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Categorization of nano-structured titanium dioxide according to physicochemical characteristics and pulmonary toxicity

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ABSTRACT

A potentially useful means of predicting the pulmonary risk posed by new forms of nano-structured titanium dioxide (nano-TiO_2) is to use the associations between the physicochemical properties and pulmonary toxicity of characterized forms of TiO_2. In the present study, we conducted intratracheal administration studies in rats to clarify the associations between the physicochemical characteristics of seven characterized forms of TiO_2 and their acute or subacute pulmonary inflammatory toxicity. Examination of the associations between the physicochemical characteristics of the TiO_2 and the pulmonary inflammatory responses they induced revealed (1) that differences in the crystallinity or shape of the TiO_2 particles were not associated with the acute pulmonary inflammatory response; (2) that particle size was associated with the acute pulmonary inflammatory response; and (3) that TiO_2 particles coated with $Al(OH)_3$ induced a greater pulmonary inflammatory response than did non-coated particles. We separated the seven TiO_2 into two groups: a group containing the six TiO_2 with no surface coating and a group containing the one TiO_2 with a surface coating. Intratracheal administration to rats of TiO_2 from the first group (i.e., non-coated TiO_2) induced only acute pulmonary inflammatory responses, and within this group, the acute pulmonary inflammatory response was equivalent when the particle size was the same, regardless of crystallinity or shape. In contrast, intratracheal administration to rats of the TiO_2 from the second group (i.e., the coated TiO_2) induced a more severe, subacute pulmonary inflammatory response compared with that produced by the non-coated TiO_2. Since alteration of the pulmonary inflammatory response by surface treatment may depend on the coating material used, the pulmonary toxicities of coated TiO_2 need to be further evaluated. Overall, the present results demonstrate that physicochemical properties may be useful for predicting the pulmonary risk posed by new nano-TiO_2 materials.

1. Introduction

The European Commission defines a nanomaterial as "a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm" [1]. Due to this small particle size, the surface area per unit mass of nanomaterials is greater than that of the corresponding bulk materials, and it is this characteristic that gives nanomaterials their unique properties.

Nanomaterials are predicted to soon become the cornerstone of the microelectronics, materials, textiles, energy, healthcare, and cosmetics industries [2]. Indeed, nano-structured titanium dioxide (nano-TiO_2) is one of the most widely used nanomaterials in the world, and the production volume of nano-TiO_2, which is increasing annually, is expected to reach nearly 2.5 million metric tons per year in 2025 [3]. Traditionally, TiO_2 fine particles have been considered to have low toxicity; however, concerns have been raised

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recently regarding the potential health risks posed by nano-TiO_2 to consumers, workers, and the environment [4]. In particular, a recently published toxicological review showed that inhalation was the primary route of nano-TiO_2 exposure and thus highlighted the need for more information on the safety of nano-TiO_2 [5]. Many researchers and regulators are now trying to understand the hazards of nano-TiO_2 and determine appropriate strategies for the assessment of the pulmonary risk associated with exposure to nano-TiO_2 materials. Nano-TiO_2 can be manufactured in various forms that have different physicochemical characteristics (e.g., crystallinity, shape, particle size, surface area, and surface modification), which can lead to nanomaterials with the same chemical formula but different pulmonary toxicities [6]. Therefore, from a regulatory standpoint, it would be beneficial to assess the pulmonary risk of all newly developed nano-TiO_2 materials; however, a program of this size is unrealistic due to the time and money that would be required.

One potential means of predicting the pulmonary risk of new forms of nano-TiO_2 is to use the associations between the physicochemical properties and pulmonary toxicity of characterized forms of TiO_2. Several researchers have already investigated the relationships between the physicochemical characteristics of different forms of nano-TiO_2 and their pulmonary toxicities in rats, including the relationship between pulmonary toxicity and particle crystallinity [7], particle size [8,9,10,11,12], particle surface area [13,10,12], and particle surface modification [14,15,16]. For example, smaller TiO_2 particles have been shown to induce a greater acute inflammatory response compared with larger TiO_2 particles after intratracheal administration in rats [8,9,11]. However, Warheit et al. [12] have also shown that the acute inflammatory and cell-injury effects of three TiO_2 materials with different particle sizes did not differ. Furthermore, in most of these previous studies only two or three test materials were examined and the studies were conducted under different test conditions, thus hampering comparison of the results.

In the present study, to clarify the associations between the physicochemical characteristics of TiO_2-based materials and their pulmonary toxicity, intratracheal administration studies were conducted in rats by using seven different characterized TiO_2. To allow a quantitative and statistical approach to be used, all physicochemical characterizations and in vivo studies were performed in the same laboratory under the same test conditions.

2. Materials and methods

2.1. Test materials and preparation of administration formulations

Seven forms of TiO_2 were selected for inclusion in this study: AMT-100, MT-150AW, and MP-100 (Tayca Co. Ltd., Japan); TTO-S-3, TTO-S-3 (Coated), and FTL-100 (Ishihara Sangyo Kaisha, LTD., Japan); and P25 (Evonik Industries, Germany). These materials were chosen to allow examination of as many different physicochemical characteristics as possible, i.e., three different types of crystallinity, three different particle shapes, surface coating (untreated or $Al[OH]_3$ coating), and a range of different particle sizes (Table 1). Representative scanning electron microscope images (S-4800, Hitachi High-Technologies Co., Japan) of each test material are shown in Fig. S1.

Administration formulations were prepared as described in Refs. [17,18] (Table S1). Briefly, 2 g of test material was dispersed in 50 mL of 2 mg/mL of disodium phosphate (food additive grade; Wako Pure Chemical Industries, Ltd., Japan), which was prepared by using endotoxin-free pure water. The suspension was sonicated in a glass bottle by using an ultrasonic bath (5510J-MT; Branson Ultrasonics Co., USA) for one to three hours and then centrifuged at 20–1000*g* for 5–40 min at 20 °C (CF16RXII and T15A41; Hitachi Koki Co., Ltd., Japan). The supernatant was collected as a stock suspension. Administration formulations were prepared by diluting the stock suspension to the appropriate concentration with 2 mg/mL disodium phosphate. The concentration of test material in the administration formulations was determined by using a weight analysis method, where the weight loss of the suspension was measured with a balance scale (AUW220D; Shimadzu Co., Japan) after drying at 200 °C in a thermostatic chamber (ON-300S; As One Co., Japan). The particle size and size distribution of the test materials in the administration formulations were measured by means of dynamic light scattering (Zetasizer Nano ZS; Malvern Instruments Ltd., UK). In principle, particle size and size distribution can be expressed in terms of mass, volume, or number of particles [19]. However, since a robust understanding of which means of expression is the best for understanding the pulmonary toxicity of TiO_2 in rats is yet to be obtained, two different values—volume average diameter and number average diameter—obtained from the dynamic light scattering analysis were used in the present study (Table 1 and Fig. S2). The particle size of FTL-100 was not determined because dynamic light scattering gives little effective information for needle-shaped particles with a large aspect ratio.

2.2. Test animals

Male F344/DuCrlCrlj rats were obtained from Charles River Laboratories Japan, Inc. Animals at 12 weeks of age with body weights of 212.3–282.1 g on the day of administration were used in the in vivo studies. The animals were housed in animal rooms equipped with a local barrier system and were maintained at 21–25 °C and 40% to 70% relative humidity with 10–15 air changes per hour and a photoperiod of 12 h of light per day (lights on, 7:00; lights off, 19:00). The study was approved by the Institutional Animal Care and Use Committee prior to the start of the study.

2.3. Test conditions

In the in vivo studies, one vehicle control group (2 mg/mL of disodium phosphate aqueous solution without test material) and three treatment groups (0.67, 2, or 6 mg/kg TiO_2) were used for each test material. The majority of previous studies using intratracheal administration of TiO_2 in rats were conducted with doses in the range of 0.75–6 mg/kg TiO_2 [8,9,20,11,12,7]. Therefore, based on the results of these previous studies, the doses in the present study were chosen so that toxic effects but not death or severe suffering were induced at the highest dose and so that adverse effects were avoided at the lowest dose. Forty rats were used for each test material (i.e., ten test animals/dose). Test animals were anesthetized by means of isoflurane inhalation and angled approximately 45° on a restraining stand. Administrations were conducted by using a stomach sonde (Natsume Seisakusho Co., Ltd., Japan) or MicroSprayer Aerosolizer (Model IA-1B-R for Rat, Penn-Century, Inc., USA) inserted transorally into the tracheal lumen at a depth of approximately 6 cm from the angle of the mouth. The volumes of the administrations were 1 mL/kg for AMT-100, MT-150AW, TTO-S-3, TTO-S-3 (Coated), P25 and MP-100, and 2 mL/kg for FTL-100, based on the animal's body weight on the day of administration. To assess the acute and subacute pulmonary toxicity of each test material, bronchoalveolar lavage fluid (BALF) examination and pathological examination were conducted at three days (acute phase) and at four weeks (subacute phase) after intratracheal administration. The examinations were not conducted earlier than three days after administration because the results would likely reflect the initial pulmonary inflammatory response to the bolus administration of liquid into the lungs [8]. On the day of exam-