Efficacy of Intravenous N-acetylcysteine as an Adjuvant Antioxidant Therapy in Acute Iron Toxicity in Rats

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KEYWORDS
Acute Iron Toxicity; Intravenous N-acetylcysteine; Antioxidant; Deferoxamine.

ABSTRACT
Iron is one of the heavy metals that is necessary for cell function. Acute and chronic exposure to high dose leads to oxidative damage. In the liver, iron overload affects hepatic mitochondrial respiration. Deferoxamine is well-established antidote for iron toxicity which acts as a chelator. N-acetylcysteine (NAC) is a safe over the counter mucolytic which is antioxidant and glutathione substitute properties. Its use as an antidote for some toxins is well-established nowadays. The aim of this work is to study the efficacy of intravenous N-acetylcysteine as an adjuvant therapy with deferoxamine in acute iron intoxication in rats. This study was carried out on twenty male albino rats, weighted 200-250 gm and divided into group I: 10 rats received 400 mg/kg elemental iron orally followed by 25 mg/kg subcutaneous deferoxamine and group II: 10 rats received 400 mg/kg elemental iron followed by 150 mg/kg IV NAC and 25 mg/kg subcutaneous deferoxamine. The results revealed that N-acetylcysteine use lowered both oxidative stress markers malondialdehyde MDA and cyclic adenosine monophosphate cAMP. On the other hand, the reduction of serum and hepatic iron levels and the elevation of Alanine Transaminase (ALT) and Aspartate Aminotransferase (AST) were statistically insignificant. It was concluded that intravenous N-acetylcysteine helps in reduction of oxidative stress caused by acute iron toxicity.

Introduction
Iron is a trace element necessary for normal cell metabolism; however it is cytotoxic in high amounts as there is no physiological mechanism to get rid of excess iron. Iron absorption is regulated to avoid accumulation. Iron overload leads to an increase of serum iron concentration. Acute toxicity presented with abdominal pain, vomiting, diarrhea and gastrointestinal bleeding in the first 6 hours after exposure. In the stage of stabilization (12 hours after ingestion) symptoms improve, as absorbed iron is removed from circulation by cellular uptake. After this mitochondrial function is affected and signs of shock, acidosis, coagulopathy, hyperglycemia or hypoglycemia and acute tubular necrosis start to develop. As a consequence, acute hepatic failure may develop within 2 days. After 2-4 weeks, delayed complications e.g. gastrointestinal scarring can occur (Skoczynska et al., 2007 and Yassin et al., 2017).

Acute iron toxicity can be diagnosed clinically together with elevated serum iron level (about 2-9 hours after exposure). Abdominal X-ray could reveal radiopaque shadows if iron tablets were ingested (Fleming...
et al., 2012). Iron toxicity is common among children due to wide availability of iron containing supplements for children or their mothers. It is rated as the most common toxicity in children less than 6 years old. Also, acute iron toxicity in adults occurs either due to suicidal attempts or iron overdose during pregnancy (Wessling - Resnick, 2017).

Acute iron intoxication leads to depletion of reduced glutathione in liver (Abu-Kishk et al., 2010). The reactive oxygen species (ROS) production and the resulting oxidative stress is a possible mechanism for tissue damage resulting from acute iron intoxication (Jaishankar et al., 2014). N-acetylcysteine (NAC) is a widely available, cheap, over-the-counter drug with antioxidant activity (Breitbart et al., 2011).

Standard treatment of iron toxicity depends on the use of deferoxamine as a chelator forming iron-deferoxamine complex which is a harmless compound excreted in urine (Umemura et al., 2017). Deferoxamine use may be associated with local or systemic side effects reactions e.g. shock, gastrointestinal disturbances, elevated liver enzymes, dizziness, paresthesia, seizures and renal failure. Hypersensitivity reactions also may occur. Moreover, Deferoxamine is pregnancy category C. Hence there is a need for discovering a safer antidote or adjunct for iron toxicity management to replace or reduce the dose of deferoxamine used (Howland, 1996 and Clajus et al., 2007).

Abu-Kishk et al. (2010) suggested that, the administration of oral NAC increases the absorption of iron through the gastrointestinal tract, causing higher serum iron levels. This explained elevated liver enzymes and hepatic destruction reported during his study. On the other hand, Breitbart et al. (2011) reported that intraperitoneal administration of NAC may decrease serum iron levels and mortality rate.

So, in this work we studied the effect of administration of intravenous NAC with IV deferoxamine on serum, hepatic tissue iron levels, and mortality rate. This work was designed to study the efficacy of intravenous NAC as adjuvant antioxidant therapy with deferoxamine in acute iron intoxication in rats.

Material and Methods

Study design and animals:

This study was carried out on 20 developing male albino rats, their weight ranged from 200-250 gram. They were housed in clean cages under standardized laboratory conditions; good lighting (12 hours' light/dark cycles) and good aeration. They were fed a standard laboratory diet and tap water. One week after accommodation, rats were randomly divided into two groups:

Group I: Ten rats received 400 mg/kg elemental iron orally (Abu-Kishk et al., 2010), followed by 25 mg/kg subcutaneous deferoxamine (Wongjaikam et al., 2016).

Group II: Ten rats received 400 mg/kg elemental iron (Abu-Kishk et al., 2010), followed by (150 mg/kg IV NAC (Breitbart et al., 2011) and 25 mg/kg subcutaneous deferoxamine) (Wongjaikam et al., 2016).

The study was carried out following approval of the medical research ethical committee of Tanta Faculty of Medicine- Tanta University (code: 33332/09/19).

Biochemical analysis:

Collection of blood samples:

At the end of the research, blood samples were collected by cardiac puncture using diethyl ether for light anesthesia (Beeton et.al, 2007). Blood was collected in sterile plain tubes then centrifuged at 3000 rpm for 15
minutes to separate serum. Serum was saved in aliquots at –80°C.

Serum was used to measure aspartate aminotransferase (AST) enzyme activity and alanine aminotransferase (ALT) enzyme activity, and iron levels colorimetrically using commercial kits (BioSystems Company, Spain kits number 11567, 11568 and 12509 respectively).

Tissue sampling:

Closely after blood sampling, animals were sacrificed by cervical dislocation under deep ether anesthesia. Liver tissues were taken, rinsed with saline to get rid of contaminating blood then dried by blotting with filter paper. They were weighed and frozen immediately at –80°C for biochemical analysis of tissue homogenate.

Preparation of tissue homogenates:

The liver tissue was homogenized using Glas-Col homogenizer. A 20% w/v homogenate was prepared in ice-cold phosphate buffer (0.01M, pH 7.4). The homogenate was centrifuged for 20 minutes at 3000 rpm for separation of the supernatant to avoid sample thawing and refreezing then kept at –80°C.

The aliquot was used for measuring MDA levels which were estimated by the double heating method to assess the lipid peroxidation (Draper et.al, 2001). This method depends on the use of spectrophotometry to measure the generated color by the reaction of thiobarbituric acid (TBA) with MDA at 532 nm. Then the absorbance coefficient of the MDA–TBA complex (absorbance coefficient of 1.56 × 105cm–1 M–1) was used to calculate the concentration of MDA and the results were expressed as mmol/mg tissue.

The CAMP level in Liver homogenates was assayed using specific Rat ELISA kit (R&D Systems, USA; Catalogs Number: KGE012B).

Iron level in liver homogenate samples was analyzed on a spectrophotometer (Mindray BA-88A) using a commercial kit (BioSystems Company, Spain kits number 12509) at 562 nm.

Statistical analysis:

Continuous variables were reported as mean ± standard deviation and compared with analysis of variance (Student’s t test). Categorical variables were expressed as frequencies and compared with $\chi^2$ test. Normality of data was determined using the D’Agostino-Pearsons test and verified using histogram plots. A two-sided P value of 0.05 was considered significant. Statistical analyses were performed using SPSS v.18 (SPSS, Chicago, IL, USA).

Results

As shown in table (1) and figure (1), serum iron level was 0.77700 mcg/dl in group I and 0.68540 mcg/dl in group II with no statistically significant difference between two groups (p=0.087).

In table (1) and figure (2) hepatic tissue iron level was 8.6375 µmol/mg in group I and 8.2320 µmol/mg in group II, with no statistically significant difference between two groups (p=0.776).

Table (1) and figures (3, 4) recorded that there was not statistically difference in alanine transaminase (ALT) and aspartate aminotransferase (AST) between group I and II (p= 0.148 and 0.115) respectively.

As shown in table (1) and figures (5, 6); malondialdehyde in group I was 2.28175 mmol/mg and in group II it was 1.65020 mmol/mg, with statistically significant
difference between two groups (p=0.002). Cyclic adenosine monophosphate in group I was 2.28175 ng/mg tissue, while in group II it was 1.65020 CAMP ng/mg tissue, with statistically significant difference between two groups (p=0.002).

Table (1): T-test comparing group I treated with iron and deferoxamine only and group II treated with iron, deferoxamine and intravenous N-Acetyl Cysteine.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>T</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron level mcg/dl</td>
<td>0.77700</td>
<td>0.68540</td>
<td>2.31</td>
<td>0.087</td>
</tr>
<tr>
<td>Hepatic tissue iron level µmol/mg</td>
<td>8.6375</td>
<td>8.2320</td>
<td>2.95</td>
<td>0.776</td>
</tr>
<tr>
<td>ALT IU/L</td>
<td>61.75</td>
<td>72.20</td>
<td>1.627</td>
<td>0.148</td>
</tr>
<tr>
<td>AST IU/L</td>
<td>264.00</td>
<td>328.00</td>
<td>1.8</td>
<td>0.115</td>
</tr>
<tr>
<td>MDA mmol/mg tissue</td>
<td>2.28175</td>
<td>1.65020</td>
<td>4.705</td>
<td>0.002*</td>
</tr>
<tr>
<td>CAMP ng/mg tissue</td>
<td>2.28175</td>
<td>1.65020</td>
<td>4.705</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Group I; Iron with deferoxamine, Group II = Iron with deferoxamine and NAC; SD; Standard Deviation, ALT; Alanine transaminase, AST; Aspartate Aminotransferase, MDA; Malondialdehyde, mmol; millimol, CAMP; Cyclic adenosine monophosphate, mcg/dl; microgram/deciliter, µmol; micromole/milligram, IU/L; international unit/liter, mmol/mg; millimol/milligram, ng/mg; nanogram/milligram.

Fig. (1): Correlation of mean of serum iron level between group I and group II.

Fig. (2): Correlation of mean of hepatic tissue iron level between group I and group II.
Discussion

Iron is considered an essential element for many cellular processes in living bodies. Elevated tissue iron results in several pathological conditions, especially in hepatic function (Fraga and Oteizab, 2002).

Acute iron toxicity is usually associated with oxidative stress. Oxidative stress results from excess release of free radicles exceeding the antioxidant mechanism capacity. Therefore, studies should be directed towards establishing novel means to limit iron-dependent damage, by minimizing the formation and release of these free radicals (Puntarulo, 2005).

In the current study, it was observed that there was no significant difference between both groups regarding serum iron level and tissue iron level, so adding of the intravenous N-acetylcysteine to the traditional antidotal therapy hasn't an additional effect on increasing iron absorption from gastrointestinal tract. In contrast to Abu-Kishk et al. (2010) who suggested that the oral administration of NAC after acute iron toxicity increases the iron
absorption from the gastrointestinal tract, leading to higher serum iron levels and hence more liver damage and mortality.

Abu-Kishk et al. (2010), Breitbart et al. (2011) and Boveris et al. (2012) discussed that hepatotoxicity induced by acute iron toxicity results from free radical generation and lipid peroxidation. Iron catalyzes hydroxyl radical creation, which is the most potent-free radical. The hydroxyl free radical triggers lipid peroxidation. Being highly reactive, free radicals produce harm at their site of origin which leads to reduced glutathione (GSH) depletion. Due to this affection of GSH system by acute iron toxicity, N-acetylcysteine is suggested as adjuvant treatment. N-acetylcysteine is a well-established potent antioxidant and glutathione substitute, which it widely used as an antidote for various intoxications reducing the lipid peroxidation and enhancing the endogenous antioxidant system (Hundekari et al., 2013).

Because of these mechanisms, it could be observed that, there was significant reduction in level of both malondialdehyde MDA and cyclic adenosine monophosphate cAMP in group II which are treated with the combination of deferoxamine and NAC. Shen et al. (2017) supports the current study by his observation about the improvement of bone marrow damage due to accumulation of reactive oxygen species (ROS) after acute iron toxicity when treated with the combination of deferoxamine and oral NAC. Also, Sripetchwandee et al. (2014) recommended the use of NAC both with chelators for restoring the brain function which affected by iron toxicity.

The current study showed insignificant elevation in both alanine transaminase (ALT) and aspartate aminotransferase (AST) in group II versus group I. Abu-Kishk et al. (2010) reported from an experimental study on rats that orally administered NAC significantly elevated liver enzymes. Also fragmentation of livers in all ten rats in group II was noticed during dissection indicating massive damage of liver. It could be explained that changing the dose of NAC could change the response regarding configuration of liver and the level of liver enzymes.

Conclusion:

Finally, it could be concluded that intravenous N-acetylcysteine helps in reduction of oxidative stress caused by acute iron toxicity which was be evident by the reduction of both oxidative stress markers malondialdehyde (MDA) and cyclic adenosine monophosphate (cAMP).

Limitation of study:

More studies must be done by different doses of intravenous N-acetylcysteine to show if there will be a significant correction of serum and hepatic tissue iron levels as well as liver enzymes Alanine Transaminase (ALT) and Aspartate Aminotransferase (AST).

References


تعرض هذه الدراسة على عشرين من ذكور الفئران التي تراوحت أوزانها بين 250-300 جم ومقسمة إلى مجموعتين. الأولى: 10 فئران تلقى 400 ملجم حديد/كم عن طريق الفم تليها 25 ملجم ديفيدروكسامين/كم تحت الجلد والمجموعة الثانية: 10 فئران تلقى 400 ملجم حديد/كم عن طريق الفم و 150 ملجم استيل سيستين/كم وردية و25 ملجم ديفيدروكسامين/كم تحت الجلد. أظهرت النتائج أن استخدام استيل سيستين قد خفض بشكل كبير علامات الإجهاد التأكسدي مالونديهيدل وسيلينك أدينوسين مونوفوسفات. و على الجانب الآخر فإن انخفاض مستوي الحديد في الدم و نسب الكبد و زيادته انزيمات الكبد الألإنين ترانس أميناز و أسبيرتات ترانس أميناز ليس له دلالة إحصائية. الخلاصة أن عطاء استيل سيستين وردية يمكن أن يساعد في الحد من الإجهاد التأكسدي الناجم عن سمية الحديد الحادة.