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Changes of serum parameters of TiO_2 nanoparticle-induced atherosclerosis in mice

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HIGHLIGHTS

- Exposure to TiO_2 NPs caused pulmonary inflamation in mice.
- Exposure to TiO_2 NPs caused atherosclerotic lesions in mice.
- Exposure to TiO_2 NPs resulted in elevation of serum lipid and cholesterol.
- Exposure to TiO_2 NPs caused alterations of serum atherosclerosis parameters.

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ABSTRACT

The evaluation of toxicological effects of nanoparticulate matter is increasingly important due to their growing occupational use and presence as compounds in consumer products. Numerous studies have shown that exposure to nanosized particles lead to systemic inflammation in experimental animals, but whether long-term exposure to nanosized particles induces atherogenesis is rarely evaluated. In the current study, mice were continuously exposed to TiO_2 nanoparticles (NPs) at 1.25, 2.5, or 5 mg/kg body weight, administered by nasal instillation for nine consecutive months, and the association between serum parameter changes and atherosclerosis in mice were investigated. The present findings suggested that chronic exposure to TiO_2 NPs resulted in atherogenesis coupling with pulmonary inflammation, increased levels of serum triglycerides, glucose, total cholesterol, low-density lipoprotein cholesterol, advanced glycation end products, reactive oxygen species, NAD(P)H oxidases 4, C-reaction protein, E-selectin, endothelin-1, tissue factor, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, monocyte chemoattractant protein-1, plasminogen activator inhibitor-1, and reduced levels of serum high-density lipoprotein cholesterol, nitric oxide and tissue plasminogen activator. Our study suggests an association of long-term exposure to TiO_2 NPs with atherosclerosis and pulmonary inflammation. This finding demonstrates the hypothesized role of TiO_2 NPs as a risk factor for atherogenesis.

1. Introduction

Long-term nanoparticule air pollution is linked to the incidence of acute cardiovascular events [1–7]. The initiation and acceleration of atherosclerosis has been hypothesized as a physiologic pathway through which particles exert cardiovascular effects [8–10]. Acute air pollution exposure has been linked to pulmonary and systemic inflammation [11,12] and repeated inflammatory responses may lead to accelerated atherosclerosis [9,13]. There is toxicologic evidence of a link between particulate matter exposure and atherosclerosis [14–16]. However, whether chronic exposure to inhalable nanoparticles contributes to atherogenesis is not reported.

As known, titanium dioxide nanoparticles (TiO_2 NPs) are widely used as a pigment due to its brightness and high refractive index. It can be found in paints, plastics, paper, inks, sunscreens, cosmetic creams, foods, medicines, and toothpaste as well as in the environmental decontamination of air, water, and soil by the destruction of pesticides [17–22]. However, numerous studies have demonstrated that chronic exposure to TiO_2 NPs resulted in the inflammation of

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lung [23–26], liver [27–31], kidney [32–34], spleen [35–38], brain [39–41], and ovary [42,43] in animals. Especially, TiO_2 NP exposure was also suggested to cause cardiac inflammation and a modest increase in plaque progression in aorta in mice [44–46] as well as endothelial inflammatory response in primary vascular endothelial cells [47,48]. Therefore, we hypothesized that the systemic inflammation and repeated inflammatory responses may lead to accelerated atherosclerosis due to chronic exposure to TiO_2 NPs.

Atherosclerosis is a complex, multifactorial disease initiated by focal lipid deposition in the arterial wall and formation of characteristic lesions known as atherosclerotic plaques. There is thus intense interest in systemic markers of inflammation in relation to both risk assessment for cardiovascular disease and the possible role of such markers in pathogenesis of atherosclerosis and atherothrombosis due to chronic exposure to TiO_2 NPs. In this study, we aimed to explore whether chronic exposure to TiO_2 NPs causes atherosclerosis in mice. The changes of serum parameters in the effect of TiO_2 NPs were also investigated.

2. Methods

2.1. Chemicals

The preparation, characteristics of TiO_2 NPs particles including the anatase structure, size, surface area, mean hydrodynamic diameter and ζ potential, have been described in our previously work [33,49,50]. The average particle size of powdered TiO_2 NPs suspended in 0.5% w/v hydroxypropylmethylcellulose (HPMC) K4M solvent after 24 h (5 mg/mL) incubation ranged from 5 to 6 nm, and the surface area was 174.8 m^2/g. The mean hydrodynamic diameter of TiO_2 NPs in HPMC solvent (5 mg/mL) ranged from 208 to 330 nm (mainly 294 nm), and the ζ potential after 24 h incubation was 9.28 mV [49,50].

2.2. Animals and treatment

160 CD-1 (ICR) female mice (20 ± 2 g body weight) were purchased from the Animal Center of Soochow University (China). All mice were housed in stainless steel cages in a ventilated animal room. Room temperature of the housing facility was maintained at $24 \pm 2\,^{\circ}C$ with a relative humidity of $60 \pm 10\%$ and a 12-h light/dark cycle. Distilled water and sterilized food were available for mice ad libitum. Prior to dosing, the mice were acclimated to this environment for 5 days. All procedures used in animal experiments conformed to the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

TiO_2 NP powder was dispersed onto the surface of 0.5% w/v HPMC and the suspension containing TiO_2 NPs was treated ultrasonically for 30 min and mechanically vibrated for 5 min. The mice were randomly divided into four groups ($N = 30$ each), including a control group treated with 0.5% w/v HPMC and three experimental groups treated with 1.25, 2.5, and 5 mg/kg TiO_2 NPs. The mice were weighed, volume of TiO_2 NP suspensions was calculated for each mouse, and the fresh TiO_2 NP suspensions were administered to the mice by nasal administration every day for nine months. Any symptoms, growth state, eating, drinking and activity, or mortality were observed and recorded carefully daily during the nine months. After the nine-month period, all mice were weighed, anesthetized with ether, blood samples were collected from the eye vein by rapidly removing the eyeball, and serum was collected by centrifuging the blood samples at 1, 200 $\times g$ for 10 min.

2.3. Assay of pulmonary inflammation

After blood collection, the lungs from the control and treated groups were immediately lavaged twice with phosphate buffer saline (PBS). An average of >90% of the total instilled PBS volume was retrieved both times and the amounts did not differ among the groups. The resulting fluid was centrifuged at 400 $\times g$ for 10 min at 4 °C to separate the cells from the supernatant containing various surfactants and enzymes. The cell pellet was used for enumeration of total and differential cell counts as described by AshaRani et al. [51]. Macrophages, lymphocytes, neutrophils, and eosinophils recovered from the BALF were counted using dark field microscopy to assess the extent of phagocytosis. The inflammatory cytokines interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-α) were measured in the primary cell-free BALF by ELISA commercial kits (R&D Systems, Minneapolis, MN, USA).

2.4. Histopathological examination of lung and coronary artery

All histopathological examinations were performed using standard laboratory procedures. Five sets of lung tissues from mice were embedded in paraffin blocks, sliced to 5-μm thickness, and placed on separate glass slides (five slices from each kidney). A section of the thoracic aorta between the distal end of the aortic arch and the diaphragm was dissected after anesthetization with an intraperitoneal injection of sodium pentobarbital (30 mg/kg). Tissue samples were immersed in 10% formaldehyde, dehydrated and embedded in optimum cutting temperature compound (OCT). Cross-sections (5 μm) were prepared every 100 μm [52,53].

After hematoxylin–eosin staining, the sections were evaluated by a histopathologist unaware of the treatments, using an optical microscope (U-III Multi-point Sensor System; Nikon, Tokyo, Japan).

2.5. Biochemical assay of myocardium function

In the present study, the levels of total cholesterol (TC), triglycerides (TG), glucose (GLU), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured by enzymatic methods according to the manufacturer's instructions using the detection kit purchased from Rongsheng Biotechnology Company Ltd. (Shanghai, China). The level of advanced glycation end products (AGEs), reactive oxygen species (ROS), NAD(P)H oxidases 4 (Nox4), C-reaction protein (CRP), E-selectin (E-sel), endothelin-1 (ET-1), tissue factor(TF), nitric oxide (NO), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), monocyte chemoattractant protein-1 (MCP-1), plasminogen activator inhibitor-1(PAI-1), tissue plasminogen activator (t-PA) in the serum was assayed by ELISA commercial kits (R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions. The absorbance was measured on a microplate reader at 450 nm (Varioskan Flash; Thermo Electron, Finland), and the concentrations of these parameters mentioned above were calculated from a standard curve for each sample.

2.6. Statistical analysis

All results are expressed as means $\pm$ SD. One-way analysis of variance (ANOVA) was carried out to compare the differences of means among the multi-group data using SPSS 19 software (SPSS, Inc., Chicago, IL, USA). Dunnett's test was performed when each dataset was compared with the solvent control data. Statistical significance for all tests was judged probability level of 0.05 ($P < 0.05$).

3. Results

3.1. Pulmonary inflammation

Lungs of TiO_2 NP-exposed mice showed emphysema, fibrotic thickening of the alveolar septae, cuffing of alveolar blood vessels, and infiltration of inflammatory cells (Fig. 1). The numbers