The Protective Effect of Selenium in Methotrexate Induced Nephrotoxicity in Rabbits

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Abstract
Methotrexate (MTX), a cytotoxic chemotherapeutic agent, is used in the treatment of acute lymphoblastic leukaemia, lymphoma, osteosarcoma, breast cancer, head and neck cancers and also in the therapy of non oncologic disorders such as rheumatic diseases and psoriasis. The aim of the present study was to examine the protective effect of selenium against the MTX-induced nephrotoxicity.

Materials and Methods:
21 rabbits were used and randomized in to three groups (7 rabbits each), all groups were maintained on standard chow diet throughout the experiment (8 weeks). Group 1 was treated with normal saline water (control), Group 2 with MTX (nephrotoxic) and Group 3 with MTX plus selenium. Induction of nephrotoxicity was carried out by administration of MTX to the rabbits in a dose of 0.25 mg/kg/day i.m for 8 weeks. Blood urea and serum creatinin were measured to asses renal function. Serum MDA levels and SOD activity also performed.

Results: Eight weeks of MTX treatment produced significant changes in renal function markers. Further it was found that serum MDA levels and SOD activity were significantly increased, while serum GSH levels were significantly decreased. Adding selenium to MTX was found to produced significant improvement in renal function.

Conclusion: selenium restored the altered renal function and possessed protection to the kidney against MTX induced nephrotoxicity.

KeyWords: Selenium , Methotrexate, Oxidative Stress

أجمال
أجريت هذه الدراسة لتحديد التأثير السمى لعقار الميثوتركسيت على الكلى بإضافته دواء السيكلين، وكان واحد وعشرون ذكرًا من الأرانب البيض (لوريند) استخدمت في هذه الدراسة. هذه الحيوانات قسمت بشكل عشوائي إلى 3جموعات، سعة أربعة أرانب في كل مجموعة. جميع الحيوانات أعطيت غداء طبيعي بيانيي طوال التجربة لمدة 8 أسابيع. الحيوانات في المجموعة الأولى أعطيت ماء مقتير عرضيًا واعتبرت مجموعة الليفين الأولى، في حين أن الحيوانات في المجموعة الثانية أعطيت عقار الميثوتركسيت 25 ملغ/كلغ/يومية، الحيوانات في المجموعة الثالثة أعطيت عقار السيكلين 0.5 ملغ/كلغ/يومية وعن طريق الفم. في نهاية الأسبوع الثامن، قلت جميع الأرانب.

وقد أخذت عينات الدم فحص المؤشرات التالية: مؤشرات وضوح الكليه (البوزارا والكرياتينين)، ومؤشرات الأكسدة (الأوميأودلي والجي). أجريت هذه القياسات وتتم التوصل إلى النتائج التالية:
1 - معالجة بعقار الميثوتركسيت أدى إلى تغييرات هامة في البوزارا والكرياتينين، مع مقارنة مع مجموعة الفم الأولي، زيادة البوزارا والكرياتينين بشكل ملمحظ (p<0.001). 
2 - تغييرات هامة في مؤشرات الأكسدة يعني مستوى مصل إم دي أي نشاط إم دي أو ومستوى جي إم إتش. حيث وجد أن مستوى مصل إم دي أي ونطاق إم دي أو زاد بشكل ملمحظ (0.001<p<0.001).
3 - إضافة السيكلين إلى إم دي إك إم دي أدى إلى انخفاض البوزارا والكرياتينين، بشكل ملمحظ (0.001)<p<0.001
4 - إضافة السيكلين إلى إم دي إك إم دي أو بشكل ملمحظ (0.001)<p<0.001 إلى انخفاض مستوى مصل إم دي أي، انخفاض نشاط إم دي أو
5 - وزيادة مستوى جي إم إتش.
Introduction

Methotrexate (MTX), a cytotoxic chemotherapeutic agent, is used in the treatment of acute lymphoblastic leukaemia, lymphoma, osteosarcoma, breast cancer, head and neck cancers and also in the therapy of non oncologic disorders such as rheumatic diseases and psoriasis (Albertioni, 1995; Bright, 1999; Jolivet, 1983; Te, 2000). High-dose methotrexate interrupts synthesis and repair of DNA and cell division by inhibiting several enzymes of the folic acid cycle. However, beside the therapeutic effects, there are also toxic effects including nephrotoxicity as well as gastrointestinal, central nervous system, hepatic, and bone marrow toxicity. The incidence of MTX-associated kidney injury is approximately 1.8% (range, 0% to 12%). Since more than 90% of MTX is excreted via the kidneys, nephrotoxicity is one of the significant reasons for restricting its use (Izzedine, 2005; Nyhlen, 1999; Vezmar, 2003). The main damages caused by MTX in the kidneys occur in a wide clinical range, varying from subclinical tubulopathy to acute renal failure Kintzel (2001). The increased production of reactive oxygen radicals is defined as one of the important causes of MTX-related renal toxicity. The use of MTX causes increased activities of malondialdehyde (MDA) and myeloperoxidase (MPO) and a decline in glutathione level in the kidneys and in other tissues Jahovic (2003). Furthermore, it is suggested that excessive nitric oxide (NO) production has an impact on renal damage developed due to MTX administration Uz (2005). These studies indicate that oxidative damage is an important mechanism involved in the pathogenesis of MTX-related nephrotoxicity. For this reason, it was thought that anti-oxidant therapy could be helpful to prevent or to ameliorate MTX nephrotoxicity. Studies have showed that several antioxidants are protective in MTX nephrotoxicity Hatfield (2006). However, MTX-related toxicity still remains one of the significant causes restricting its use in desired doses. Hence several trials have thus far been performed to ameliorate MTX nephrotoxicity. These studies indicate that selenium is an essential trace element, the importance of selenium (Se) in humans is well established, and its deficiency has caused serious health effects in humans, such as Keshan disease. Foods are major natural source of Se, and its levels generally depend on soil Se levels. Since its discovery as an important component of antioxidant enzymes, such as glutathione peroxidase (GPx), thioredoxin reductase (TrxR) and iodothyronine deiodinases (IDD), there has been an increased interest in the study of other Se-containing proteins (selenoproteins) or enzymes (selenoenzymes) Hatfield (1996). Selenium may also have immunomodulatory, anticarcinogenic and anti-atherogenic activities. The antioxidant activity of selenium is mainly accounted for by virtue of its role in the formation and function of the selenium-dependent glutathione peroxidases (Schwarz, 1957; Surai, 2002).

The goal of our study is to ascertain biochemically whether selenium has any protective effect on MTX induced nephrotoxicity in rabbits.

Materials and Methods

Animals and drugs:
A total of 21 New Zealand White Male Rabbits aged 3–4 months with weight of 1.5 – 2 kg, were used in the study. The animals were placed in the animal house, in a group caging system, at controlled temperature (25±2 °C) and ambient humidity. Lights were maintained on a 12-h light/dark cycle. The animals had free access to water. All the experimental studies were conducted in the Department of Pharmacology, College of Medicine, AL-Qadisyia University, in accordance with the guidelines for the Care and Use of Laboratory Animals; the experimental study was approved by the Ethical Committee of AL-Qadisyia College of Medicine.

Methotrexate (EBEWE Pharma, AUSTRIA) was used in a dose of 0.25 mg/kg/day i.m were given to the rabbits according to body weight once daily Novaes (1996).

Selenium was used in a dose of 0.05 mg/kg/day orally Van vleet (1980). A 100 mcg tablet (Jamieson laboratories Canada.941767) dissolved in D.W Abdo (1994) and the dose was given to the rabbit according to body weight once daily through stomach tube.

**Induction of nephrotoxicity:**

Induction of nephrotoxicity was carried out by administration of MTX to the rabbits in a dose of 0.25 mg/kg /day i.m for 8 weeks Novaes (1996).

**Experimental plan:**

After two week acclimatization period, the animals were randomly separated in to three groups (7 rabbits each); **Group 1** was treated with normal saline water (control or placebo), **Group 2** with methotrexate (MTX, nephrotoxic) and **Group 3** with MTX plus selenium (MTX+Se). The duration of treatment was 8 weeks. On end of 8th week morning the animals were sacrificed. The blood was collected in clean dry test tubes and allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 3000 rpm for 10 min. The blood, collected a centrifuge tube that contained sodium citrate, as anticoagulant, was centrifuged at 2500 rpm for 10 min and the plasma separated was used for estimations.

**Biochemical evaluation and measurement of antioxidants activity:**

Blood samples were collected and analyzed for blood urea nitrogen (BUN) and serum creatinine (Scr) by using the commercial kits Burtis (1999), Henry (1974). Serum MDA and GSH levels were determined using methods of Buegue et al (1978) and Beutlar (1975) respectively. Serum SOD activity was determined according to Winterbourn et al (1975).

**Statistical analysis:**

Two software programs were used for the analysis of data. These were SPSS version 16 and Microsoft Office Excel 2007. One way ANOVA test was used for comparison of mean of blood urea and serum creatinin among the three study groups Data were presented as mean ±SEM. P-value would be considered significant if it was less than 0.05.

**Results:**

Treating a MTX to rabbits for eight weeks resulted in significant changes in renal function tests, as compared to placebo group. Blood urea and serum creatinin were significantly increased (p< 0.001). Table (1)

| Table (1): Changes in serum renal function markers of rabbits treated with MTX for 8 weeks, (N=7). |  |  |
As compared to nephrotoxic group, a combination drug treatment resulted in the following changes in renal function parameters:

Adding Selenium to MTX was found to be significantly (p< 0.001) reduced blood urea (by a mean of 36.857) and serum creatinin (by a mean of 1.228) Tables (1,2). Figures(1,2).

In comparison with placebo group, MTX treatment for eight weeks resulted in significant changes in oxidation parameters namely serum MDA level, SOD activity and GSH level. It was found that serum MDA levels and SOD activity were significantly increased (p< 0.001) while serum GSH levels were significantly decreased (p< 0.001). Table (3)

Adding Selenium to MTX was found to be significantly (p< 0.001) reduced MDA(by a mean of 1.431 Mmol/L), SOD (by a mean of 0.122 U/ml) and increased GSH (by a mean of 2.381 Mmol/L).Table (4) Figures(3,4,5).

Table (2): Changes in serum renal function markers of rabbits treated with MTX +Se for 8 weeks, (N=7).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>MTX</th>
<th>MTX+Se</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood urea (mg/dl)</td>
<td>placebo</td>
<td>26.857 ± 3.712</td>
<td>68..714 ± 2.740</td>
<td>p&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>MTX</td>
<td>68.714 ± 2.740</td>
<td>36.857 ± 2.086</td>
<td></td>
</tr>
<tr>
<td>Serum creatinin (mg/dl)</td>
<td>placebo</td>
<td>0.8 ± 0.0617</td>
<td>1.614±0.116</td>
<td>P=0.001</td>
</tr>
<tr>
<td></td>
<td>MTX</td>
<td>1.614±0.116</td>
<td>1.228±0.080</td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Changes in serum oxidation parameters of rabbits treated with MTX only for 8 weeks, (N=7).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>MTX</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA μmol/L</td>
<td>Control</td>
<td>0.363 ± 0.0539</td>
<td>2.164 ± 0.1848</td>
</tr>
<tr>
<td></td>
<td>MTX</td>
<td>2.164 ± 0.1848</td>
<td></td>
</tr>
<tr>
<td>SOD U/ml</td>
<td>Control</td>
<td>0.998 ± 0.0665</td>
<td>2.055 ± 0.0541</td>
</tr>
<tr>
<td></td>
<td>MTX</td>
<td>2.055 ± 0.0541</td>
<td></td>
</tr>
<tr>
<td>GSH μmol/L</td>
<td>Control</td>
<td>5 ± 0.17</td>
<td>1.7 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>MTX</td>
<td>1.7 ± 0.18</td>
<td></td>
</tr>
</tbody>
</table>

Table (4): Changes in serum oxidation parameters of rabbits treated with MTX and MTX+Selenium for 8 weeks, (N=7).

| Group           | Parameters      | MDA Mmol/L | SOD U/ml | GSH Mmol/L |
|-----------------|-----------------|------------|----------|------------|------------|

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MTX only | 2.164±0.1848 | 2.055±0.0541 | 1.7±0.18
MTX+Se | 0.733±0.0255* | 1.933±0.0256ns | 4.1±0.11*

The data expressed as means ± SEM  *P<0.05  ns=P>0.05

Figure 1: Histogram shows the difference in mean of blood urea among the 3 treatment groups

Figure 2: Histogram shows the difference in mean of serum creatinin among the 3 treatment groups
Figure 3: Histogram shows the difference in mean of serum MDA (Mmol/L) among the 3 treatment groups.

Figure 4: Histogram shows the difference in mean of serum SOD (U/ml) among the 3 treatment groups.
Discussion

The major findings of the present study are that long term renal function may be decreased by treatment with methotrexate. The glomerular filtration rate was measured as creatinin clearance these results in agreement with Peter Seideman, Roland Muiller-Suur (1993). Nephrotoxicity arises through two primary mechanisms. The first is crystal nephropathy, which occurs when MTX and its metabolites precipitate within the renal tubules. In the early stages of crystal precipitation, urine microscopy demonstrates renal tubular epithelial cells and, rarely, MTX crystals Perazella (2010).

By comparison, drug crystals are not present in urine with an alkaline pH, as alkalinization greatly increases the solubility of MTX. Crystal nephropathy initially manifests with asymptomatic elevations in serum creatine levels and then progresses to tubular necrosis and more severe renal injury. The second mechanism of HDMTX-related renal injury is direct tubular toxicity; MTX induces the formation of oxygen radicals in the kidney, with subsequent cellular injury Perazella (2010), Oktem (2006). Methotrexate leads to a reduction in antioxidant enzymatic defense capacity and causes lipid peroxidation in renal tissue , which might be one of the reasons for MTX-induced nephrotoxicity Oktem (2006). Lipid peroxidation, which is caused by reactive oxygen species, has been implicated in the pathogenesis of MTX induced kidney injury. The most widely used marker of lipid peroxidation is formation of MDA. Serum MDA level was significantly higher in MTX treated than in placebo rabbits. Similar findings were reported by Kaplowitz et al. (1996); Kaplowitz et al. (2000) and Suleyman Uraz et al. (2008). MDA level is widely utilized as a marker of lipid peroxidation. Thus, the increase in MDA level is an indication of elevated oxidative stress condition. The finding of elevated lipid peroxidation product (MDA) in our study suggests that lipid peroxidation might be an
important contributing factor for the development of MTX mediated tissue damage Xia et al (2003).

Antioxidant enzymes, mainly SOD and GSH are the first line of defense against free radical induced oxidative stress. SOD is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to hydrogen peroxide Kaplowitz (1996). ROS such as hydrogen peroxide, super oxide anion and hydroxyl radicals are generated under normal cellular conditions and are immediately detoxified by endogenous antioxidants like GSH and SOD but excessive ROS accumulation by MTX causes antioxidant status imbalance and lead to lipid peroxidation with GSH depletion. The observed increase in SOD activity is an adaptive mechanism to oxidative stress. High levels of serum SOD activity indicated the presence of high level of superoxide anion, which represents an indicator to high degree of oxidative stress, therefore increased amounts of substrate of superoxide results in a stimulation to increase synthesis of serum SOD in order to provide protection OHTA Yoshiji et al (2006).

Selenium can enhance antioxidant ability by enhancing the activities of antioxidant enzymes and by increasing contents of the antioxidants. Se is crucial in several enzymes with physiological antioxidant properties, including GPx and thioredoxin reductase. GPx scavenges H2O2 and lipid hydroperoxides, using reducing equivalents from glutathione and protecting membrane lipids and macromolecules from oxidative damage. Therefore, this trace element could be useful as a free radical scavenger compound against stress conditions in several tissues, including the kidney McCord (1969), Watanabe et al (1997). These findings are in agreement with Xia et al. (2003).

Hence, in the present study, MTX-induced increase in serum creatinine and urea levels was significantly blocked by Se administration. The protective effect of Se on creatinine and urea concentrations can be attributed to its antioxidant properties, as it has been found that ROS may be involved in the impairment of GFR Pedraza-Chaverri et al (2000).

So the present study demonstrates the antioxidant properties of Se that possesses free radical scavenging property against MTX induced nephrotoxicity in rabbitts.

References


