JOURNAL OF FOOD AND DRUG ANALYSIS 22 (2014) 95–104

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Review Article

Genotoxicity of titanium dioxide nanoparticles☆

*Tao Chen**, *Jian Yan*, *Yan Li*

Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR, USA

ARTICLE INFO

Article history:
Received 30 September 2013
Received in revised form
16 December 2013
Accepted 21 December 2013
Available online 5 February 2014

Keywords:
Ames test
Carcinogenicity
Comet
Genotoxicity
Hypoxanthine-guanine phosphoribosyl transferase gene
Micronucleus
Phosphatidylinositol glycan
Class A gene
Sister chromatid exchange
Titanium dioxide nanoparticles
Wing point mutation

ABSTRACT

Titanium dioxide nanoparticles (TiO_2-NPs, <100 nm) are increasingly being used in pharmaceuticals and cosmetics due to the unique properties derived from their small sizes. However, their large surface-area to mass ratio and high redox potential may negatively impact human health and the environment. TiO_2-NPs can cause inflammation, pulmonary damage, fibrosis, and lung tumors and they are possibly carcinogenic to humans. Because cancer is a disease involving mutation, there are a large number of studies on the genotoxicity of TiO_2-NPs. In this article, we review the results that have been reported in the literature, with a focus on data generated from the standard genotoxicity assays. The data include genotoxicity results from the Ames test, *in vitro* and *in vivo* Comet assay, *in vitro* and *in vivo* micronucleus assay, sister chromatid exchange assay, mammalian cell hypoxanthine-guanine phosphoribosyl transferase gene assay, the wing somatic mutation and recombination assay, and the mouse *phosphatidylinositol glycan*, class A gene assay. Inconsistent results have been found in these assays, with both positive and negative responses being reported. The *in vitro* systems for assessing the genotoxicity of TiO_2-NPs have generated a greater number of positive results than the *in vivo* systems, and tests for DNA and chromosome damage have produced more positive results than the assays measuring gene mutation. Nearly all tests for measuring the mutagenicity of TiO_2-NPs were negative. The current data indicate that the genotoxicity of TiO_2-NPs is mediated mainly through the generation of oxidative stress in cells.

1. Introduction

Titanium dioxide (TiO_2) is the naturally occurring oxide of titanium. It has several different crystalline structures. Rutile is the most common natural form of TiO_2, whereas anatase and brookite are two more rare polymorphs. TiO_2 has been used widely in pigments, accounting for 70% of the total production volume of pigments worldwide. It provides whiteness and opacity to products such as paints, plastics, papers, inks, foods, and toothpastes. It can also be found in pharmaceuticals and cosmetic products such as sunblock [1] due to its

☆ The views presented in this paper are those of the authors and do not necessarily reflect official positions or policies of the US Food and Drug Administration.

* *Corresponding author*. Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, US Food and Drug Administration, 3900 NCTR Road, Jefferson, AR 72079, USA.
E-mail address: tao.chen@fda.hhs.gov (T. Chen).

http://dx.doi.org/10.1016/j.jfda.2014.01.008

photocatalytic, biocidal, and/or antiproliferative properties [2]. Until recently, the use had been limited to coarse and fine (both diameters > 100 nm) TiO_2 particles. Coarse and fine particles of TiO_2 have been investigated and declared biologically inert in humans and animals [3,4]. More recently, TiO_2 nanoparticles (TiO_2-NPs, <100 nm) have increasingly been used in pharmaceuticals and cosmetics due to the unique properties derived from their small sizes [5,6]. These new applications of TiO_2-NPs, however, call into question their biological inertness.

TiO_2-NPs have a large surface-area to mass ratio [7] and a high redox potential, which can cause undesirable effects on human health and the environment. Recent studies have revealed that exposure to TiO_2-NPs can cause inflammation, pulmonary damage, fibrosis, and lung tumors in rodents [7–9]. TiO_2-NPs are possibly carcinogenic to humans (Group 2B) based on sufficient evidence in experimental animals and inadequate evidence from epidemiology studies, according to a report from the International Agency for Research on Cancer [10]. The National Institute for Occupational Safety and Health (NIOSH) [11] also concluded that TiO_2-NPs were a potential occupational carcinogen, acting through a secondary genotoxicity mechanism primarily related to particle size and surface area.

Genotoxicity data are important for nanotechnology regulation and risk assessment. Recently, the genotoxicity of TiO_2-NPs has been intensively studied due to their carcinogenicity. Although a large number of reports on the genotoxicity of TiO_2-NPs and its underlying mechanisms have been published, there has been no review article specific to the genotoxicity of TiO_2-NPs. The purpose of this review is to present up-to-date knowledge regarding the genotoxicity of TiO_2-NPs, with a focus on results from standard genotoxicity assays.

2. *In vitro* studies

Results on genotoxicity studies on TiO_2-NPs were identified through Medline database searches. The data from the studies using the standard genotoxicity assays including the Ames test, Comet assay, micronucleus assay, sister chromatid exchange (SCE) assay, and mammalian cell gene mutation assay, are summarized in Table 1.

2.1. *Ames test*

The Ames test is formally called the *Salmonella typhimurium* reversion assay. This test is used worldwide as an initial screen to determine the mutagenic potential of agents and the assay identifies point mutagens [12,13].

Four different types of TiO_2-NPs have been evaluated by the Ames assay and all of them were negative in the standard mutation assay. However, two showed positive responses when evaluated with a modified fluctuation test procedure (Table 1).

Jomini et al [14] used the standard fluctuation test and a modified fluctuation test procedure with the *S. typhimurium* strains TA97, TA98, TA100, and TA102 to measure the mutagenic potential of two types of TiO_2-NPs. The test was negative when the normal assay was used. However, when they applied a simple pre-exposure of bacteria to the NPs in a low ionic strength solution (NaCl, 10 mM) at a pH below the nanoparticles isoelectric points (pH 5.5), the results were positive. They concluded that a simple pre-exposure step in a low ionic-strength solution, at a pH below the nanoparticle isoelectric points (NaCl, 10 mM, pH 5.5) could increase bacterial uptake of the nanoparticles and improve the accuracy of the test.

In another two tests, TiO_2-NPs were negative in different *Salmonella* strains. Landsiedel et al [15] evaluated several TiO_2-NPs used for sunscreen products using Ames test. *S. typhimurium* TA1535, TA100, TA1537, TA98, and TA102 were treated with the NPs at 20–5000 μg/plate both with or without metabolic activation. No mutagenicity was found. In the other study, the bacteria were preincubated with eight different concentrations of 10 nm anatase TiO_2-NPs up to 5000 μg/plate. No mutation induction was found. Analyses with transmission electron microscopy and energy-dispersive X-ray spectroscopy show that the TiO_2-NPs are not able to enter the bacterial cells [16].

2.2. *Comet assay*

The Comet assay is a method for measuring DNA strand breaks in eukaryotic cells. The Comet assay is also called the single-cell gel electrophoresis assay due to its working principle. After treatment, single cell suspensions are embedded in agarose on a microscope slide and lysed. Electrophoresis at high pH results in structures resembling comets when observed by fluorescence microscopy. The intensity of the comet tail relative to the head is proportional to the number of DNA breaks. For detecting oxidative DNA damage, cells embedded in agarose on microscope slides can be further treated with nucleases such as formamidopyrimidine DNA-glycosylase (Fpg), endonuclease III (Endo III), and 8-hydroxyguanine DNA-glycosylase to generate secondary DNA breaks at the sites with oxidative DNA adducts. The Comet assay has been widely used to assess genotoxicity of nanomaterials due to its sensitivity and simplicity. The results from *in vitro* Comet assays on TiO_2-NPs are summarized in Table 1.

Among 24 Comet assay tests, 17 of them showed positive responses to treatments of different types of TiO_2-NPs (Table 1). Bottlenose dolphin leukocytes were treated with smaller than 25 nm anatase TiO_2-NPs and the Comet assay was performed to measure the genotoxicity of the NPs. The results showed that the NPs were genotoxic for the cells after exposure to concentrations of 50 μg/mL and 100 μg/mL for 24 hours and 48 hours, respectively [17]. AGS human gastric epithelial cells treated with 21 nm TiO_2-NPs caused DNA damage. The tail intensity increased 1.88-fold in 150 mg/mL of TiO_2-NPs treated cells compared to the control cells [18]. Human peripheral blood lymphocytes and cultured human embryonic kidney (HEK293) cells were treated with 1 μg/mL, 10 μg/mL, and 100 μg/mL of 2.3 nm TiO_2-NPs and the DNA breaks were measured using the Comet assay with or without the Fpg and Endo III enzymes. The 100 μg/mL of TiO_2-NPs significantly increase the DNA damage with or without the Fpg and Endo III enzymes in both the cell lines [19]. The Comet assays were conducted using human bronchial epithelial BEAS 2B cells to