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Short communication

Vasomotor dysfunction in human subcutaneous arteries exposed *ex vivo* to food-grade titanium dioxide

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ABSTRACT

Animal studies have shown that titanium dioxide (TiO_2) exposure affects arterial vasomotor function, whereas little is known about the effects in arteries from humans. This study investigated vasomotor responses after direct exposure of human subcutaneous arteries to food-grade TiO_2 (E171) (14 or 140 μg/ml) for 30 min and 18 h. Vasomotor responses to bradykinin, 5-hydroxytryptamine (5-HT), sarafotoxin 6c (S6c) and nitroglycerin were recorded in wire-myographs. Vasoconstrictor responses to 5-HT were increased in arteries exposed to E171 for 18 h ($P < 0.05$). Furthermore, an increase in S6c responses was seen in low concentration E171 exposed arteries (30 min exposure; $P < 0.05$). The vasorelaxation response to nitroglycerin was increased in low concentration E171 exposed arteries (30 min exposure; $P < 0.05$). Vasorelaxation responses to bradykinin were unaffected after treatment with E171. There was no difference in gene expression levels of intercellular cell adhesion molecule 1, vascular cell adhesion molecule 1, 5-hydroxytryptamine receptor 1B, 5-hydroxytryptamine receptor 2A, endothelin receptor A and endothelin receptor B in E171 exposed arteries after exposure to TiO_2 for 30 min or 18 h. In conclusion, this study shows that the same type of vasomotor dysfunction is found in artery segments of rats and humans following *ex vivo* exposure to E171.

1. Introduction

Studies employing experimental animal models have shown that exposure to particles produces vasomotor dysfunction, displayed as increased vasoconstriction and/or decreased vasodilation of arteries, although there may be differences in potency between types of materials and mode of exposure (Møller et al., 2016). The same effect on vasomotor function has been observed in humans after pulmonary exposure to air pollution particles using mainly indirect methods such as flow-mediated vasodilation or plethysmographic responses to arterial infusion of vasoactive compounds (Møller et al., 2011). In contrast, there is little information about the effect of non-combustion particles or direct effects of any type of particles on human arteries. Titanium dioxide (TiO_2) is one of the most widely used and investigated types of particles. It has been shown that occupational exposure to TiO_2 is associated with cardiopulmonary effects, notably elevated levels of intercellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) in serum (Zhao et al., 2018). Expression of adhesion molecules on endothelial cells is an indicator of activation and atherogenesis. Several studies have shown that exposure to various

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HT1B, 5-hydroxytryptamine receptor 1B; 5-HT2A, 5-hydroxytryptamine receptor 2A; EDNRA, endothelin receptor A; EDNRB, endothelin receptor B; ICAM-1, intercellular cell adhesion molecule 1; L-NAME, N^G-nitro-L-arginine methyl ester; NTG, nitroglycerin; S6c, sarafotoxin 6c; TiO_2, titanium dioxide; VCAM-1, vascular cell adhesion molecule 1

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types of TiO_2 increases the expression of ICAM-1 and VCAM-1 on the surface of cultured endothelial cells (Alinovi et al., 2015; Danielsen et al., 2015; Han et al., 2013; Mikkelsen et al., 2013; Montiel-Dávalos et al., 2012; Ramos-Godínez et al., 2013).

TiO_2 is used as a white pigment in a number of food items. The European Food and Safety Authority (EFSA) has estimated the average daily intake of food-grade TiO_2 to 1.8–10.4 mg/kg in children (95% percentile: 4.9–32.4 mg/kg per day), whereas it is lower in the elderly (mean: 0.4–4.5 mg/kg/day; 95% percentile: 1.2–10.7 mg/kg/day). Children have a higher dose than elderly because they ingest higher amounts of certain food items with E171 (e.g. candies) and their body weight is lower. In comparison, inhalation exposure to pigment-grade TiO_2 at the occupational maximal exposure limit (US EPA: 2.4 mg/m^3) only amounts to a deposited dose of 0.02 mg/kg/day (assuming inhalation of 16 m^3/day, 8-h working day and 10% deposition). TiO_2 is considered not to penetrate beyond the epidermis layer of the skin and therefore have little health consequences by the dermal route of exposure (Warheit and Donner, 2015). E171 is authorized as a food additive in Europe, allowed in *quantum satis* levels (EFSA, 2016; European Commission, 2012). This material consists mostly of larger sized particles, but a minor fraction of the mass is nanosized (Warheit et al., 2015a). Yang et al., 2014 reported that five different samples of E171 contained 17–35% nanosized particles, based on the size distribution. Humans are predominantly exposed to E171 through food intake and to a lesser extent via inhalation of particles, e.g., during the manufacturing process.

The current literature on TiO_2-induced vasomotor function responses has produced conflicting results. It has been shown that pulmonary exposure to fine and nanosized pigment-grade TiO_2 affects the vasodilation response in the microcirculation of rats (Nurkiewicz et al., 2009). Other studies have shown that intratracheal instillation of nanosized TiO_2 did not alter the acetylcholine-induced vasorelaxation response in intrapulmonary arteries of rats (Courtois et al., 2010) or aorta segments from dyslipidemic ApoE knockout mice (Mikkelsen et al., 2011). We have found similar changes in the vasomotor function of coronary arteries of rats exposed intragastrically to E171 and after direct *ex vivo* exposure of rat aorta segments to E171 (Jensen et al., 2018).

The purpose of the present study was to investigate if direct exposure to E171 affects the vasomotor function and endothelial cells in isolated human subcutaneous arteries, which could be a promising model system for vascular particle toxicology. Subcutaneous arteries were isolated from tissue surgically removed from patients undergoing elective plastic surgery for excessive abdominal skin after massive weight loss due to bariatric surgery or lifestyle changes. Gene expression levels of endothelin and 5-hydroxytryptamine 1B (5-HT1B) or 2A (5-HT2A) receptors were measured to assess direct effects of TiO_2-exposure on receptor-mediated vasocontraction responses. In addition, the expression levels of ICAM-1 and VCAM-1 were assessed in arterial segments to investigate a possible initiation of endothelial damage on the artery segments.

2. Material and methods

2.1. Particle characterization

E171 was purchased at Bolsjehuset (Albertslund, DK, www.bolsjehuset.dk), which is a supplier of ingredients to candies in Denmark. EFSA, 2016, states that E171 is insoluble in water, hydrochloric acid, dilute sulfuric acid and organic solvents, whereas it dissolves slowly in hydrofluoric acid and hot concentrated sulfuric acid. The sample contained 99.8% and 0.2% anatase and rutile form of TiO_2, respectively (X-ray diffractometer measurement, Danish Technological Institute, Taastrup, DK). The raw material and identical suspensions have been characterized in a previous study (Jensen et al., 2018). In brief, the primary particle size, assessed by transmission electron microscopy, showed three size groups of 135 ± 6 nm, 305 ± 61, 900 ± 247 nm. The specific surface area was 1935 m^2/g. The zeta potential was −37.2 ± 2.0 mV. The hydrodynamic particles size distribution in Dulbecco's Modified Eagle's medium (DMEM, Sigma-Aldrich), measured by Nanoparticle Tracking Analysis, showed a mean particle size of 203 ± 75 nm and 90% of the particles with a size below 363 ± 126 nm. The hydrodynamic particle size (in PBS) was also measured by Dynamic Light Scattering; it showed that 94% of the particles were below 880 ± 390 nm and 1% were below 19 nm.

A particle stock solution was made immediately before exposure to vessel segments. E171 was suspended in DMEM to a concentration of 1.4 mg/ml. The stock suspension was sonicated for 16 min on ice (Vibra Cell Vc50t Ultrasonic Processor, Sonics & Materials, 20 kHz).

2.2. Subcutaneous tissue

Human subcutaneous resistance arteries from abdominal adipose tissue were used to study vasomotor responses after *ex vivo* exposure to E171. A description of tissue removal and collection has been described previously (Dreier et al., 2016). The type of arteries is branches from peri-umbilical perforators. Ten patients (all women) were included in the study and gave full written consent before participation. The study was approved by the Committee of Health Research Ethics in the Capital Region of Denmark (H-150056617). All patients had excessive abdominal skin removed after massive weight loss due to either previous bariatric surgery (n = 7) or lifestyle changes (n = 3). None of the patients had elevated blood pressure or clinical manifestations of cardiovascular disease. It has been shown that patients with massive weight loss had unaltered levels of pro-inflammatory cytokines (interleukin 1, interleukin 6 and tumor necrosis factor α) in superficial inferior epigastric arteries, whereas there was a thickening of the vessel wall (Katzel et al., 2016). The surgery was done at the Department of Plastic Surgery, Herlev Hospital, Denmark. A whole-piece abdominal skin was removed during surgery. Bleeding vessels in the specimen were closed with haemoclips rather than electric cautery to minimize tissue damage and also to facilitate quick identification of the vessels in the laboratory. After removal of the abdominal skin, the surgical specimen was immediately transferred to an ice-cold physiological saline solution (5 °C, 9 g/l NaCl) and transported to the laboratory, where vessel isolation and myograph studies were performed. Time from the removal of tissue from the patient to delivery at the laboratory was approximately one hour.

2.3. Isolation of arteries and particle exposure

Immediately after tissue arrival at the laboratory, the abdominal skin biopsy was placed into ice-cold pre-aired (5% CO_2/95% O_2) physiological buffer (117.8 mM NaCl, 4.0 mM KCl, 2.0 mM $CaCl_2$, 0.9 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 20.0 mM $NaHCO_3$, and 5.0 mM glucose). Arteries (303–930 μm in diameter) were dissected free from the adipose tissue under a stereomicroscope and cut into ring segments (length 1–2 mm). Six segments were used for wire myograph experiments, and six segments were used for gene expression analysis. After isolation, the segments were placed in single wells in a culture plate containing DMEM (2 ml/well). Six of the arterial segments were immediately exposed to E171 for 18 h (two segments in exposure group). The other six segments were maintained in DMEM for 18 h (37 °C supplemented with 5% CO_2) and subsequently exposed to E171 for 30 min (two segments in each exposure group). The exposure periods were chosen to fit into doable working days; the preparation of particle suspension and assessment of vasomotor response in myograph experiments had a total duration up to 10 h. We considered the 30 min to 18 h period relevant because previous studies of direct particle exposure have been shown to have an effect on vasomotor responses within 30 min (Hansen et al., 2007; Jensen et al., 2018; Vesterdal et al., 2012). The late time point is similar to a typical 24 h exposure in studies on the expression of cell