The Ameliorative Effect of Fimasartan against Methotrexate-Induced Nephrotoxicity in Rats

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Abstract

Drug-induced acute kidney injury is a serious disorder. Oxidative stress has a key role in its initiation and progression. In this study, the possible ameliorative effect of fimasartan against methotrexate-induced nephrotoxicity was investigated in comparison with α-tocopherol in rats. Wistar rats were allocated into six groups and treated as follows: group I received water on a daily basis for 8 successive days; group II received methotrexate (20 mg/kg) on day 1, followed by water for 7 successive days; group III received fimasartan (3 mg/kg/day) for 7 successive days; group IV received α-tocopherol (1 g/kg/day) for 7 successive days; group V received methotrexate (20 mg/kg) on day 1, followed by fimasartan (3 mg/kg/day) for 7 successive days; and group VI received methotrexate (20 mg/kg) on day 1, followed by α-tocopherol (1 g/kg/day) for 7 successive days. Finally, after euthanization of each animal by diethyl ether, the samples were collected for analysis.

Administration of fimasartan and α-tocopherol resulted in a significant decline in serum creatinine and urea, a reduced production of reactive oxygen species (ROS) in renal tissue, improved renal histology, and reduced tubular obstruction via precipitation of MTX and its metabolites within the renal tubules. Fimasartan and α-tocopherol had comparable ameliorative effects in rats. Conclusion, fimasartan has ameliorative effects, comparable to those of α-tocopherol, on methotrexate-induced nephrotoxicity in rats.

Keywords: Nephrotoxicity, Methotrexate, Oxidative stress, Fimasartan, α-Tocopherol.

Introduction

Acute kidney injury (AKI) is a serious medical problem characterized by a rapid and reversible decline in renal function (1). It is associated with a high risk of irreversible renal injury, poor prognosis, and high healthcare costs (2). Many important medications have been reported to cause AKI, which limits their clinical usefulness (3). Among these agents, methotrexate (MTX), which is a widely used antimetabolite, has been reported to cause AKI in about 12% of patients receiving high dose-MTX (HDMTX) (4). HDMTX therapy, defined as the administration of MTX in doses exceeding (500 mg/m²), is generally used in chemotherapy against various malignancies (5). MTX-induced AKI is a complex process that arises from tubular obstruction via precipitation of MTX and its metabolites within the renal tubules (5,6), as well as direct tubular toxicity linked to inflammation, mitochondrial dysfunction, and increased production of reactive oxygen species (ROS) in renal tissue (4-8).

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Given that oxidative stress (OS) has a key role in the development and progression of MTX-induced renal injury, many studies have been directed to identify interventions that promote the antioxidant defences of the cells in order to circumvent MTX-induced AKI development or its complications, and the results are encouraging (7, 9-11). Previous researches have proved that α-tocopherol (α-Toc), which is the predominant and most biologically active form of vitamin E, can mitigate renal injury and inhibit renal fibrosis due to its potent antioxidant and anti-inflammatory properties (12-14). Furthermore, high doses of α-tocopherol were shown to protect the renal and hepatic tissues against oxidative damage induced by various drugs (14). Fimasartan (FMS) is an efficacious and potent angiotensin II receptor blocker (ARB) that was recently developed and approved in Korea as an antihypertensive medication (15). It is metabolically stable and chiefly excreted via the bile, and its use has proved that α-tocopherol can mitigate renal injury and inhibit renal fibrosis due to its potent antioxidant and anti-inflammatory properties (15, 18, 19). Besides, Cho et al. (2018) found that fimasartan preserves kidney structure and function in a murine model of ischemia-reperfusion injury through its anti-inflammatory and antiapoptotic properties (20). All these factors make fimasartan an attractive candidate to be examined as a renoprotective adjuvant to the standard MTX chemotherapy.

In view of the above considerations, this study was conducted to examine the possible ameliorative activity of fimasartan, in comparison to α-tocopherol, against MTX-induced renal injury in rats, pointing to its ability to suppress OS.

**Materials and Methods**

**Chemicals, drugs, and kits**

(+)-α-Tocopherol was obtained from Santa Cruz Biotechnology Inc. (Texas, USA). Fimasartan potassium trihydrate was purchased from Novochemistry (Loughborough, UK). Methotrexate (50mg/2ml) solution for injection was supplied from Mylan S.A.S. (Saint-Priest, France). Diethyl Ether (ROMIL LTD, Cambridge, UK) and phosphate-buffered saline (EuroClone, S.p.A., Milan, Italy) were also used in the study. All enzyme-linked immunosorbent assay (ELISA) kits utilized in the study were obtained from MyBioSource, Inc. (California, USA) and include: rat blood urea nitrogen (BUN) ELISA kit, rat creatinine (Cr) ELISA kit, rat malondialdehyde (MDA) ELISA kit, and rat superoxide dismutase [Cu-Zn] (SOD-1) ELISA kit.

**Animal selection**

Thirty-six adult Wistar rats (8 weeks old) of both sexes, weighing 150-240 g, were used in the present study. They were obtained from and maintained in the Animal House at the College of Pharmacy/University of Baghdad under conditions of controlled temperature, humidity and light periodicity (12-hour light/dark cycle). They were fed commercial pellets and tap water ad libitum throughout the experimental period. To get adapted, these rats were routinely handled and acclimatized for 7 days in the above-stated conditions before drug administration.

**Experimental protocol**

This study was approved by the Scientific and Ethical Committees of the College of Pharmacy/University of Baghdad. The rats employed in this study were randomly divided into six groups of six rats each, as follows:

**Group I (negative control group):** Rats received sterile water for injection in a volume of (6 ml/kg) intraperitoneally (21) for 8 days starting from day 1.

**Group II (MTX group):** Rats received a single dose of MTX (20 mg/kg) intraperitoneally on day 1, followed by daily intraperitoneal (IP) administration of sterile water for injection (6 ml/kg) for 7 days starting from day 2 (22).

**Group III (FMS group):** Rats received a daily IP injection of fimasartan (3 mg/kg/day) for 7 successive days. A solution of (0.05% w/v) FMS was prepared by dissolving fimasartan potassium trihydrate in water on the day of administration (18, 23, 24).

**Group IV (α-Toc group):** Rats received α-tocopherol (1 g/kg/day) orally for 7 successive days (14).

**Group V (MTX plus FMS group):** Rats received a single dose of MTX (20 mg/kg) intraperitoneally on day 1, followed by daily IP injection of fimasartan (3 mg/kg/day) for 7 successive days starting from day 2.

**Group VI (MTX plus α-Toc group):** Rats received a single dose of MTX (20 mg/kg) intraperitoneally on day 1, followed by daily administration of α-tocopherol (1 g/kg/day) orally for 7 successive days starting from day 2.

**Samples collection and preparation of kidney tissue homogenate**

After twenty-four hours from the final drug administration, blood samples were withdrawn from the carotid artery (at the neck) and collected in gel activated tubes and allowed to stand for 30 minutes to clot. Then, it was centrifuged at 3000 rpm for 15 minutes using a centrifuge (EBA 20, Andreas Hettich GmbH & Co. KG, Germany) to obtain serum (25). The obtained sera were utilized for the estimation of urea and creatinine levels.

Next, all rats were sacrificed by cervical dislocation under diethyl ether anaesthesia and kidney tissues were isolated and processed for analysis (25). Briefly, the kidneys were rapidly excised, cleaned from fatty tissues, and washed with a pre-cooled PBS (pH=7.4, 4°C) to rinse away any residual blood. Then, each kidney was blotted on
filter paper, weighed, and chopped into fine pieces. For each rat, the left kidney was used to prepare the kidney tissue homogenate by adding 0.4 g of the minced tissue and 3.6 ml of PBS (pH=7.4, 4°C) into a tube (26). Homogenization was then accomplished using a tissue homogenizer (Dyna-Passion® WT130, Success Technic Industries, Selangor, Malaysia) at set 3 for 1 minute at 4°C. Samples were kept on ice throughout all the above-mentioned steps. The resultant suspension was then subjected to a freeze-thaw cycle and centrifuged in a refrigerated centrifuge (HERMLE Labotechnik GmbH, Germany) at 10,000 rpm for 10 minutes at 4°C. The resultant supernatant was immediately collected and stored at −20°C until the day of analysis when it was used for the estimation of MDA and SOD-1 levels (26,27).

Biochemical analysis

Serum levels of kidney function biomarkers, blood urea nitrogen (BUN) and creatinine (sCr), were measured using ELISA kits according to the manufacturers’ instructions. Moreover, to assess the oxidant/antioxidant status in the tissue, the concentrations of MDA and SOD-1 were quantified in the renal tissue homogenate by sandwich ELISA method according to the kit manufacturers’ instructions (11, 22).

Measurements of the relative kidney weight (kidney index)

On the morning of sacrifice day, the bodyweight of each rat was measured. Then, the rats were euthanized and the weights of right and left kidneys were measured immediately after harvesting the kidneys from the rat carcasses. Then, the kidney-to-body weight ratio, also called the relative kidney weight or kidney index (KI), had been calculated for each rat by dividing the total right and left kidney weights by the total body weight of the rat, then multiplying the result by 100 (9).

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Analysis was carried out using Statistical Package for Social Sciences (SPSS, version 25) software. The differences between the groups were evaluated by a two-way analysis of variance (ANOVA). The differences among the groups were considered statistically significant at a P value of less than 0.05 (P<0.05).

Results

Effects on serum markers of kidney function

(Table 1) and (Figures 1 and 2) revealed that administration of MTX in group II resulted in a significant increase of BUN and sCr levels compared to the negative control group (group I) (P<0.05). At the same time, there were no significant differences in the FMS and α-Toc groups when compared to group I (P>0.05). Besides, comparing the FMS group and α-Toc group with the MTX group revealed significant differences between them (P<0.05).

Interestingly, rats in the MTX plus FMS group showed a significant decrease in BUN and sCr levels compared to the MTX group (P<0.05). Similarly, rats in MTX plus α-Toc group showed significantly lower levels compared to the MTX group (P<0.05), as shown in (Table 1) and (Figures 1 and 2).

By comparing the levels of BUN and sCr in rats treated with MTX alone (group II), FMS alone (group III), and MTX followed by FMS (group V), the obtained results showed that there were significant differences between the three groups (P<0.05). Likewise, when we compare BUN and sCr levels among the MTX group, α-Toc group, and MTX plus α-Toc group, statistically significant differences between them can be noted (P<0.05), as shown in (Table 1) and (Figures 1 and 2).

The same table also showed a significant difference in BUN levels when we compare the MTX plus FMS group to the MTX plus α-Toc group (P<0.05), which has significantly lower BUN levels compared to the MTX plus FMS group. However, there is a nonsignificant difference in sCr levels when we compare the MTX plus FMS group to the MTX plus α-Toc group (P>0.05).

Table 1. Effects of fimasartan on the serum levels of BUN and creatinine.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum BUN (mmol/L)</th>
<th>Serum Cr (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Negative control group</td>
<td>1.79±0.46</td>
<td>1.03±0.48</td>
</tr>
<tr>
<td>II. MTX group</td>
<td>2.86 ± 0.79*</td>
<td>2.73±0.61*</td>
</tr>
<tr>
<td>III. FMS group</td>
<td>1.78±0.62b</td>
<td>1.20±0.69b</td>
</tr>
<tr>
<td>IV. α-Toc group</td>
<td>1.74±1.27c</td>
<td>1.42±0.32c</td>
</tr>
<tr>
<td>V. MTX + FMS group</td>
<td>2.05±0.34c</td>
<td>1.79±0.92c</td>
</tr>
<tr>
<td>VI. MTX + α-Toc group</td>
<td>1.69±0.24b</td>
<td>1.83±0.48b</td>
</tr>
</tbody>
</table>

• The data are expressed as mean ± SD, number of rats in each group = 6
• Superscript (*) indicates significant differences when groups II, III and IV are compared to the negative control group (P<0.05)
• Superscript (ψ) indicates significant differences when group III and IV are compared to the MTX group (P<0.05)
• Superscript (δ) indicates a significant difference when group V is compared to group II (P<0.05)
• Superscript (β) indicate a significant difference when group VI is compared to group II (P<0.05)
Notably, the rats in MTX plus FMS group showed a significant decrease in MDA along with a significant increase in SOD-1 levels as compared to the MTX group \( (P<0.05) \). Similarly, rats in MTX plus \( \alpha \)-Toc group showed significantly decreased MDA and increased SOD-1 levels as compared to the MTX group \( (P<0.05) \), as shown in (Table 2) and (Figures 3 and 4).

By comparing the levels of MDA and SOD-1 in the renal tissue homogenate of rats in the MTX group, FMS group, and MTX plus FMS group, the obtained results showed that there were significant differences between the three groups \( (P<0.05) \). Likewise, when we compare the renal MDA and SOD-1 levels among the MTX group, \( \alpha \)-Toc group, and MTX plus \( \alpha \)-Toc group, statistically significant differences between them can be noted \( (P<0.05) \), as shown in (Table 2) and (Figures 3 and 4).

The same table also showed a nonsignificant difference in renal MDA levels when we compare the MTX plus FMS group to the MTX plus \( \alpha \)-Toc group \( (P=0.05) \). However, MTX plus FMS group has significantly higher renal SOD-1 levels compared to MTX plus \( \alpha \)-Toc group \( (P<0.05) \).

### Table 2. Effects of fimasartan on the renal MDA and SOD-1 levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Renal MDA (nmol/ml)</th>
<th>Renal SOD-1 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Negative control group</td>
<td>8.44±1.22</td>
<td>149.11±11.35</td>
</tr>
<tr>
<td>II. MTX group</td>
<td>16.58±1.03</td>
<td>109.66±19.21</td>
</tr>
<tr>
<td>III. FMS group</td>
<td>9.07±0.94</td>
<td>151.87±23.87</td>
</tr>
<tr>
<td>IV. ( \alpha )-Toc group</td>
<td>8.32±1.265</td>
<td>148.51±18.82</td>
</tr>
<tr>
<td>V. MTX + FMS group</td>
<td>11.69±0.99</td>
<td>158.36±16.86</td>
</tr>
<tr>
<td>VI. MTX + ( \alpha )-Toc group</td>
<td>10.82±0.59</td>
<td>135.23±20.83</td>
</tr>
</tbody>
</table>

- The data are expressed as mean ± SD, number of rats in each group = 6
- Superscript (*) indicates significant differences when groups II, III and IV are compared to the negative control group \( (P<0.05) \)
- Superscript (ψ) indicates significant differences when group III and IV are compared to the MTX group \( (P<0.05) \)
- Superscript (δ) indicates a significant difference when group V is compared to group II \( (P<0.05) \)
- Superscript (β) indicate a significant difference when group VI is compared to group II \( (P<0.05) \)

Table 2 and Figures 3 and 4 revealed that administration of MTX in group II resulted in a significant increase in renal MDA levels, coupled with a significant decrease in renal SOD-1 contents, as compared to the negative control group (group I) \( (P<0.05) \). At the same time, there were no significant differences in the levels of MDA and SOD-1 in the FMS and \( \alpha \)-Toc groups as compared to group I \( (P>0.05) \). Besides, comparing the FMS group and \( \alpha \)-Toc group with the MTX group revealed significant differences between them \( (P<0.05) \).
- Small letter superscripts (a, b, c) indicate significant differences among the groups (II, III, V) ($P<0.05$)
- Capital letter superscripts (A, B, C) indicate significant differences among the groups (II, IV, VI) ($P<0.05$)
- Superscript (♣) indicates a significant difference when group V is compared to group VI ($P<0.05$)

By comparing the KI of rats in the MTX group, FMS group, and MTX plus FMS group, the obtained results showed that there were significant differences between the three groups ($P<0.05$). Likewise, when we compare among MTX group, α-Toc group, and MTX plus α-Toc group, statistically significant differences between them can be noticed ($P<0.05$), as shown in (Table 3) and (Figure 5).

The same table also showed a nonsignificant difference in KI when we compare the MTX plus FMS group to the MTX plus α-Toc group ($P>0.05$).

**Table 3. Effects of fimasartan on the kidney index (KI).**

<table>
<thead>
<tr>
<th>Groups</th>
<th>KI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Negative control group</td>
<td>0.69±0.074</td>
</tr>
<tr>
<td>II. MTX group</td>
<td>1.12±0.29 A,B</td>
</tr>
<tr>
<td>III. FMS group</td>
<td>0.69±0.04 B</td>
</tr>
<tr>
<td>IV. α-Toc group</td>
<td>0.74±0.03 A</td>
</tr>
<tr>
<td>V. MTX + FMS group</td>
<td>0.71±0.12 B,C</td>
</tr>
<tr>
<td>VI. MTX + α-Toc group</td>
<td>0.725±0.11 B,C</td>
</tr>
</tbody>
</table>

- The data are expressed as mean ± SD, number of rats in each group = 6
- Superscript (*) indicates significant differences when groups II, III and IV are compared to the negative control group ($P<0.05$)
- Superscript (ѱ) indicates significant differences when group III and IV are compared to the MTX group ($P<0.05$)
- Superscript (δ) indicates a significant difference when group V is compared to group II ($P<0.05$)
- Superscript (β) indicate a significant difference when group VI is compared to group II ($P<0.05$)
- Small letter superscripts (a, b, c) indicate significant differences among the groups (II, III, V) ($P<0.05$)
- Capital letter superscripts (A, B, C) indicate significant differences among the groups (II, IV, VI) ($P<0.05$)
- Superscript (♣) indicates a significant difference when group V is compared to group VI ($P<0.05$)
Fimasartan and methotrexate-induced nephrotoxicity

Importantly, the present study showed that fimasartan ameliorated the renal injury induced by MTX, evidenced by the significant decrease in the serum levels of renal function parameters and KI, coupled with a significant decrease in renal MDA and a significant increase in renal SOD-1 contents, reflecting a restoration of the cellular redox balance in the kidney. These results are consistent with previous researches that reported the renoprotective effects of fimasartan and other ARBs in various drug-induced renal injury models (18,20,35,36). A study by Kim et al. (2015) in a mice model of unilateral ureteral obstruction showed that the ameliorating effect of fimasartan against renal oxidative stress, inflammation, and fibrosis was mediated through upregulation of the antioxidant enzymes (including SOD-1) along with counteracting the effects of angiotensin II (Ang II), which is a major component of the renin-angiotensin-aldosterone-system (18). They found that the locally expressed Ang II in the kidneys contributed to the oxidative stress by enhancing NADPH oxidases, and blockade of Ang II/AngII type 1 receptor signalling by fimasartan reduced the oxidative stress and inflammation induced by Ang II (18). Similarly, in another preclinical study, fimasartan was found to preserve renal structure and function in an ischemia/reperfusion injury model by preventing apoptosis induced by the inflammatory pathway (20).

Notably, the mechanisms offered by fimasartan and other renoprotective ARBs are the same, where it was reported that irbesartan displayed a protective role in gentamicin-induced nephrotoxicity via its antioxidant effect (56). Similarly, losartan showed a renoprotective effect through counteracting oxidative stress in an ischemic renal injury model (37). Consequently, the beneficial effects of fimasartan against renal injury can be attributed to its antioxidant activity mediated by the enhancement of the endogenous antioxidants, with the resultant attenuation of lipid peroxidation in renal tissue.

On the other hand, α-tocopherol appears to have antioxidant effects which contribute to its renoprotective effects. In agreement, many investigators verified that α-tocopherol can mitigate the nephrotoxicity induced by several agents, and the protective effect was credited to its antioxidant and anti-inflammatory properties (12,38,39). However, fimasartan appears to be a more powerful antioxidant than α-tocopherol, since it resulted in a more significant increase in the renal SOD-1 levels.

Conclusion

In conclusion, the present study revealed that treatment of rats with fimasartan have ameliorative effects, that are comparable to those of α-tocopherol, against MTX-induced nephrotoxicity through boosting the antioxidant defences in the kidneys; with fimasartan being more effective than α-tocopherol since it increased the renal SOD-1 levels to a greater extent.
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