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Original Article

The impact of gallic acid on the methotrexate-induced kidney damage in rats

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ARTICLE INFO

Article history:
Received 29 July 2016
Received in revised form
22 April 2017
Accepted 6 May 2017
Available online 31 May 2017

Keywords:
Gallic acid
Methotrexate
Nephrotoxicity
Oxidative stress
Pathology

ABSTRACT

Prolonged use of an antineoplastic agent methotrexate (MTX), can cause numerous side effects such as nephrotoxicity. The aim of this study was to examine the effects of MTX on kidneys and demonstrate the protective effects of gallic acid (GA). Twenty-four, male, rats distributed into three groups. Each groups consisted eight rats and only saline was administered to the control group. The MTX group received a single dose (20 mg/kg) MTX intraperitoneally. The MTX + GA group received same dose MTX and 100 mg/kg GA orally during the 7 days. Renal functions, oxidative stress markers, histopathological and immunohistochemical changes were evaluated at the end of the experiment. Blood urea nitrogen, creatinine, uric acid levels and tissue oxidative stress markers, total oxidant status and oxidative stress index levels significantly increased and total antioxidant status levels significantly decreased in MTX group compared with the control group. At the histopathological examination hemorrhages, tubular cell necrosis, glomerulosclerosis, inflammatory cell infiltrations and proteinous materials in tubules were noticed in MTX group. Immunohistochemical examination revealed that increased expressions of serum amyloid A (SAA), tumor necrosis factor alpha (TNF-α), prostaglandin E2 (PGE-2) and C-reactive protein (CRP) in tubular epithelial cells of kidneys in this group. There were no immunoreaction with SAA and CRP, only small number of PGE-2 and TNF-α positive tubular epithelial cells were observed in MTX + GA group. In conclusion, all evidence suggested that oxidative stress caused MTX-induced nephrotoxicity and GA prevent the kidney from the nephrotoxicity due to its antioxidant and anti-inflammatory activities.

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http://dx.doi.org/10.1016/j.jfda.2017.05.001

1. Introduction

Methotrexate (MTX) is an antineoplastic agent and it may be used in the treatment of several maladies, such as cancer and inflammatory diseases. Prolonged use of this agent led to causes many side effects on different organs including kidney, liver, lung, testis, bone marrow and brain. Because of drug excretion from the kidneys by glomerular filtration and active transport, nephrotoxicity occurs more than other side effects. Mainly, this side effect restricts the use of MTX for treatment.

The most common mechanism of MTX induced damages is oxidative stress which triggered by inflammation due to producing reactive oxygen species (ROS) [1–3]. The levels of the various classical inflammatory mediators, such as an acute phase protein C-reactive protein (CRP), proinflammatory cytokine tumor necrosis factor-α (TNF-α) and Prostaglandin E2 (PGE-2) are important for monitoring the severity of inflammation during the damage [4]. Additionally, serum amyloid A (SAA) is the major acute-phase indicator of inflammation, which is secreted in inflammation, trauma or infection [5]. Besides, it expresses by vitamin D-binding protein isoform-1 precursor, plasma kallikrein, and apolipoprotein A-I in a malignant tumor, multiple myeloma (MM), in which methotrexate is used for treatment [6].

There are several agents that used to ameliorate the potential side effects of MTX due to their antioxidant and anti-inflammatory activities [2,7]. Gallic acid (3,4,5-trihydroxybenzoic acid, GA), a natural endogenous product, presented in red wine, green tea, strawberries, pineapples, bananas, lemons, gallnuts, sumac, witch hazel, tea leaves, oak bark, and apple peels [8]. GA, strong chelating agent, protects human cells or tissues against oxidative stress, by its biological activities, including anti-oxidant and anti-inflammatory effects [9–12]. It does not only protect the integrity of plasma membrane, but at the same time increases the regenerative and reparative capacity of the liver and kidney [13]. Additionally, GA and its derivations have anticancer activities due to several mechanisms. For example, in one study, lauryl gallate induced acute myeloid leukemia cell apoptosis, resulted in down-regulation of anti-apoptotic proteins (Bcl-2, Mcl-1, and Bcl-xL); and in another study, matrix metalloproteinases-2 and matrix metalloproteinases-9 downregulation in GA treated human leukemia K562 cells are mediated through the suppression of Jun N-terminal kinases-1(JNK-1) mediated c-Jun/Activating transcription factor 2 (Akt-2) and Akt/ERK-mediated c-Jun/c-Fos pathways [14,15].

The aim of this study was to focus on the knowledge of the effects of MTX on the kidneys, and demonstrate the protective effects of GA through the CRP, TNF-α, PGE-2 and SAA pathways.

2. Materials and methods

2.1. Experimental conditions

All experiments were performed in accordance with the guidelines for animal research from the National Institutes of Health, and were approved by the Committee on Animal Research of Suleyman Demirel University, Isparta.

Twenty-four, male, Wistar Albino rats, weighing 300–350 g, were placed in a temperature (21–22 °C) and humidity (60 ± 5%) controlled room in which a 12:12 h light/dark cycle was maintained. All the rats were fed with standard commercial chow diet (Korkuteli yem, Antalya, Turkey). The rats were distributed into three groups that consisted eight rats:

(I) Control group; 0,1 ml saline by oral gavage for 7 days, and only intraperitoneally (i.p.) on the second day;
(II) MTX group; 20 mg/kg MTX (i.p., Methotrexate 50 mg/ml flk, Kocak, Turkey) in a single dose on the second day and 0,1 ml saline by oral gavage for 7 days [16];
(III) MTX + GA group; 20 mg/kg MTX (i.p.) in a single dose on the second day and 100 mg/kg GA by oral gavage for 7 days [17].

Twenty-four hours after the last GA administration, all rats were anesthetized by intraperitoneal injection of 90 mg/kg ketamine (Alfamin, Alfasan IBV, Turkey) and 10 mg/kg xylazine (Alfazin, Alfasan IBV, Turkey). Blood samples were collected for blood urea nitrogen (BUN), uric acid and creatinine analyses. Both kidneys were quickly removed and cut in two parts, one half of the kidneys was fixed in 10% neutral formalin solution for histopathological and immunohistochemical examinations. The other half of the kidneys was placed into the liquid nitrogen and stored at −20 °C until for biochemical analyses of Total Antioxidant Status (TAS), Total Oxidant Status (TOS) and Oxidative Stress Index (OSI).

2.2. Biochemical analyses

Kidneys were homogenized in ice-cold phosphate buffer (pH 7.4) to produce 10% homogenate. Tissues were homogenized in a motor-driven tissue homogenizer (IKA Ultra-Turrax T25 Basic; Labortechnic, Staufen, Germany) and sonicator (UW–2070 Bandelin Electronic, Germany) with phosphate buffer (pH 7.4). Unbroken cells, nuclei and cell debris were sedimented by centrifugation at 10000*g* for 10 min at 4 °C. Protein levels in the homogenate were determined according to the method of Bradford et al. [18]. This tissue homogenate was used for to determination of TAS and TOS levels [19,20]. The TAS levels of samples were measured spectrophotometrically at the 660 nm absorbance. The results were expressed as mmol Trolox Eq/mg protein. The color intensity is related to the total amount of oxidant (TOS) molecules in the samples. The results are expressed in terms of mM hydrogen peroxide equivalent per g liter (mmol H2O2 Equiv/L, mmol H_2O_2 Equiv/mg protein). Determination of OSI, which is an indicative parameter of oxidative stress level and the ratio of TOS to TAS was calculated using the following formula [21]:

OSI (arbitrary unit) = [(TOS, μmol/L) / (TAS, μmol Trolox equivalent/L) X 100]

TAS and TOS were measured by the automated chemistry analyzer Beckman Coulter AU5800 (Japan). Serum BUN, uric acid and creatinine levels were determined using the Olympus