Protective Role of Vitamin E on Methotrexate-Induced Renal Injury in Rabbits

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Abstract

Background: Methotrexate (MTX), a folic acid antagonist, is widely used as a treatment for malignancies as well as in the treatment of various inflammatory and autoimmune disorders. Nephrotoxicity is an important side-effect of this drug. Prevention and treatment of MTX-induced renal dysfunction are essential to prevent potentially life-threatening MTX-associated toxicities. The present study was undertaken to determine whether vitamin E could ameliorate methotrexate-induced oxidative renal injury in rabbit. Materials and methods: Twenty eight rabbits were randomly assigned into four groups (n=7): Group 1 (control group), Group 2 (Received 20mg/kg MTX), Group 3 (Received MTX plus vitamin E 100 mg/kg orally), and group 4 (vitamin E 100 mg/kg orally). On the 6th day rabbits were anesthetized and renal tissue was taken for pathologic and biochemical assessment. Results: Data showed that renal tissue injury index and malondialdehyde (MDA) level were lower in MTX+ vitamin E group comparing to MTX group significantly (P<0.05). Renal tissue injury index and MDA were higher in MTX+ vitamin E group comparing to control group significantly (P<0.05). Conclusion: These findings suggest that vitamin E by improving cellular anti-oxidant defense system, reduction in lipid peroxidation, and tissue damage, appears to have a protective role in the MTX-induced oxidative injury in renal tissue. However, further studies are essential to elucidate the exact mechanisms of MTX-induced renal toxicity, and protective effect of vitamin E. [GMJ.2014;3(3):194-99]

Keywords: Kidney; Methotrexate; vitamin E; Rabbit

Introduction

Methotrexate (MTX) is widely used in the treatment of clinical conditions such as breast cancer, lymphoma, stomach cancer, urinary bladder cancer, psoriasis, and rheumatoid arthritis [1]. It has been shown to promote cell death through apoptosis of both cancerous and non-transformed cells. The efficacy of MTX is often limited by severe side effects and toxic sequelae such as intestinal injury, hepatotoxicity, and suppression of bone marrow. In fact, MTX depletes folate species and the lack of folate affects several biochemical pathways including purine metabolism. These metabolic alterations are responsible for both the therapeutic and the toxic effects of MTX [2]. Furthermore, MTX can cause increased serum creatinine levels, uremia, and hematuria and its administration in high doses has been reported to cause acute renal failure. Therefore, nephrotoxicity is an important side-effect...
of this drug. The mechanism of MTX-induced renal toxicity has been proposed as follows: the direct toxic effect of MTX [3], inhibition of several enzymes related to DNA synthesis [4], and enhancement of the production of reactive oxygen species (ROS)[5]. Multiple factors are known to induce renal tubular cell apoptosis. These factors can be divided into several categories, one of which is also related to ROS [6]. It has been established that MTX inhibits cytosolic nicotinamide adenine diphosphate (NADP)-dependent dehydrogenase and NADP-malic enzyme, resulting in decreased availability of NADPH in cells. NADPH is normally used by glutathione reductase (GSH-Rd) to maintain cell glutathione (GSH), known as an important protective agent against ROS, in its reduced state. Thus, when the antioxidant defense system is reduced, cells begin to sensitize to ROS-related injury [5]. There is increasing evidence to support the view that the kidneys are susceptible to oxidative stress through exogenous drugs and chemicals. MTX, primarily cleared by renal excretion, may also lead directly to renal dysfunction and damage [7]. Prevention and treatment of MTX-induced renal dysfunction are essential to prevent potentially life-threatening MTX-associated toxicities, especially myelosuppression, mucositis, and dermatitis. In addition to conventional treatment approaches, dialysis-based methods have been used to remove MTX with limited effectiveness. Regarding mentioned problems, finding agents in which side effects of MTX can be prevented or decreased is necessary. Antioxidant defense mechanisms can effectively protect cells and tissues from free radical mediated deleterious effects. Vitamin E is fat-soluble and acts as a free radical scavenger to prevent lipid peroxidation of polyunsaturated fatty acids and block nitrosamine formation [8]. Vitamin E is a major antioxidant in biological systems acting as a powerful chain-breaking agent through the scavenging of peroxyl radicals [9]. Thus, a number of studies have been carried out to determine the protective effects of vitamin E in different biological models of injury [10]. Currently, there is considerable interest in the role of vitamin E in the protection of membrane lipids against oxidative stress [11]. Administration of Vitamin E alone or in combination with other vitamins, such as vitamin C, increases the activities of antioxidant enzymes such as SOD, catalase, and glutathione-S-transferase in rats. In the present study, we aimed to investigate the effect of vitamin E on renal injury after MTX administration.

Materials and Methods

**Animals**

Twenty eight male rabbits were obtained from Laboratory Animal Care Center of Tabriz University of Medical Sciences (Tabriz, Iran). The experiments were performed according to the guidelines of our Ethics Committee. After an adaptation period of 3 days, rabbits (n=28) were assigned to 4 groups of 8 animals each: Group 1: control group (0.9% saline); Group 2: MTX injected with 20 mg/kg intraperitoneally (i.p) [12]; Group 3: MTX+Vitamin E; MTX injected i.p. (20 mg/kg) + Vitamin E (100 mg/kg) orally [13] dissolved in olive oil. Group 4: MTX+olive oil. The administration of Vitamin E was initiated 3 days before MTX and continued for 6 days. At 6th day, rabbits were anesthetized with an intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey) and were sacrificed and the renal tissue were quickly removed and was stored at −20°C for biochemical analysis such as malondialdehyde (MDA).

**Histopathological Analyses**

The specimens fixed in 10% formalin were embedded in paraffin. Sections of 4 μm were prepared, stained with hematoxylin and eosin, and then examined by a pathologist under a light microscope. The histopathologic scoring analysis was performed according to previously described methods, the assessment was expressed as the sum of the individual score grades from 0 (no findings), 1 (mild), 2 (moderate), to 3 (severe) for each of the following 4 parameters from kidney sections: tubular cell swelling, cellular vacuolization, pyknotic nuclei, and medullary congestion (The Microscope power was 40X and 10 fields were used in each specimen) [14].
Biochemical Analysis
Measurement of BUN and Cr levels
Serum creatinine (Cr) and blood urea nitrogen (BUN) levels were determined using quantitative kits (Pars Azmoon, Iran).

MDA level
Tissue malondialdehyde was determined by the method of Uchiyama and Mihara [15]. 3-mL aliquot of 1% phosphoric acid and 1 mL of 0.6% thiobarbituric acid solution were added to 0.5 mL of 10% tissue homogenate. The mixture was heated in boiling water for 45 minutes. After cooling, the color was extracted into 4 mL of n-butanol. The absorbance was measured in a spectrophotometer (Amersham Pharmacia Biotech UK Ltd., Little Chalfont, Buckinghamshire, UK) at 532 nm (ε = .56 × 105 mol/L−1 cm−1). The amounts of lipid peroxides calculated as thiobarbituric acid reactive substances (TBARS) of lipid peroxidation were expressed as nMol/ml [16]. The study protocol was approved by the medical ethics committee of Tabriz medical school and the animals were treated according to international ethical rules.

Statistical analyses
Data were expressed as means ± SD. Differences among various groups were tested for statistical significance using the one-way ANOVA test and Tukeys post hoc test. P value of less than 0.05 denoted the presence of a statistically significant difference.

Results
Lipid Peroxidation Assay
The level of MDA, which is a major degradation product of lipid peroxidation, was significantly elevated in the renal tissue of rabbits treated with MTX (P<0.05). However, this elevation was significantly suppressed by vitamin E treatment during MTX administration (P<0.05) (Table-1).

Effects of Vitamin E on Serum BUN and Cr changes After MTX Treatment
For clarifying MTX administration effect on serum BUN and Cr, we measured these factors. MTX treatment elevated serum BUN and Cr levels. BUN and Cr levels were higher in MTX group comparing to MTX+ Vitamin E group significantly (P<0.05). BUN and Cr levels were significantly lower in MTX group comparing to MTX+vitamin E group significantly (P<0.05).

Renal Tissue Injury
Semi-quantitative assessments of histological lesions produced significantly higher scores for MTX treated groups than the normal controls at day 6th. Renal tissue injury index was higher in MTX group comparing to MTX+ Vitamin E group significantly, and also it was higher in MTX+ Vitamin E group comparing to control group significantly(P<0.05).

Discussion
Our study demonstrates that MTX administration causes oxidative tissue damage, as assessed by increased lipid peroxidation, while vitamin E treatment prevents the oxidative damage. Antitumor drugs are being increasingly utilized as adjuvant therapy for patients at high risk for recurrent disease [17]. Recent advances showed that oxygen radicals and hydrogen peroxides are linked with the development of several pathological processes asso-

Table 1. Renal Tissue MDA and Histological Parameters and Serum Biochemical Parameters. Values are shown as mean±SD.

<table>
<thead>
<tr>
<th>MDA (nmol/ mg protein)</th>
<th>BUN(U/ml)</th>
<th>Cr(U/ml)</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.62±0.37</td>
<td>14.66±1.82</td>
<td>0.48±0.06</td>
</tr>
<tr>
<td>MTX</td>
<td>2.85±0.59</td>
<td>27.55±7.01</td>
<td>0.77±0.04</td>
</tr>
<tr>
<td>MTX+Vit E</td>
<td>2.21±0.35</td>
<td>20.87±2.79</td>
<td>0.59±0.08</td>
</tr>
<tr>
<td>MTX+Olive oil</td>
<td>2.63±0.40</td>
<td>28.77±3.99</td>
<td>0.73±0.080</td>
</tr>
</tbody>
</table>
associated with chemotherapy including adverse effects of antitumor drugs [18,19]. The delayed therapeutic and toxic effect of MTX is due to its conversion to polyglutamated form which has a longer metabolic half life [20]. MTX competitively and irreversibly inhibits dihydrofolate reductase (DHFR), an enzyme that participates in the tetrahydrofolate synthesis [21]. DHFR catalyses the conversion of dihydrofolate to the active tetrahydrofolate. MTX has about 1000-fold greater affinity for DHFR compared to that of folate [22]. Since folic acid is needed for the de novo synthesis of the nucleosides, by interacting with DHFR, MTX contributes to inhibition of nucleic acid synthesis. Specifically, MTX exhibits its cytotoxic action during the S-phase of the cell cycle [23,24]. The mechanisms of MTX-induced renal toxicity have not been exactly established, yet. However, free radicals are expected to play a role in MTX-induced renal toxicity. We used MDA levels to show damage to the kidney caused by lipid peroxidation in our study. Elevated MDA levels in the present study suggest that lipid peroxidation, mediated by oxygen free radicals, which is believed to be an important cause of destruction and damage to cell membranes, was an important contributing factor to the development of MTX-mediated tissue damage [25]. However, MTX-induced lipid peroxidation was prevented by vitamin E implicating an antioxidant effect of this molecule.

The renal proximal tubule is both the segment of the nephron responsible for the active excretion of toxic chemicals, and an important target tissue for many of those same chemicals. Thus, it should not be surprising to find a convergence of these aspects of proximal tubule function and dysfunction [26]. Acute renal failure (ARF) is the most common form of renal toxicity due to high dose of MTX (HDMTX) and is seen in 2–10% of treatment cycles [27]. The most commonly described pathophysiology of ARF is the precipitation of methotrexate and its metabolites in the acidic environment of the urine. Other possible causes of ARF have been previously described [28]. HDMTX-induced ARF is almost universally reversible and, given adequate recovery time, rarely will patients require discontinuation of HDMTX or dose reductions upon future cycles. Maintenance of adequate urine flow and urinary alkalinization while receiving HDMTX is essential to prevent this adverse effect [27]. Progressive renal dysfunction, defined as steady increase in baseline serum creatinine, with each subsequent cycle of HDMTX has not been reported in the literature [29]. Kidney tissue, which presented severe glomerular congestion and degeneration, dilatation in Bowman’s space, inflammatory cell infiltration in interstitium, and tubular degeneration in the MTX-treated group, showed mild glomerular and tubular degeneration, and mild inflammatory cell infiltration in the interstitium of the vitamin E+MTX-treated group.

Beside histologic impairment, MTX administration impaired renal functional test like BUN and Cr. BUN and Cr levels in plasma were increased after MTX administration while vitamin E administration decreased this elevation and there was significant difference in BUN and Cr levels in control group and MTX+ vitamin E group. Cetiner et al. [30] reported that MTX administration doesn’t elevate BUN and Cr significantly, although there were tendency to increase. On the other hand, Kolli et al. [30] have indicated that MTX administration increased plasma BUN and Cr levels significantly in which is in accordance with our results.

**Conclusion**

In conclusions, it has been suggested that vitamin E may be a promising drug against MTX-induced renal damage and oxidative renal stress. Further studies are warranted to define the exact mechanism of the protecting effect of vitamin E on MTX-induced nephrotoxicity and the optimum dosage of this compound. In our study, the increased level of tissue MDA may be suggested that the underlying mechanism is related to direct toxicity of MTX rather than blockage in folate synthesis in rabbit kidneys. Vitamin E administration also attenuated renal tissue injury and suppressed elevation of BUN and Cr levels.
However, further studies are essential to elucidate the exact mechanisms of MTX-induced renal toxicity, and protection and the effect of Vitamin E.

Conflicts of interest
Nothing declared

Acknowledgments
Authors wanted to appreciate the scientific writing and editing of the manuscript by scientific writing committee of Sadra-tech™ Company, Shiraz University of Medical Sciences, Shiraz, Iran, and also Ms. Keivanshokooh and Research Improvement Center of Shiraz University of Medical Sciences

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