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Toxicity of titanium dioxide nanoparticles: Effect of dose and time on biochemical disturbance, oxidative stress and genotoxicity in mice

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ABSTRACT

The toxic impact of titanium dioxide nanoparticles (TiO_2NPs) on human health is of prime importance owing to their wide uses in many commercial industries. In the present study, the effect of different doses and exposure time durations of TiO_2NPs (21 nm) inducing oxidative stress, biochemical disturbance, histological alteration and cytogenetic aberration in mice liver and bone marrow was investigated. Different doses of (TiO_2NPs) (50, 250 and 500 mg/kg body weight) were each daily intrapertioneally injected to mice for 7, 14 and 45 days. Aspartate and alanine aminotransferases (AST &ALT), gamma glutamyl transpeptidase (GGT), total protein, total antioxidant capacity (TAC), malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and nitric oxide (NO) levels were measured. The work was extended to evaluate the liver histopathological pattern and the chromosomal aberration in mice spinal cord bone marrow. The results revealed severe TiO_2NPs toxicity in a dose and time dependent manner with positive correlation (r = 0.98) for most investigated biochemical parameters. The same observation was noticed for the histological analysis. In case of cytogenetic study, chromosomal aberrations were demonstrated after injection of TiO_2NPs with 500 mg/kg b. wt. for 45 days. In conclusion, the selected biochemical parameters and the liver architectures were influenced with dose and time of TiO_2NPs toxicity, while the genetic disturbance started at the high dose of exposure and for long duration. Further studies are needed to fulfil the effect of TiO_2NPs on pharmaceutical and nutritional applications.

1. Introduction

Recently, the field of nanotechnology has greatly emerged owing to the wide applications of nano-sized particles in many medical and industrial products [1]. The chemical composition and particle size of nanoparticles influence their accumulation in liver causing cellular damage and oxidative stress [2–4].

Particularly, TiO_2NPs are produced on a large scale and are being used in a variety of products such as cosmetics, sunscreens, pharmaceutical additives and food colorants [5,6]. The adverse effects of TiO_2NPs on human health have been raised and their carcinogenic effect especially by inhalation has been reported [7,8]. Uptake of TiO_2NPs can also occur through different routes, including inhalation, ingestion and transdermal, the latter being associated with the use of sunscreen and cosmetics, while there is no evidence demonstrating that TiO_2NPs can penetrate into normal skin [9]. TiO_2NPs is used in food industries as a coloring agent that facilitates its oral intake to humans.

Following its oral uptake in the gastrointestinal tract, Wang et al. [10] reported that when mice were orally administered TiO_2NPs, these particles may travel with blood circulation and be distributed to different organs such as liver, kidney, or lungs. The accumulation of these nanoparticles in liver causing toxicity which was demonstrated by disturbance of liver function indices (ALT/AST) and hydropic degeneration around the central vein with spotty necrosis of hepatocyte [10].

Both liver toxicity and inflammatory responses induced by TiO_2NPs are complicated multifactorial disease processes. Nanoparticles that enter the rat liver induce oxidative stress locally resulted in depletion of reduced glutathione and oxidized glutathione, while the inflammatory cytokines cascade may cause inflammatory cell chemotaxis and apoptosis, resulting in serious liver injury [11]. These cellular damage and oxidative stress of nanoparticles in the liver cells were related to the particle size and chemical compositions of nanoparticles [11].

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Little information on the toxicological effects of TiO_2NPs has thus far been reported. A long-term study of its accumulation and the detailed mechanisms of its biological effects are clearly needed. Therefore, the aim of the present study is to evaluate the effect of dose and time relationship of titanium dioxide nanoparticles (21 nm) exposure on liver function enzymes, oxidative stress markers, liver histology and chromosomal architecture in spinal cord bone marrow in mice.

2. Materials & methods

2.1. Chemicals

Titanium dioxide nanoparticles (21 nm) was purchased from Sigma-Aldrich Co (St. Louis, MO, USA). All other chemicals are of highest analytical grade; product of Sigma (US) and Merck (Germany).

2.2. Animals & ethics

Male Swiss albino mice (18–25 g) were obtained from the animal house of National Research Center. Animals were housed in cages kept at standardized conditions ($22 \pm 5\,^{\circ}C$, $55 \pm 5\%$ humidity, and 12 h light/dark cycle). They were allowed free access to water and standard mice chow diet. Anesthetic procedures and handling with animals complied with the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt (Approval no: 13 045).

2.3. Experimental design

After 1 week of acclimatization, 180 mice were randomly divided into twelve groups, of 15 mice each. The selected doses were recommended by the work of Chen et al. [12]

Groups 1–3: Normal control mice; intraperitoneally (IP) injected with hydroxyl propyl methyl cellulose (HPMC) and sacrificed after 7, 14 and 45 days of injection, respectively.

Groups 4–6: IP injected daily for 7 days with doses of 50, 250 and 500 mg/ kg body weight of TiO_2NPs, in HPMC respectively.

Groups 7–9: IP injected daily for fourteen days with doses of 50, 250 and 500 mg/ kg body weight of TiO_2NPs in HPMC, respectively.

Groups 10–12: IP injected daily for 45 days with doses of 50, 250 and 500 mg/ kg body weight of TiO_2NPs in HPMC.

2.4. Sample preparations

Serum sample: Blood was collected from each animal by puncture the sublingual vein in a clean and dry test tube, left 10 min to clot and centrifuged at 3000 r.p.m for serum separation. The separated serum was stored at $-80\,^{\circ}C$ for further determinations of liver function enzymes.

Liver homogenates: Liver tissue was homogenized in normal physiological saline solution (0.9% NaCl) (1:10 w/v). The homogenate was centrifuged at $4\,^{\circ}C$ for 15 min at 3000 rpm [11] and the supernatant was stored at $-80\,^{\circ}C$ for further estimation of hepatic oxidative stress markers and hepatic total protein contents.

2.5. Biochemical assays

Malondialdehyde was assayed in hepatic tissue according to the method of Buege and Aust [13]. Malondialdehyde; a product of polyunsaturated fatty acids oxidation, was calculated using the extinction coefficient value 1.56×105 M–1 cm-1 and read at 535 nm.

Hepatic glutathione (GSH) was assayed according to the method of Moron et al. [14] using dithiobis-2-nitrobenzoic acid (DTNB) in PBS. The reaction color was read at 412 nm.

Hepatic total protein was assayed by the method of Bradford [15], where Coomassie Brilliant Blue dye reacted with Bradford reagent and gave a blue complex at 595 nm.

Serum aspartate and alanine amintransferases were estimated by the method of Reitman and Frankel [16] using Diagnostic kit (Biodiagnostic, Egypt), where the transfer of amino group from aspartate or alanine formed oxalacetate or pyruvate, respectively and the developed color was measured at 520 nm.

Serum GGT was estimated by the method of Szasz [17], where GGT enzyme reacted with L-g-glutamyl-3-carboxy-*p*-nitroanilide and glycyl-glycine to give L-g-glutamyl-glycyl-glycine and 5-amino-2-nitrobenzoate. The decrease in absorbance was read at 450 nm at 1 min intervals for 3 min.

Nitric oxide was assayed in serum by the method of Moshage et al. [18]. The assay is based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Giess Reaction.

Total antioxidant capacity (TAC) in hepatic tissue was measured using the diagnostic Abcam Assay Kit (Cambridgeshire, UK), which can measure by either the combination of both small molecule antioxidants and proteins or small molecules alone in the presence of Protein Mask. Cu^{2+} ion is converted to Cu+ by both small molecule and protein. The Protein Mask prevents Cu^{2+} reduction by protein, enabling the analysis of only the small molecule antioxidants. The reduced Cu+ ion is chelated with a colorimetric probe giving a broad absorbance peak around 570 nm, proportional to the total antioxidant capacity.

2.6. Chromosome aberrations

Mice were injected i.p. with colchicine 2 h before sacrifice. Somatic chromosome from bone marrow cells were made according to the technique described by Yosida and Amano [19]. A group of fifteen mice was used for each treatment and 100 well-spread metaphases were analyzed per animal scoring different kinds of abnormalities. Gaps, breaks, fragments, deletions and metaphases were recorded in bone marrow cells.

2.7. Histopathological study

Liver slices were fixed in 10% paraformaldehyde and embedded in paraffin wax blocks. Sections of 4 μm thick were stained with hematoxylin & eosin (H&E) and Masson's trichrome, then examined under light microscope for determination of pathological changes [20].

A semiquantitative scoring system, ranging between zero and four, was used for grading both histopathological changes (oedema, vascular congestion, hemorrhage, inflammation and fibrosis) and chromosomal aberration (fragments and degradation) in all of the tissue samples (liver or bone marrow). The five different histopathologically assessed parameters and the two chromosomal aberration indexes were scored as follows: 0: absent, 1: mild, 2: moderate, 3: common, and 4: severe. The pathological score was calculated based on the mean ± SD of the scores of these parameters in each group under examination [21].

2.8. Statistical analysis and calculations

All data were expressed as mean ± SD of 15 mice in each group. Statistical analysis was carried out by independent student *t*-test using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) Computer Program. Significance difference