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Innovative perception on using Tiron to modulate the hepatotoxicity induced by titanium dioxide nanoparticles in male rats

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ABSTRACT

The extensive application of titanium dioxide nanoparticles (TiO_2 NPs) in the food industry arouses a debate regarding the probable risk associated with their use. Several recent studies reported that most nanoparticles (NPs) have adverse actions on the liver. The objective of this study is to examine whether Tiron plays a modulatory role against apoptotic damage induced by TiO_2 NPs in rat livers. Forty rats were randomly divided into 4 groups; a control group received phosphate-buffered saline, an intoxicated group received 100 mg/kg/day of TiO_2 NPs for 60 days, a treated group received 470 mg/kg/day of Tiron for the last 14 days after TiO_2 NPs administration, and a Tiron group received Tiron only as previously mentioned. Oral administration of TiO_2 NPs significantly increased serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). In the liver, TiO_2 NPs increased oxidative stress through increasing lipid peroxidation and decreasing GSH concentration and the levels of the SOD and GPx enzymes. TiO_2 NPs significantly upregulated the proapoptotic Bax gene and downregulated the antiapoptotic Bcl-2 gene. Histopathological examination of hepatic tissue reinforced the previous biochemical results. Apoptotic lesions were also obvious in this group. Treatment with Tiron as an antioxidant significantly decreased serum biochemistry, ameliorated oxidative stress in hepatic tissue, upregulated Bcl-2, decreased Bax expression and attenuated the histopathology of hepatic injury. These findings indicate that Tiron effectively diminishes the hazardous effects of TiO_2 NPs on rat liver.

1. Introduction

Industrial use of metal oxide nanoparticles (i.e., titanium oxide, iron oxide, silicon, etc.) has rapidly grown during the past decade. This has led to an increase in the occupational and environmental exposure of humans and other species to nanoparticles (NPs) [1]. The safety of nanomaterials has been a focus of worldwide concern because of the lack of information available regarding their potential risks for workers and the general population. Among the various commercial nanomaterials, titanium dioxide nanoparticles (TiO_2 NPs) are one of the most frequently used in industrial applications. Their potential toxicity to humans and the environmental impact of TiO_2 NPs have attracted considerable attention with their increased use in industrial applications. The food industry is starting to use various NPs as food additives or to improve food packaging in an attempt to optimize their product [2]. In the IARC [3] (2006), pigment-grade TiO_2 was classified as possibly carcinogenic to humans (Group 2B), depending on an appropriate demonstration of carcinogenicity in animals and insufficient evidence for human tumour promotion. Concern has been raised regarding the possible adverse health effects of ingested NPs. TiO_2 is well known to be a common food additive used for whitening and brightening foods, especially confectioneries, white sauces and dressings, and certain powdered foods. Recently, food-grade TiO_2 (referred to as E171) test results suggested that approximately 36% of the particles are less than 100 nm in at least one dimension [4]. Many in vivo studies suggested that nanoparticles can accumulate in several tissues such as the liver and kidneys and can generate various inflammatory responses. The liver is a main organ that executes a wide array of functions including biotransformation, metabolism and excretion of endogenous and exogenous compounds [5]. The toxic effects of TiO_2 NPs in liver

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tissue were reported previously. TiO_2 NPs have been reported to damage liver function and induce oxidative stress and lipid peroxidation in the rodent liver [6]. Tiron (4,5-dihydroxy-1,3-benzene disulfonic acid) is a water-soluble, cell-permeable analogue of vitamin E and functions as a direct hydroxyl radical and superoxide scavenger [7]. It is both a beneficial protective antioxidant and an efficient nontoxic metal chelator that has been commonly used in oxidative-stress-associated studies [8]. Tiron is a mitochondrial localized antioxidant that can permeabilize the organelle membrane and accumulate inside [9]. For example, it can reverse the reactive oxygen species (ROS)-induced cell apoptosis or act as a nontoxic chelating agent to alleviate acute toxic metal overload [10]. Tiron has been reported to be able to inhibit apoptosis in human lung cancer cells [11]. In this paper, we present an overview of the antioxidant role and the possible therapeutic effect of Tiron against TiO_2-NPs-induced hepatotoxicity in the rat liver.

2. Materials and methods

2.1. Materials

Titanium dioxide (TiO_2): Anatase form, white in colour, density of 3.9 g/ml, particle size of 10 nm, surface area > 150 m^2/g, and purity of 99.9% was purchased from Sigma Aldrich Chemical Co., Germany. Nanosized TiO_2 was suspended in a phosphate-buffered saline solution (PBS) at a concentration of 1 mg/ml, shaken and dispersed via sonication for 10 min before use. Tiron (a metal chelator and antioxidant), a white odourless powder, was purchased from Sigma Aldrich Chemical Co.

2.2. Animals and experimental design

Forty adult male albino rats weighing 180–200 g were housed in separate well-ventilated cages under standard conditions, with free access to a standard diet and water. The design and experiments were approved by the institutional committee, and the protocol conforms to the guidelines of the National Institutes of Health (NIH).

The rats were allowed a two-week adjustment period and were then divided into 4 groups (n = 10 rats for each group) as follows: control, intoxicated, treated and Tiron groups.

The control group received PBS orally, the intoxicated group received TiO_2 NPs at an oral dose of 100 mg/kg bw/day for 60 days [12], the treated group received TiO_2 NPs at 100 mg/kg bw/day for 60 days and 470 mg/kg bw/day of Tiron during the last 14 days of the experimental period [13], and the Tiron group received 470 mg/kg bw/day of Tiron during the last 14 days of the experimental period. The selected dose in this study was chosen after consulting a World Health Organization report from 1969. According to the report, the LD50 of TiO_2 for rats is over 12,000 mg/kg bw after oral administration. In addition, the quantity of TiO_2 NPs should not exceed 1% of the food weight according to the Code of Federal Regulations of the US Government. All symptoms and deaths were carefully recorded daily. At the end of the experiment, the rats were weighed and euthanized, and then, both blood and liver samples were collected for subsequent investigation. Blood samples were collected from the eye vein. Serum was collected by centrifuging blood at 3000 × *g* for 10 min.

2.3. Relative liver weight (RLW)

The relative weight of liver was calculated as the ratio of liver (wet weight, mg) to body weight (g) for each rat [14].

2.4. Serum parametric analysis

The liver functions were evaluated based on the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) according to the instructions provided by the manufacturers.

Table 1
Therapeutic influence of tiron on the liver function tests and relative liver weights inTiO2 NPs intoxicated rats.

Parameter	Control	Intoxicated group	Treated group	Tiron group
ALP	17.39 + 1.3[a]	68.2 + 3.2[b]	33.2 + 0.9[ac]	22.2 + 0.8[ad]
ALT	42.7 + 3.05[a]	182.6 + 2.4[b]	70.6 + 2.9[c]	33.6 + 0.5[d]
AST	92.7 + 0.9[a]	235.8 + 3.4[b]	156.8 + 2.3[c]	103.5 + 3.4[d]
RLW(g)	6.86 + 0.08	6.98 + 0.07	6.923 + 0.02	7.04 + 0.09

Data were expressed as means ± S.E.M (n = 10). Groups having different letters are significantly different from each other, RLW: relative liver weight, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase.

2.5. Estimation of lipid peroxidation and antioxidants in liver tissue

Liver specimens were homogenized in cold PBS (pH 7.4) using a Teflon homogenizer. The homogenates were centrifuged at 14,000 × *g* for 15 min at 4 °C. The supernatant was used to measure the MDA [15], superoxide dismutase (SOD) activity [16], reduced glutathione (GSH) concentration [17], and glutathione peroxidase enzyme (GPx) activity [18] and to estimate the protein content [19].

2.6. DNA laddering assay

To evaluate the degree of DNA damage, genomic DNA was extracted from liver tissue according to Ibrahim et al. [20]. DNA samples (10 µg) were separately loaded into 1.5% agarose gel electrophoresis for 45 min at 80 V. The migration of fragmented DNA on the agarose gel results in a characteristic laddering pattern, which is considered a distinctive feature of apoptotic DNA damage. The degree of fragmentation was determined by gel image analysis and quantitation software; Quantitiy one 4.6.6 BIO-RAD software.

2.7. Quantitative real-time PCR for Bax and Bcl-2 genes

Total RNA was isolated using an RNeasy Mini Kit (Qiagen). The Reverse transcription reaction was completed using a Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific) following the manufacturer's instructions.

The mRNA expression levels of the antiapoptotic gene Bcl-2 and preapoptotic gene Bax in the liver tissue were determined using real-time quantitative polymerase chain reaction (qRT-PCR). Sybr green master mix (Thermo Scientific) was used according to standard protocol, and the primers were designed using Primer 3 software; Bcl-2 forward primer: 5′-GAGGATTGTGGCCTTCTTTG-3′, reverse: 5′-CGTTA TCCTGGATCCAGGTG-3′ and Bax forward: 5′-ACCAAGAAGCTGAGCG AGTG-3′, reverse: 5′-CCAGTTGAAGTTGCCGTCTG-3′. The cDNA was amplified by 40 cycles of denaturation at 95 °C for 45 s, annealing at 57 °C for the Bcl-2 gene and 55 °C for the Bax gene for 45 s and extending at 72 °C for 45 s. The GAPDH gene was amplified during the same reactions to serve as a reference gene [21]. Duplicate plates were tested, and cycle threshold (Ct) 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 ΔΔCt method.

2.8. Histopathological examination

The liver tissues from various groups were fixed in 10% neutral buffer formalin then managed to obtain 4 µm paraffin embedding sections. The tissue sections were stained with haematoxylin and eosin (H &E) [22].