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Potential protective effect of etanercept and aminoguanidine in methotrexate-induced hepatotoxicity and nephrotoxicity in rats

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ABSTRACT

Methotrexate (MTX), a chemotherapeutic and immunosuppressant drug, is generally well-tolerated by most patients. However, its cytotoxic nature contributes to life-threatening side effects including hepatotoxicity and nephrotoxicity. The present study investigated the possible role of tumor necrosis factor-alpha (TNF-α) inhibitor, etanercept and inducible nitric oxide synthase (iNOS) inhibitor, aminoguanidine, on MTX-induced hepatotoxicity and nephrotoxicity in rats. Rats were divided into 7 groups: control group, etanercept group, aminoguanidine group, MTX group, MTX+etanercept group, MTX+aminoguanidine group, and MTX+etanercept+aminoguanidine group. MTX caused hepatotoxicity and nephrotoxicity as evidenced biochemically by significant increase in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine, respectively as well as by histopathological changes. Such effects were associated with significant changes in oxidative stress markers (malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), catalase, and glutathione (GSH)) as well as by upregulation of TNF-α, iNOS and caspase-3 expressions in hepatic and renal tissues. Etanercept and aminoguanidine significantly attenuated MTX-hepatotoxicity and nephrotoxicity. The protective effect of either agent was associated with significant improvement in oxidative stress parameters as well as by downregulation of TNF-α, iNOS and caspase-3 expressions in hepatic and renal tissues. The study suggested that inhibitors of either TNF-α and/or iNOS have protective effect in MTX-induced hepatotoxicity and nephrotoxicity. The protective effect of either agent relies, at least partially, on their antioxidant effects and decreased TNF-α, iNOS, and caspase-3 expressions.

1. Introduction

Methotrexate (MTX), a folic acid antagonist, is widely used in cancer therapy and several autoimmune disorders including rheumatoid arthritis, psoriasis, and inflammatory bowel diseases (Wu and Schiff, 2004). With this enlarged spectrum of clinical use; its hepatotoxicity and nephrotoxicity has gained more attention in clinical research (Akbulut et al., 2014; Tousson et al., 2014). However, the mechanism of MTX-induced toxicity is not fully explored.

Several mechanisms have been suggested as a possible explanation for MTX-toxicity including oxidative stress (Mukherjee et al., 2013; Tousson et al., 2014) and release of inflammatory mediators including tumor necrosis factor-alpha (TNF-α) (Cetiner et al., 2005) and inducible nitric oxide synthase (iNOS) (Leitão et al., 2011). TNF-α is a key player in liver and kidney homeostasis. It activates pro-apoptotic (mainly caspases) and anti-apoptotic (mainly nuclear factor kappa B (NF-κB)) pathways (Liedtke and Trautwein, 2012; Rao et al., 2010). Increased expression of TNF-α was reported in a model of MTX- induced hepatic, renal and intestinal damage (Cetiner et al., 2005). The pro-inflammatory effect of TNF-α is mediated through NF-κB regulated proteins, such as iNOS, and cyclooxygenase-2 (Aggarwal et al., 2012).

Nitric oxide (NO) is a free radical molecule with a multitude of physiological functions. This highly reactive molecule was synthesized from L-arginine by group of isoenzymes collectively termed NO synthases (NOS). NOS existed as three distinct isoforms, endothelial (eNOS), neuronal (nNOS), and the inducible NOS variants (iNOS). The role of NO has been implicated in the pathogenesis of MTX-induced toxicity (Leitão et al., 2011).

The nature of the role of TNF-α and iNOS is not fully understood. Therefore, the current study was conducted to investigate the effect of TNF-α inhibitor, etanercept and iNOS inhibitor,

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aminoguanidine on MTX-induced hepatotoxicity and nephrotoxicity in rats. The mechanisms of their effects were investigated.

2. Materials and methods

2.1. Animals

The present study was conducted on adult male albino rats weighing 150–250 g. Rats were obtained from the animal house, El-Giza, Egypt. Rats were fed a standard diet of commercial rat chow and tap water and left to acclimatize to the environment for 2 weeks prior to inclusion in the experiments.

2.2. Chemicals and antibodies

Etanercept was obtained as solution in pre-filled syringes from Wyeth Medica, Ireland. Aminoguanidine powder was purchased from Sigma, U.S.A. Methotrexate vials were purchased from Maine, Australia. Caspase-3 (Catalog number: 1197P1302D), iNOS (Catalog number: 9242P1204L) and TNF-α (Catalog number: 145288) polyclonal antibodies were purchased from Thermo Fisher Scientific Inc. /Lab Vision Corporation (Fremont, CA, U.S.A).

2.3. Experimental protocol

All experimental protocols were approved by board of faculty of medicine, Minia University, Egypt. The rats were assigned into 7 groups (8–10 rats per each group) and treated for 7 days (duration of the study) as follows: Normal control group was injected with normal saline 0.5 ml/ rat, i.p. daily for 7 days. Etanercept group was injected with etanercept (5 mg/kg. twice/week, s.c.) at first and fifth days of the experiment (Chio et al., 2013). Aminoguanidine group was administered with aminoguanidine (10 mg/kg, i.p.) daily for 7 days (Leitão et al., 2011). MTX group was injected with a single dose of MTX (20 mg/kg, i.p.) at the third day of the experiment to serve as positive control group (Akbulut et al., 2014). MTX+etanercept group was co-administered with MTX plus etanercept (as mentioned before). MTX+aminoguanidine group was co-administered with MTX plus aminoguanidine (as mentioned before). MTX+etanercept+aminoguanidine group was co-administered with MTX plus etanercept plus aminoguanidine (as mentioned before). Doses and schedule of the experiment were selected according to our preliminary study and based on previous studies.

2.4. Sample collection and storage

At the end of experimental period (7 days), rats were fasted for 12 h, anesthetized by urethane (1.5 g/kg, i.p.), and killed. Blood samples were collected from neck vessels by decapitation and centrifuged at 5000 rpm for 10 min for collection of sera. Sera were kept at −80 °C until assessment of ALT, AST, urea and creatinine. Liver and kidney of each rat were cleaned from blood by saline, dried on filter paper and weighed. Liver and kidney samples were kept in 10% formalin for histopathological examination and immunohistochemical studies. Other liver and kidney samples were kept at −80 °C and used for the measurement of biochemical parameters.

2.5. Biochemical analysis

2.5.1. Serum liver enzymes

Measurement of serum levels of alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were done by enzymatic colorimetric kits (Catalog numbers: 264001 and 264002) according to previous method (Reitman and Frankel, 1957).

Table 1
Effect of etanercept and aminoguanidine on serum levels of ALT, AST, urea, and creatinine.

Groups	ALT (U/l)	AST (U/l)	Urea (mg/dl)	Creatinine (mg/dl)
Control	8.5 ± 1.2	14.8 ± 1.1	24.2 ± 2.4	0.5 ± 0.02
Etan	10.3 ± 2.7	18.3 ± 0.9	29.9 ± 2.1	0.6 ± 0.05
Amino	9.2 ± 1.0	15.9 ± 1.2	27.8 ± 1.4	0.7 ± 0.03
MTX	65 ± 9.7[a]	38.4 ± 1.8[a]	155.3 ± 18.6[a]	1.3 ± 0.09[a]
Etan+MTX	13.6 ± 1.8[b]	23.3 ± 1.7[a,b]	73.7 ± 2.8[a,b]	0.8 ± 0.03[a,b]
Amino+MTX	13 ± 1.5[b]	3 ± 1.6[a,b]	57.9 ± 1.3[a,b]	0.7 ± 0.03[b]
Etan+Amino+MTX	15.3 ± 3.7[b]	24.2 ± 1.8[a,b]	58.0 ± 4.5[a,b]	0.7 ± 0.02[b]

All parameters are expressed as means ± S.E.M (n=6–8). MTX: methotrexate; Etan: etanercept; Amino: aminoguanidine; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

[a] Significantly different (at $P<0.05$) from normal control group.
[b] Significantly different (at $P<0.05$) from MTX groups.

Table 2
Effect of etanercept and aminoguanidine on liver and renal histopathological scores.

Groups	Liver histopathological score			Renal histopathological score
	Cellular necrosis	Hepatitis	Steatosis	
Control	0 ± 0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Etan	0.4 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
Amino	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.0 ± 0.0
MTX	3 ± 0.2[a]	2.3 ± 0.2[a]	1.6 ± 0.2[a]	2.3 ± 0.1[a]
Etan+MTX	0.7 ± 0.2[b]	0.2 ± 0.2[b]	0.2 ± 0.2[b]	1.3 ± 0.2[a,b]
Amino+MTX	0.2 ± 0.2[b]	0.2 ± 0.2[b]	0.2 ± 0.2[b]	1.4 ± 0.4[a,b]
Etan+Amino+MTX	0.7 ± 0.3[b]	0.0 ± 0.0[b]	0.3 ± 0.2[b]	0.8 ± 0.2[a,b]

All parameters are expressed as means ± S.E.M. (n=6–8). MTX: methotrexate; Etan: etanercept; Amino: aminoguanidine.

[a] Significantly different (at $P<0.05$) from normal control group.
[b] Significantly different (at $P<0.05$) from MTX group.

2.5.2. Serum urea and creatinine

Serum levels of urea and creatinine were measured by enzymatic colorimetric kits (Catalog numbers: 318001 and 13006, respectively) and according to the methods described by Fawcett and Scott (1960) and Schirmeister et al. (1964), respectively.

2.5.3. Oxidative stress parameters in hepatic and renal tissues (malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase, and NO)

Hepatic and renal tissue content of MDA, a lipid peroxidation determent, was evaluated by a spectrophotometric method that based on MDA reaction with thiobarbituric acid (Buege and Aust, 1978). The thiobarbituric-MDA adducts form colored complexes when extracted with n-butanol/pyridine. Its absorbance was measured at 532 nm. The resulting concentration was calculated from a standard curve using 1, 1, 3, 3-tetramethoxypropane as a standard.

GSH was measured using commercially available kit (Catalog number: GR2511). The method is based on that the sulfhydryl group of GSH reacts with 5, 5′-dithio-*bis*- 2-nitrobenzoic acid, Ellman's reagent, that yields a yellow colored 5-thio-2-nitrobenzoic acid. It was measured at 405 nm using Beckman DU-64 UV/VIS spectrophotometer.

The activity of SOD in hepatic and renal tissues was estimated