diluted in enough water (~ 10ml) and gavage for the patints.

An external control study was conducted in same center to provide a robust comparison group. All critically ill patients who underwent mechanical ventilation and recieved oxygen with Fio2 more than 50% for at least 3 days and PEEP < 5 cm H2O were enrolled in control group. Exclusion criteria 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 – 80^0C 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 (SPO_2), 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 Π (APACHE Π) 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 fentanyle. A flexible fibroptic bronchoscope (Olympus, type 20D,New Hyde Park, NY,USA) was passed through endothracheal tube of the ventilated patients after preoxygenation(Fio2=1). After wedging into the right middle lobe, 4 successive 20ml liquates 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

Table 1. Demographic characteristics of patients.

	Control (n=12)	Deferasirox (n=12)	P Value
Age, year	53±17	52.5±6.7	0.31
Male, No.	1	6	0.027
APACHE II score	22.5±0.76	16.5±0.76	<0.01
SOFA score	7±0.36	5.8±0.53	0.08
Reason of admission			
Cerbrovascular accident	3	2	
Multiple trauma	4	3	
Intracranial hemorrahge	2	2	
Neurological etiology	2	3	
Others	1	2	

P values <0.05 considered as significant.
Data shown are mean ± SE_{mean}.
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.

	Control (n=12)		Deferasirox (n=12)		
	Baseline	t_{72}	Baseline	t_{72}	p Value
Iron (mcg/dl)	44±7.2	29.8±2.7	170±19.55	120±15.7	<0.001
Ferritin (ng/dl)	135.9±28	137.27±52	385±105.53	206.53±68.29	0.73

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

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.

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

	Control(n=12)		Deferasirox(n=12)		
	Baseline	t_{72}	Baseline	t_{72}	p Value
Iron (mcg/dl)	817.75±116.8	681±112.18	786.5±194	367.83±68.46	0.18
Ferritin(ng/d)	1077.08±211.48	1028.68±190.3	677.3 ± 158	547.5 ± 86.5	0.056
Trans(mg/dl)	106.67±9.2	90.58±6.6	73.17±8	66.08±11.06	0.018

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

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

	Control(n=12)		Deferasirox(n=12)		
	Baseline	Day 7	Baseline	Day 7	p Value
APACHE II	22.5±0.76	21.83±0.86	16.5±0.76	14.33±1.87	0.006
SOFA	7±0.36	7.1±0.47	5.8±0.53	5.2±0.27	0.08

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 availibility 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

1. Patel d, Goel A, Agrawal SB, Gary P, Lakhani K. Oxygen toxicity. JIAMC 2003; 4(3):234-7.
2. Fink M. Role of reactive oxygen and nitrogen species in ARDS. Curr Opin Crit Care 2002; 8(1):6-11.
3. Chow Ch, Abreu M, Suzuki T, Downey G. Oxidative stress and acute lung injury. Am J Respir Cell Mol Biol 2003; 29:427-31.
4. Lung J, McArdle Ph, O'reilly Ph, Matalon S. Oxidant-antioxidant balance in acute lung injury. Chest 2002; 122:S314-S320.
5. Nash G, Blennerhassett J, Pontoppidan H. Pulmonary lesion associated with oxygen therapy and artificial ventilation. N Engl J Med 1967; 276:368-74.

6. Zaher T, Miller E, Morrow D, Javdan M, Mantell L. Hyperoxia-induced signal transduction pathways in pulmonary epithelial cells. Free Radic Biol Med 2007; 42(7):897-908.
7. Altemeir W, Sinclair S. Hyperoxia in the intensive care unit: Why more is not always better. Curr Opin Crit Care 2007; 13:73-8.
8. McCord JM. Iron, free radicals and oxidative injury. Semin Hematol 1998; 35(1):5-12.
9. Emerit J, Beaumont C, Trivin F. Iron metabolism, free radicals and oxidative injury. Biomed Pharmacother 2001; 55:333-9.
10. Turi J, Yang F, Garrick M, Piantadosi C, Ghio A. The iron cycle and oxidative stress in the lung. Free Radic Biol Med 2004; 36(7):850-7.
11. Legan A, Melley D, Evans T, Quinlan G. Pathogenesis of the systemic inflammatory syndrome and acute lung injury: role of iron mobilization and decompartmentalization. Am J Physiol Lung Cell Mol Physiol 2008; 294:L161-L174.
12. Mateos F, Brock J, Perez-Arellano J. Iron metabolism in the lower respiratory tract.Thorax 1998; 53:594-600.
13. Drossos G, Lazou A, Panagopoulos P, Westaby S. Deferoxamine cardioplegia reduces superoxide radical production in human myocardium. Ann Thorac Surg 1995; 59:169-72.
14. Nakamura T, Keep R, Hua Y, Schallert T, Hoff J, Xi G. Deferoxamine-induced attenuation of brain edema and neurological deficits in a rat model of intracerebral hemorrhage. Neurosurg Focus 2003; 15(4):1-7.
15. Paraskevaidis L, Lliodromitis EK, Vlahakos D, Tsiapras D, Nikolaidis A, Marathias A, et al. Deferoxamine infusion during coronary artery bypass grafting ameliorates lipid peroxidation and protects the myocardium against reperfusion injury: immediate and long-term significance. Eur Heart J 2005; 26:263-70.
16. Prass K, Ruscher K, Karsch M, Isaev N, Megow D, Priller J, et al. Desferrioxamine induces delayed tolerance against cerebral ischemia in vivo and in vitro. J Cerebral Blood flow and Metabolism 2002; 22: 520-5.
17. Mousavi S, Mojtahedzadeh M, Abdollahi M. Place of iron chelators like Desferrioxamine and Deferasirox in management of hyperoxia-induced lung injury: A systematic review. Int J pharmacol 2010; 6:1-12.
18. Stumpf J. Deferasirox. Am J Health-syst Pharm 2007; 64:606-16.
19. Vanorden H, Hagemann T. Deferasirox.An oral agent for chronic iron overload. Ann Phamacother 2006; 40:1110-7.
20. Pantarulo S. Iron, oxidative stress and human health. Mol Aspects Med 2005; 26:299-312.
21. Lamb N, Gutteridge J, Baker C, Evans T, Quinlan G. Oxidant damage to proteins of bronchoalveolar lavage fluid in patients with ARDS. Crit Care Med 1999; 27(9):1738-44.
22. Sinclair S, Altemeir W, Matute-Bello G, Chi E. Augmented lung injury due to interaction between hyperoxia and mechanical ventilation. Crit Care Med 2004; 32:2496-501.
23. Ghio A, Carter J, Richards J, Richer L, Grissom C, Elstad M. Iron and iron related protein in the lower respiratory tract of patients with ARDS. Crit Care Med 2003; 31(2):395-400.
24. D'angio CT, Lomonaco MB, Chaudhry SA, Paxhia A, Ryan RM. Discordant pulmonary proinflammatory cytokines expression during acute hyperoxia in the newborn rabbit. Exp Lung Res 1999; 25:443-65.
25. Bailey T, Martin E, Zhao L, Veldhuizen R. High oxygen concetrations predispose mouse lungs to the deleterious effects of high stretch ventilation. J App Physiol 2003; 94:975-82.
26. Baleeiro C, Wilcoxen S, Morris S, Standiford Th, Paine R. Sublethal hyperoxia impaires pulmonary innate immunity. J Immunol 2003; 171:955-63.
27. Tateda K, Deng J, Moore Th, Neustead M, Paine R, Kobayashi N, et al. Hyperoxia mediates Acute lung injury and increased lethality in Murine Legionella pneumonia:The role of apoptosis. J Immunol 2003; 170: 4209-16.
28. Yang F, Coalson J, Bobb H, Carter J, Banu J, Ghio A. Resistance of hypotransferrinemic mice to hyperoxia-induced lung injury. Am J Physiol Lung Cell Mol Physiol 1999; 277:1214- 23.
29. Kontoghioghes GJ, Kolnagou A, Peng CT, Shah SV, Aessopos A. Safety issues of iron chelation therapy in patients with normal range iron stores including thalassaemia, neurodegenerative, renal and infectious diseases. Expert Opin Drug Saf 2010; 9(2):201-6.
30. Hershko Ch. Iron chelators in medicine. Mol Aspects Med 1992; 45: 276-81.
31. Ritter C, Dacunha AA, Isaber C, Adrades M, Reinke A, Lucchiari N, et al. Effects of N-acetylcysteine plus deferoxamine in lipopolysaccharide-induced acute lung injury in the rat. Crit Care Med 2006; 34(2):471-7.
32. Lalonde C, Ikegani K, Demling R. Aerosolized deferoxamine prevents lung and systemic injury caused by smoke inhalation. J App Physiol 1994; 77(5):2057-64.
33. Pinho R, Silveria P, Silva L, Streck E, Dal-Pizzol F, Moreira J. N-acetylcysteine and deferoxamine reduce pulmonary oxidative stress and inflammation in rats after coal dust exposure. Environ Res 2005; 99(3):355-60.
34. Mousavi S, Abdollahi M, Ahmadi A, Najafi A, Pazoki M, Hadjibabaei M, et al. The dilemma of hyperoxia following positive pressure mechanical ventilation: role of iron and the benefit of iron chelation with deferasirox. Eur Rev Med Pharmacol Sci 2011; 15(10):1141-48.