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The potential protective role of taurine against 5-fluorouracil-induced nephrotoxicity in adult male rats

Hany N. Yousef*, Hanaa R. Aboelwafa

Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Roxy, Cairo, Egypt

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ABSTRACT

Nephrotoxicity is common with the use of the chemotherapeutic agent 5-Fluorouracil (5-FU). The current study aimed to investigate the probable protective effect of taurine (TAU) against 5-FU-induced nephrotoxicity in rats using biochemical, histological and ultrastructural approaches. Twenty-four rats were equally divided into control, TAU, 5-FU and 5-FU + TAU groups.

5-FU significantly elevated levels of blood urea nitrogen (BUN), creatinine, and uric acid; while it reduced activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). Also, 5-FU induced significant elevation in malondialdehyde (MDA) levels accompanied with marked decline in γ-glutamyltranspeptidase (GGT) and alkaline phosphatase (AP) levels in kidney tissues. These biochemical alterations were accompanied by histopathological changes marked by destruction of the normal renal structure, in addition to ultrastructural alterations represented by thickened and irregular glomerular basement membranes, congested glomerular capillaries, damaged lining fenestrated endothelium, mesangial cells hyperplasia with expanded mesangial matrix, and distorted podocyte's processes. Also, the proximal (PCT) and distal (DCT) convoluted tubules showed thickened basement membranes, destructed apical microvilli and loss of basal infoldings of their epithelial cells.

Administration of TAU to 5-FU-treated rats reversed most of the biochemical, histological, and ultrastructural alterations. These results indicate that TAU has a protective effect against 5-FU-induced nephrotoxicity.

1. Introduction

5-Fluorouracil (5-FU) is one of the most commonly used chemotherapeutic drugs in the treatment of various types of human malignancies, like breast, head, neck, stomach, gastrointestinal, liver and skin cancers (Sausville and Longo, 2001; Xiao et al., 2001; Yoshikawa et al., 2001; Liu et al., 2002; Miura et al., 2010; Kocar et al., 2016).

5-FU is a pyrimidine analog and it is converted intracellularly to active metabolites, including fluorodeoxyuridine monophosphate, fluorodeoxyuridine triphosphate, and fluorouridine triphosphate. 5-FU exerts its anticancer effects by integrating its toxic metabolites into RNA and DNA and inhibiting the nucleotide synthetic enzyme thymidylate synthase (Chibber et al., 2011; David et al., 2011).

Like other chemotherapeutic drugs, 5-FU is non-targeted in action and results in RNA and DNA damage and cell death leading to extensive side effects like myelotoxicity, leukopenia, gastrointestinal toxicity, diarrhoea, vomiting, mucositis, alopecia and cardiotoxicity (Kinhult et al., 2003; Tsibiribi et al., 2006; David et al., 2011; Chang et al., 2012; Lamberti et al., 2012). Besides, it was reported that 5-FU is catabolised into dihydrouracil in the liver which is cleaved into a-fluoro-b-alanine, urea, ammonia, and carbon dioxide, thereby leading to hepatotoxicity and nephrotoxicity (Ali, 2012; Rashid et al., 2014).

Taurine (TAU; 2-aminoethanesulfonic acid) is one of the most abundant free amino acids in animal cells and tissues. It is found in high concentrations in the liver, brain, heart and kidneys of mammals. It is synthesized in the liver from cysteine and methionine and ingested directly in certain foodstuffs as meats, seafood, and milk (Huxtable, 1992). Several studies demonstrate that TAU has antioxidant, anti-inflammatory, antitumorigenic and hepatorenal protective potential (Tabassum et al., 2007; Kim et al., 2013; Marcinkiewicz and Kontny, 2014).

In literature, there are few studies related to the effect of TAU on the biochemical, histological and ultrastructural alterations that may occur in kidney tissues of mammals as a result of 5-FU exposure. Therefore, the current study was conducted to

* Corresponding author.
E-mail address: hany_barsoum@edu.asu.edu.eg (H.N. Yousef).

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investigate the probable protective influence of TAU against the toxic effects induced in the kidney tissues of adult male albino rats as a consequence of 5-FU administration.

2. Materials and methods

2.1. Pharmacological materials

5-FU is manufactured by EBEWE Pharma Ges.m.b.H. Nfg. KG. A-4866 Unterach, AUSTRIA. It is available in packages enclosing five ampoules, each containing 250 mg/5 ml of 5-FU. TAU was purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals used in the present study were of analytical grade of Merck quality.

2.2. Experimental animals

Twenty-four adult male albino rats (*Rattus norvegicus*) of similar age (3–4 months) and weight (160- 180 g) were obtained from the animal house of Theodor Bilharz Research Institute (TBRI), El-Giza, Egypt. They were housed in clear plastic cages (2 animals/cage) with wood chips as bedding in a room with a temperature range of 25 ± 2 °C, relative humidity of 55 ± 5% and a 12-h light/dark cycle. Standard laboratory rodent chow and tap water were provided ad-libitum. The animals were acclimatized for a period of one week prior to the commencement of the experiment. All animal experiments were performed under protocols approved by the local Institutional Animal Ethics Committee of Ain Shams University.

2.3. Experimental design

The rats were divided randomly into four groups of six animals each as follows:

Group I (Control group): Rats were given the normal saline parallel to the treated groups throughout the course of the study.

Group II (TAU group): Rats were orally received TAU (50 mg/kg bw/day) dissolved in distilled water (1 ml per animal) by gastric tube for 7 days. This dose was based on previously published studies which showed that this dose was effective against the toxicity induced by various xenobiotics (Çetiner et al., 2005; Sener et al., 2005a,b,c).

Group III (5-FU group): Rats were intraperitoneally (i.p.) injected with 5-FU (20 mg/kg bw/day) for 7 days. This dose was chosen according to the work accomplished by Takizawa and Horii (2002), El-Sayyad et al. (2009) and Ali (2012).

Group IV (5-FU + TAU group): Rats were orally administered TAU (50 mg/kg bw/day) alone for 7 days pre-treatment with 5-FU, subsequently they were administered TAU for another 7 days parallel with i.p. injection with 5-FU (20 mg/kg bw/day), after that they were orally given TAU (50 mg/kg bw/day) alone for another 7 days (post treatment with 5-FU).

2.4. Collection of blood and tissue samples

At the end of the experimental time period, control and treated animals were fasted overnight and then anesthetized under light ether anesthesia. Blood samples were collected by cardiac puncture then centrifuged at 1500*g* for 10 min at 4 °C to obtain sera which were immediately stored at −80 °C until use. Kidneys of rats were dissected out and washed immediately with ice-cold physiological saline (0.9% NaCl). Samples from the kidneys were stored frozen at −80 °C for further biochemical analyses, whereas other kidney samples were used for histological and ultrastructural studies.

2.5. Preparation of tissue homogenates

Tissue homogenates were prepared from kidney samples by homogenizing the tissue in ice-cold 0.9% NaCl to obtain a 10% solution using Ultra Turrax tissue homogenizer. Samples were immediately centrifuged (10,000*g* for 15 min) at 4 °C to remove debris, and the clear supernatant fluids were separated and used for the biochemical estimations.

2.6. Biochemical assessment

2.6.1. Renal functions assessment

Specific markers related to renal function including levels of blood urea nitrogen (BUN), creatinine and uric acid in the sera were estimated spectrophotometrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt. Ltd, Baroda, India).

2.6.2. Assay of tissue biomarkers

Lipid peroxidation in tissue homogenates was estimated based on the formation of thiobarbituric acid reactive substances (TBARS) and expressed as the extent of malondialdehyde (MDA) production following the method of Buege and Aust (1978).

Superoxide dismutase (SOD) activity was determined as described previously by Sun et al. (1988) and expressed as units/mg protein using standard calibration curve. Catalase (CAT) activity was determined by the method of Greenwald (1985) and expressed as nmol H_2O_2 consumed/min/mg protein. Glutathione peroxidase (GSH-Px) activity was assayed spectrophotometrically following the method of Mohandas et al. (1984) and expressed as μmol/min/mg protein.

Kidney tissue γ-glutamyltranspeptidase (GGT) and alkaline phosphatase (AP) activities were measured by using the method of Tate and Meister (1985) and Tenenhouse et al. (1980), respectively. Protein contents in the kidney homogenates were estimated according to the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.7. Histological preparations

Samples from the kidneys of the control and experimental animals were rapidly fixed in aqueous Bouin's fixative for 24 h. Then, they were subjected to the normal procedures for paraffin sectioning. After routine processing, 4 μm sections were cut, stained with haematoxylin-eosin (H & E), dehydrated in ascending series of ethyl alcohol, cleared in xylene and mounted in DPX (Bancroft and Gamble, 2002). The stained sections were examined with a light microscope and photomicrographs were made as required.

2.8. Ultrastructural preparations

Small pieces of the renal cortices from the control and treated rats were immediately fixed in cold 4F1G (4% formalin + 1% glutaraldehyde adjusted at pH 2.2) for 24 h, then were post fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.3). After fixation, they were subjected to the normal procedures for ultrastructural evaluation by transmission electron microscopy as described previously by Dykstra et al. (2002). After routine processing, the stained grids were examined and photographed by JEOL.JEM-1400-EX-ELECTRON MICROSCOPE at the Electron Microscopy Department of TBRI, El-Giza, Egypt.