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Tiron ameliorates oxidative stress and inflammation in titanium dioxide nanoparticles induced nephrotoxicity of male rats

Ashraf Morgan[b], Mona K. Galal[a,*], Hanan A. Ogaly[a], Marwa A. Ibrahim[a], Reham M. Abd-Elsalam[c], Peter Noshy[b]

[a] Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[b] Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[c] Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

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ABSTRACT

Although the widespread use of titanium dioxide nanoparticles (TiO_2 NPs), few studies were conducted on its hazard influence on human health. Tiron a synthetic vitamin E analog was proven to be a mitochondrial targeting antioxidant. The current investigation was performed to assess the efficacy of tiron against TiO_2 NPs induced nephrotoxicity. Eighty adult male rats divided into four different groups were used: group I was the control, group II received TiO2 NPs (100 mg\Kg BW), group III received TiO2 NPs plus tiron (470 mg\kg BW), and group IV received tiron alone. Urea, creatinine and total protein concentrations were measured in serum to assess the renal function. Antioxidant status was estimated by determining the activities of glutathione peroxidase, superoxide dismutase, malondialdehyde (MDA) level and glutathione concentration in renal tissue. As well as Renal fibrosis was evaluated though measuring of transforming growth factor-β1 (TGFβ1) and matrix metalloproteinase 9 (MMP9) expression levels and histopathological examination. TiO_2 NPs treated rats showed marked elevation of renal indices, depletion of renal antioxidant enzymes with marked increase in MDA concentration as well as significant up-regulation in fibrotic biomarkers TGFβ1 and MMP9. Oral administration of tiron to TiO_2 NPs treated rats significantly attenuate the renal dysfunction through decreasing of renal indices, increasing of antioxidant enzymes activities, down-regulate the expression of fibrotic genes and improving the histopathological picture for renal tissue. In conclusion, tiron was proved to attenuate the nephrotoxicity induced by TiO_2 NPs through its radical scavenging and metal chelating potency.

1. Introduction

As nanotechnology is developing rapidly, more concerns on the potential health hazards about exposure to nanoparticles (NPs) have been arising. NPs, according to the European Union, are natural or manufactured particles with one or more external dimensions in the size range 1–100 nm [1]. Owing to the extremely small sizes and large surface areas, NPs possess dramatic differences in the physicochemical properties compared to their fine size analogues [2]. Because of these peculiar features, NPs are able to penetrate cells and interfere with various subcellular mechanisms [3]. Some nanoparticles interact directly with cell macromolecules including proteins, lipids and DNA [4]. In the recent decades, nano-sized titanium dioxide (TiO2 NPs) have been mass-produced and used as a common additive in a wide range of applications including food-related industries, materials for air pollution control, pharmaceuticals, household products, cosmetics and other personal care products [2,5]. As a consequence, the level of human exposure to TiO2 NPs through multiple media and pathways has been increased. Inhalation and dermal exposure of industrially released TiO2 NPs are considered as the main routes of TiO2 NPs exposure, whereas oral exposure may occurs by direct consumption of products elaborated with high amounts of TiO2 NPs as nano-food or nanomedicine [5]. Administration of TiO_2 NPs through different routes results in their accumulation in the various tissues including liver, kidney, brain and spleen with potential toxicological impacts [6,7]. As kidney is a frequent target for toxic effects of xenobiotics, kidney is found to be one of the major targets of TiO2 NPs deposition, even in low level of TiO2 NPs exposure [7]. Subchronic TiO_2 NPs toxicity led to chronic nephritis

* Corresponding author.
E-mail addresses: ashrafmorgan550@yahoo.com (A. Morgan), monagalal12@gmail.com (M.K. Galal), hananogaly@cu.edu.eg (H.A. Ogaly), marwa199@gmail.com (M.A. Ibrahim), reham_pathology@hotmail.com (R.M. Abd-Elsalam), peter_medicine@hotmail.com (P. Noshy).

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with several pathological lesions such as proximal cell death [8], renal cell necrosis [9] and renal fibrosis [10]. Recently, the molecular mechanisms responsible for the renal toxicity of TiO2 NPs have been elucidated. Generation of intracellular reactive oxygen species (ROS) by TiO2 NPs and its subsequent oxidative damage play the key role in the mechanism underlying the TiO2 NPs-induced toxicity [12]. The implication of oxidative stress and inflammation in the etiology and progression of several clinical disorders has led to the suggestion that agents with antioxidant properties may have great health benefits. Some studies have disclosed that antioxidant pretreatment or co-treatment can reverse the toxicity of metallic NPs [4,13,14]. Tiron (4,5-dihydroxy-1,3-benzene disulfonic acid), a water soluble synthetic analog of vitamin E, has been known to be a potent antioxidant to rescue ROS-evoked oxidative damage [15,16]. Additionally, tiron is a non-toxic chelator that is effective in alleviation of various metal intoxications such as aluminum, lead and vanadium [17]. The renal protective influence of tiron against metal toxicity and diabetic nephropathy was reported previously [15–17]. Trion acts as a direct hydroxyl radical, superoxide scavenger as well as a metal chelator [18].

In this study, we present an overview on possible influences of tiron as antioxidants and metal chelator against nephrotoxicity and renal fibrosis in TiO2 NPs induced nephrotoxicity in rats.

2. Materials and methods

2.1. Chemicals

TiO_2 NPs: Anatase form, white in color, density 3.9 g\ml, particle size (10 nm), surface area (>150 m^2\g), purity 99.9 was purchased from Sigma Aldrich Chemical Co., Germany. The nanosized TiO_2 was suspended in phosphate buffer saline solution at a concentration of 1 mg\ml, shaken and dispersed via sonication for 10 min before use. Tiron: White odorless powder was purchased from Sigma Aldrich Chemical Co., Germany.

2.2. Animals

Eighty adult (average 55 days old) male albino rats weighting 180–200 g were obtained from department of Toxicology and Forensic Medicine's animal house, Faculty of Veterinary medicine Cairo University were used. All animals were subjected to two weeks of acclimatization. The animals were housed in separate well-ventilated cages, under standard conditions, with free access to standard diet and water. The local Committee approved the design of the experiments and the protocol conforms to the guidelines of the National Institutes of Health.

2.3. Experimental design

For two months the rats were divided into four equal groups. Group I (control group) injected IP with normal saline in last two weeks of the experimental period, Group II (TiO_2 NPs treated group) received 100 mg\Kg BW of TiO_2 via oral gavage once daily [19]. Group III (TiO_2 NPs + Tiron) this group received 100 mg\kg BW TiO_2 NPs once daily and the same rats injected IP with tiron 470 mg \kg BW [20] daily for the last two weeks of experimental period, group IV (tiron group) it was received tiron 470mg\kg BW only in the last two weeks. All symptoms and deaths were carefully recorded daily.

2.4. Sampling

At the end of the experiments, the rats were weighted, scarified and the blood and kidney tissues were collected. Blood samples were collected from the eye vein and the serum was collected by centrifuging blood at 3000 x g for 10 min for serum analysis. While kidney tissues were washed and stored for subsequent investigations.

2.5. Relative weight of kidney

After weighing the body and kidney of each animal, the relative weight of kidney was calculated as the ratio of kidney (wet weight, mg) to body weight (g).

2.6. Serum analysis

Kidney indices were evaluated by measuring of serum levels of blood urea according to Tietz [21], creatinine Tietz [22] and total protein concentration Tietz [23] using commercial Kits.

2.7. Titanium content analysis

The frozen kidneys tissues were thawed and ~0.1 g samples were weighed, digested, and analyzed for titanium content according to method described by Gui et al. [24]. The detection limit of titanium was 0.074 ng/ml.

2.8. Malondialdehyde and antioxidants biomarkers determinations

Specimens from kidney tissue was weighted and homogenized in cold phosphate buffered saline (pH 7.4) using Teflon homogenizer. The homogenates were centrifuged at 14,000 × g for 15 min at 4 °C. The supernatant was used to measure the MDA level [25], superoxide dismutase (SOD) activity [26], glutathione peroxidase (GPX) activity [27], reduced glutathione (GSH) concentration [28] and protein content [29].

2.9. Quantitative real-time PCR for matrix metalloproteinase 9 (MMP9) and transforming growth factor β1 (TGFβ1)

Approximately 100 mg of kidney tissue was used for total RNA extraction using Qiagen Rneasy Mini Kit following the manufacturer's protocol. The RNA yields and purity were determined using Spectrophotometer (Thermo Scientific, USA). Ten μg of TRNA were treated with DNase I for 20 min at 37 °C. The cDNA synthesis was carried out using reverse transcriptase (Invitrogen) and oligo-dT following the manufacturer protocol. After initial heat denaturation of 1 μg of total RNA (65 °C for 5 min), the reactions (20 μl) were incubated for 60 min at 42 °C. cDNA was added to a SYBR Green qPCR Master Mix (Qiagen) containing 30 pg/ml of each primer with the following sequence MMP9 forward CACTGTAACTGGGGGCAACT, reverse CACTTCTTGTCAGCGTCGAA, TGFβ1 forward GGACTCTCCACCTGCAAGAC, reverse CTCTGCAGGCGCAGCTCTG. The cDNA was amplified by 40 cycles of denaturation at 95 °C for 45 s, annealing at 57 °C for 45 s for MMP9 and 60 °C for 45 s for TGFβ1 and extension at 72 °C for 45 s. The size of all amplicons was confirmed by 2% agarose gel electrophoresis stained with SYBR Safe DNA gel stain (Invitrogen). The GAPDH gene was amplified in the same reaction to serve as the reference gene. Each measurement was repeated 3 times, and the values were used to calculate the gene/GAPDH ratio, with a value of 1.0 used as the control (calibrator). The normalized expression ratio was calculated using the method described by Khalaf et al., [14].

2.10. Histopathological examination

The kidneys tissues from the different groups were fixed in 10% neutral buffer formalin then managed for obtain 4 μm paraffin embedding sections. The tissue sections were stained with