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Amelioration of titanium dioxide nanoparticles-induced liver injury in mice: Possible role of some antioxidants

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ABSTRACT

This study investigates the efficacy of idebenone, carnosine and vitamin E in ameliorating some of the biochemical indices induced in the liver of titanium dioxide nanoparticles (TiO_2 NPs) intoxicated mice. Nano-anatase TiO_2 (21 nm) was administered (150 mg/kg/day) for 2 weeks followed by the aforementioned antioxidants either alone or in combination for 1 month. TiO_2 NPs significantly increased serum liver function enzyme activities, liver coefficient and malondialdehyde levels in hepatic tissue. They also suppressed hepatic glutathione level and triggered an inflammatory response via the activation of macrophages and the enhancement of tumor necrosis factor-α and interleukin-6 levels. Moreover, the mRNA expression of nuclear factor-erythroid-2-related factor 2, nuclear factor kappa B and Bax was up-regulated whereas that of Bcl-2 was down-regulated following TiO_2 NPs. Additionally, these NPs effectively activated caspase-3 and caused liver DNA damage. Oral administration of idebenone (200 mg/kg), carnosine (200 mg/kg) and vitamin E (100 mg/kg) alleviated the hazards of TiO_2 NPs with the combination regimen showing a relatively higher effect. The histopathological examination reinforced these findings. In conclusion, oxidative stress could be regarded as a key player in TiO_2 NPs-induced liver injury. The study also highlights the anti-inflammatory and the anti-apoptotic potentials of these antioxidants against the detrimental effects of TiO_2 NPs.

1. Introduction

Owing to the advantageous combination of physicochemical and biological properties of titanium dioxide nanoparticles (TiO_2 NPs), they are extensively used for a wide range of implanted medical devices as cardiovascular stents, dental implants, joint replacements and spinal fixation devices. However, under mechanical stress or altered physiological conditions such as low pH, titanium (Ti)-based implants can release large amounts of nanoparticles (NPs) debris (Cunningham et al., 2002).

The distinct properties of NPs, such as small size, high number per given mass, large specific surface area, have aroused global concern regarding their fate in biological systems. Previous studies have indicated that NPs can penetrate cell nuclei and directly interfere with DNA structure, causing several adverse effects represented in increased production of reactive oxygen species (ROS), induction of apoptosis, genotoxicity and DNA damage (Wang et al., 2007).

Additionally, NPs can be accumulated in liver, kidney, spleen, lung, heart, and brain, generating various inflammatory responses (Borm et al., 2002). According to Ma et al. (2009), TiO_2 NPs stimulate hepatocytes and induce inhibitory proteins, such as inhibitory kappa B (IκB), which is phosphorylated and degraded, leading to activation of nuclear factor-kappa B (NF-κB) with subsequent gene transcription of proinflammatory cytokines in the mouse liver. Beforehand, high doses of nano-TiO_2 (25 and 80 nm) were reported to increase the ratio of alanine aminotransferase (ALT) to aspartate aminotransferase (AST), the activity of lactate dehydrogenase (LDH) and the liver weight, and to mediate necrosis of hepatocytes (Wang et al., 2007).

Nowadays, antioxidants have gained great interest because of their potential role as therapeutic agents in many diseases (Lakho and Rohra, 2006). Idebenone (ID) [2,3-dimethoxy-5-methyl-6-(10-hydroxydecyl)-1,4 benzoquinone] is a synthetic analogue of coenzyme Q10, the vital cell membrane antioxidant and the

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essential constituent of the ATP-producing mitochondrial electron transport chain. Idebenone has the ability to operate under low oxygen tension. It protects cell membranes and mitochondria from oxidative damage by inhibiting lipid peroxidation. Idebenone has been proved to be an effective remedy against cerebral ischemia, nerve damage in the central nervous system (Parnetti, 1995) and bile acid-induced hepatocellular injury in rats (Shivaram et al., 1998).

Carnosine (CR) [β-alanyl-L-histidine] is a dipeptide of the amino acids β-alanine and histidine. Carnosine is considered as a mobile organic pH-buffer. It can chelate divalent metal ions and scavenge ROS as well as unsaturated aldehydes formed from the peroxidation of cell membrane fatty acids during oxidative stress (Reddy et al., 2005). Carnosine was also found to inhibit mRNA expression of apoptosis-inducing factor (AIF) and caspase-3, to increase superoxide dismutase (SOD) activity and to decrease malondialdehyde (MDA) level when given to mice (Renner et al., 2010).

Vitamin E (α-tochopherol) is a well-known chain breaking antioxidant that precludes the propagation of oxidative stress especially in biological membranes (Schaffer et al., 2005). It is able to modulate the activity of several enzymes involved in signal transduction via its antioxidant properties (Zingg, 2007). It is worthy noted that the anti-inflammatory and the anti-carcinogenic activities of vitamin E in the lung and colon are associated with the reduction of oxidative damage and trapping of reactive nitrogen species (Qureshi et al., 2011). Likewise, vitamin E could protect from lipid peroxidation and oxidative DNA damage in human hepatocellular carcinoma cell lines (Fantappiè et al., 2004).

Based on this background, the present study was conducted to further stretch the confirmatory role of these antioxidants (idebenone, carnosine and vitamin E), either alone or in combination, against TiO_2 NPs-induced liver injury in mice.

2. Materials and methods

2.1. Chemicals

Nano-anatase TiO_2 (particle size 21 nm), idebenone, carnosine and vitamin E were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Kits used for the determination of liver function and oxidative stress biomarkers were obtained from Randox Company (UK). ELISA kits of caspase-3, tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) were provided from R&D Systems (MN, USA). Primers used in real time-PCR were purchased from Shine Gene (China). All other chemicals are of highest analytical grade.

TiO_2 NPs were suspended in 1% Tween 80 and dispersed by ultrasonic vibration for 15 min. The size distribution of the NPs in the suspension (hydrodynamic size) and the zeta potential were analyzed with a Brookhaven 90 Plus particle size analyzer. Scanning electron microscopy (SEM) was used to evaluate the size of TiO_2 NPs.

2.2. Animals

Male albino mice, weighing 20–25 g, obtained from the animal house of National Research Center were used in this study. Animals were housed in cages kept at standardized conditions (22 ± 5 °C, 55 ± 5% humidity, and 12 h light/dark cycle). They were allowed free access to water and pelleted standard chow diet.

All procedures relating to animal care and treatments strictly adhered to the ethical procedures and policies approved by Animal Care and Use Committee of National Research Center (12-038) and Faculty of Pharmacy, Cairo University, and complied with the Guide for Care and Use of Laboratory published by the US National Institute of Health.

2.3. Experimental design

After 1 week of acclimatization, animals were randomly divided into six groups (each group ranges from 10 to 14 animals) according to the following schedule:

Group1: animals received Tween 80 and served as a normal control group.

Groups from 2 to 6: animals were given a daily oral dose of TiO_2 NPs (150 mg/kg) for 2 weeks, then the following regimen was applied:

- Group 2: the TiO_2 NPs-intoxicated animals were left untreated.
- Group 3: theTiO_2 NPs-intoxicated animals were treated with a daily oral dose of idebenone (200 mg/kg) (Seznec et al., 2004).
- Group 4: the TiO_2 NPs-intoxicated animals were treated with a daily oral dose of carnosine (200 mg/kg) (Zhang et al., 2011).
- Group 5: the TiO_2 NPs-intoxicated animals were treated with a daily oral dose of vitamin E (100 mg/kg) (Ishrat et al., 2009).
- Group 6: the TiO_2 NPs-intoxicated animals were given idebenone (200 mg/kg), carnosine (200 mg/kg) and vitamin E (100 mg/kg) in daily oral doses. Treatment was carried throughout a period of 1 month after TiO_2 NPs-intoxication.

It is worthy to note that the selected dose of TiO_2 NPs was previously reported to be the most effective in inducing liver damage (Ma et al., 2009; Li et al., 2010a).

2.4. Blood sampling and liver tissue preparation

At the end of the experimental period, mice were weighed, slightly anesthetized and blood samples were collected from the sublingual vein. Sera were separated by centrifugation at 4000 rpm for 10 min and were kept at −80 °C for subsequent estimation of aminotransferases activities.

Animals were then sacrificed by cervical dislocation and liver tissues were carefully separated, blotted dry, weighed and then divided into four portions. The first portion was homogenized in 4 volumes of phosphate buffer, pH 7.4, using Teflon homogenizer (Glass-Col homogenizer, Terre Haute, USA). An aliquot of this homogenate (20% w/v) was centrifuged at 4000 rpm at 4 °C for 15 min and the supernatant was used for MDA analysis. Another aliquot was mixed with 7.5% sulfosalicylic acid, centrifuged at 3000 rpm for 15 min, and the resulting protein-free supernatant was used for the estimation of reduced glutathione (GSH) level. The last aliquot was used for the determination of TNF-α and IL-6 levels as well as caspase-3 activity.

The second portion of the liver was used for the estimation of Nrf2, NF-κB, Bax and Bcl-2 mRNA expression levels, whereas the third portion was used for the detection of DNA damage. The remaining portion was kept in 10% formaldehyde, and then embedded in paraffin for subsequent immnunohistochemical and histopathological examinations.

2.5. Measured parameters

2.5.1. Coefficient of liver

After weighing the body and liver, the coefficient of liver to body weight was calculated as the ratio of tissue (wet weight, mg) to body weight (BW, g) (Peters et al., 2006).

2.5.2. Serum alanine and aspartate aminotransferases (ALT & AST) activities

ALT and AST activities were estimated spectrophotometrically using commercially available kits provided from Randox Company. In brief, L-alanine reacts with oxoglutarate in presence of ALT to form pyruvate and L-glutamate. On the other hand, in presence of