The effect of deferoxamine on survival of rats with induced peritonitis

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Received: 12/26/2010    Revised: 12/14/2011    Accepted: 01/10/2012

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Journal of Jahrom University of Medical Sciences, Vol. 10, No. 1, Spring 2012

Abstract

Introduction:
Oxidative damage is one of the major factors that lead to cell damage, organ dysfunction and mortality in sepsis. Thus, an attractive candidate for pharmacologic treatment of the septic syndrome is deferoxamine (DFX), an antioxidant iron chelator used for the removal of iron and a potential free radical scavenger. In this study, the impact of DFX administration on the survival of septic animals was evaluated.

Material and Methods:
Sepsis was induced in 20 rats, using the Cecal Ligation and Puncture method (CLP) and 10 rats randomly received subcutaneous DFX (total dose: 40mg/kg) twice and the other 10 rats received placebo (normal saline). All the rats had equal health status, environment and nutrition. The time of the death of the rats was recorded.

Results:
Deferoxamine increased the survival period in rats and the difference was statistically significant.

Conclusion:
Treatment with DFX significantly increases the survival of septic rats.

Keywords: Deferoxamine, Survival, Rats, Peritonitis

Introduction:
Infectious diseases have been always accompanied human beings and there is no historical era without them. Before detection and recognition of pathogens and development of modern methods for diagnosis and treatment, these diseases caused severe epidemics and even pandemics which imposed high casualties and economic and human damages on governments. Nowadays most of the etiologic microorganisms of infectious diseases are determined, their transmission routes are revealed and proper treatments of them are recognized. These days we don’t witness epidemic eruptions of infectious diseases which could slay millions of people. However infectious diseases are still a great important challenge for medicine (1). Severe sepsis and septic shock are medical
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Emergencies and early invasive treatment with empiric broad spectrum antibiotics must be established based on common bacterial species on the region (2). One of the pathologic processes during sepsis is oxidative stress which researchers have paid high attention to it in recent years. Oxidant materials transfer electrons of atoms of human body and deteriorate the situation. One of these materials is iron. \( \text{O}_2^- \) can combine with some proteins responsible for transferring metal groups such as iron or sulphur which might affect cell functions. Meanwhile deferoxamine (DFX) with the trade name of Desferal is an iron chelator used to decrease iron overload in thalassemic patients who receive cyclic blood transfusions and therefore have high serum iron levels (3).

In a study pulmonary damage was induced with injection of lipopolysaccharide to rats’ airways. These rats were divided into two groups and one of them received N-acetylcysteine and subcutaneous DFX. Those rats had less oxidative stress and mitochondrial dysfunction and their inflammatory responses and alveolar pathologies following administration of lipopolysaccharide were minimized (4). In another study, peritonitis was induced in rats by Cecal Ligation and Puncture (CLP) and DFX was administered as an iron chelator. Compared to the control group, those rats which received DFX had longer survival and lower levels of inflammatory and oxidative mediators (5). To the best of our knowledge these effects of DFX have not been studied in Iran. While one of the most important characteristics of every scientific hypothesis is its repeatability and regarding the point that administration of DFX has not been approved for treatment of sepsis in human; we performed this study to evaluate the effect of DFX on treatment of sepsis in rats.

Material and Methods:
This experimental study was performed to evaluate the effect of DFX on survival of rats with induced peritonitis. 40 male healthy rats with weight of about 240 to 250 grams were divided into 4 groups. According to the Cohen’s Chart minimum number of samples in each group was 8 ones. CLP was performed and peritonitis was induced. We used 75 mg/kg of intraperitoneal ketamine to sedate the rats. All ethical principles were respected all through the study. The study was performed in animal laboratory of Hamedan University of Medical Sciences in winter 2010. The rats were divided into 4 groups. No surgical or pharmacological interventions were performed in the first group (the control group).

The second group (the incision group) undergone surgery without CLP and didn’t receive any medications. This group was involved in the study to evaluate the effect of stress of surgery and incision on survival of rats. The third group (the DFX group) received two doses of 20 mg/kg of DFX; one immediately following CLP and the other dose 6 hours later. The fourth group (the placebo group) received placebo (normal saline) immediately after surgery and also 6 hours later. We chose normal saline because it is analogous to physiologic serum of the body and also other similar studies had used normal saline too. To prevent any biases we used the double blind method all through the study and the staff was not aware which sample belonged to which group.

At the end of the study and to evaluate the effect of DFX on survival time of rats with induced peritonitis, mean survival time of rats in each group was measured and compared with each other by One Way ANOVA test in SPSS software.
**Results:**
Results of the study demonstrated that the survival time of rats in the first group was not different from normal healthy rats (normal lifetime of healthy rats is 21600 hours) which revealed that there were not any confounding factors in the study such as diseases or nutritional problems. Moreover the rats in the second group which had only undergone surgical incision had the same survival time compared to normal healthy rats which revealed that the incision had not decreased rats’ survival time and was not a confounding factor in data analysis. 40% of rats in the third group which had undergone incision and CLP and had received DFX had normal lifetime and the other ones had died after 72 to 96 hours (figure 1). Survival time of rats in the fourth group which had undergone incision and CLP and had received placebo was 30 to 42 hours (figure 2). We used One Way ANOVA test to compare mean survival times between four groups. Statistical significance was recognized at F=36.138 and P=0.001 (table 1). To evaluate the difference between mean survival times we utilized Scheffe post hoc test. The differences between mean survival times in the DFX and the control group and also between the DFX group and the incision group were -12909.300 which were statistically significant (P=0.001). Furthermore the difference of means of the DFX group and the placebo group was 8655.600 and statistically significant (P=0.014) which evidenced that DFX could significantly increase the survival time of rats with induced peritonitis.

**Discussion:**
Sepsis resulted from peritonitis is among the most important challenges in medicine. Sepsis syndrome can impair functions of most of the human body’s organs and indeed it is a multi-organ malfunction syndrome. Inappropriate management of these urgent conditions might lead to initiation of the coagulation cascade and several vicious cycles which cause high mortality and morbidity (1). Several researchers are studying on developing novel pharmacological and non-pharmacological methods for controlling complications of septic processes and to reduce their high mortality (2).
DFX as an iron chelator is used in the states of iron overload. It can also impede the production of hydroxyl free radicals which could cause tissue and cell damages. Senoglu and colleagues induced septic shock by CLP in rats and reported that the levels of preinflamatory cytokines like TNF-α and IL-6 were significantly lower in rats which had received N-acetylcysteine or beta-glucan. In addition to that levels of superoxide dismutase and catalase (anti oxidant indices) in their liver were higher and taken as a whole, in those rats the severity of sepsis syndrome was less (6). de Souza and his colleagues cultured macrophages of septic rats after performing CLP and reported that levels of thiobarbituric acid reactive substances (TBARS) and carbonyl protein (which increases during sepsis) were lower in the group which had received N-acetylcysteine and DFX (along with basic supports) compared to the group which had only received normal saline and antibiotics as basic supports (7). Ritter and colleagues also performed CLP and induced sepsis in rats. In their study first group of rats received N-acetylcysteine, second group received DFX, third group received both N-acetylcysteine and DFX and the last group received normal saline along with ceftriaxone and clindamycin. They evaluated levels of tissue myeloperoxidase (index of neutrophilic infiltration), reactive thiobarbituric acid (oxidative stress marker), activity of catalase and superoxide dismutase (anti oxidant enzymes) and mitochondrial superoxide and found that in those rats which received both DFX and N-acetylcysteine, levels of myeloperoxidase and thiobarbituric acid were lower and balance of anti oxidant enzymes was much better. Survival rate of that group was 47%; while it was 10% in rats which received no treatments and 40% in rats which received antibiotics. They concluded that anti oxidant medications are beneficial in decreasing oxidative stress, improving anti oxidant profile and increasing survival of septic rats (8).

In Moch and colleagues’ study, rats had undergone CLP and sepsis too. One group received hydroxyethyl starch and the other group received hydroxyethyl starch plus DFX. They evaluated different organs of rats in both groups and reported that there was tissue damage due to oxygen radicals immediately after sepsis induction. Histological and biochemical studies revealed that treatment with iron scavengers such as hydroxyethyl starch and DFX could significantly decrease systemic oxidative stress. Their effects were more considerable in lungs and kidneys (9).

Teixeira and colleagues performed an experimental study on animals with induced pulmonary damage due to bleomycin injection to their lungs. They evaluated the inflammatory and anti inflammatory reactions by measurement of indices including LDH, complete blood count, neutrophil count, thiobarbituric acid, catalase, superoxide dismutase. Compared to the groups which had received DFX or N-acetylcysteine, the group which had received both DFX and N-acetylcysteine had lower levels of oxidative substances and higher levels of anti oxidant enzymes (10).

Ozdulger studied the effect of N-acetylcysteine on rats following CLP. Histopathological study on rats’ lungs demonstrated that in the group of rats which had received N-acetylcysteine levels of
myeloperoxidase and malondialdehyde were lower and frequency of apoptosis was less (11).

Results of these studies suggest beneficial effects of DFX and N-acetylcysteine in decreasing oxidative damage in animals; however in some studies the relation had not been statistically significant.

**Conclusion:** Results of this study about increased survival of rats in DFX group compared to placebo group are consistent with preceding studies. We involved the first and second groups in the study to control confounding factors and their results had no role in analysis of the effect of DFX on rats survival. We concluded that the DFX medication can significantly increase the survival time of rats with induced peritonitis.

Towards the end regarding results of preceding and our studies about characteristics of DFX as anti oxidant and iron scavenger, we suggest that DFX could be considered as a therapeutic option in treatment of sepsis syndrome and septic shock while approved by competent authorities.

**Acknowledgments:** This study was an approved research project in Hamedan University of Medical Sciences and the authors wish to acknowledge the invaluable cooperation and support of the University.

**References:**