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TiO_2 nanoparticles and bulk material stimulate human peripheral blood mononuclear cells ☆

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ABSTRACT

Nanomaterials are increasingly produced and used throughout recent years. Consequently the probability of exposure to nanoparticles has risen. Because of their small 1–100 nm size, the physicochemical properties of nanomaterials may differ from standard bulk materials and may pose a threat to human health. Only little is known about the effects of nanoparticles on the human immune system. In this study, we investigated the effects of TiO_2 nanoparticles and bulk material in the *in vitro* model of human peripheral blood mononuclear cells (PBMC) and cytokine-induced neopterin formation and tryptophan breakdown was monitored. Both biochemical processes are closely related to the course of diseases like infections, atherogenesis and neurodegeneration. OCTi60 (25 nm diameter) TiO_2 nanoparticles and bulk material increased neopterin production in unstimulated PBMC and stimulated cells significantly, the effects were stronger for OCTi60 compared to bulk material, while P25 TiO_2 (25 nm diameter) nanoparticles had only little influence. No effect of TiO_2 nanoparticles on tryptophan breakdown was detected in unstimulated cells, whereas in stimulated cells, IDO activity and IFN-γ production were suppressed but only at the highest concentrations tested. Because neopterin was stimulated and tryptophan breakdown was suppressed in parallel, data suggests that the total effect of particles would be strongly pro-inflammatory.

1. Introduction

The use of nanomaterials in consumer products, electronics, sporting goods and medicine has grown enormously in the past years (Kaida et al., 2004; Thomas et al., 2006). In parallel, the potential exposure of humans to nanoparticles has increased. However, aside from many positive properties of nanomaterials, which make them desirable for many applications, they represent an unknown risk factor for human health, which needs to be investigated in more detail. Because of the small 1–100 nm size, which causes a huge increase of total surface area, the physicochemical properties of nanoparticles may considerably differ from bulk materials and may pose a threat to human cells. However it is still important to investigate possible harmful effects and potential risks of these materials on biochemical processes especially those mediated by the immune system.

Titanium dioxide (TiO_2) crystallizes in three major different structures: anatase, rutile and brookite. However, only rutile and anatase play any role in the applications of TiO_2 (Diebold, 2003). Anatase is more chemically reactive than rutile, and it has been suggested that anatase has a greater toxic potential than rutile (Warheit et al., 2007b; Sayes et al., 2006; Xue et al., 2010; Petkovic et al., 2011). TiO_2 normally presents as a mixture of anatase and rutile crystal forms. The principal parameters of the particles affecting their physicochemical properties are shape, size, surface characteristics and inner structures. TiO_2 fine particles are poorly soluble and have only little toxic features. However, smaller sized nanoparticles may exert different physicochemical properties with increased bioactivity compared to fine particles. Based on this fact, toxicity can rise and exhibit harmful effects on human health, associated with the decreased size of particles (Shi et al., 2013; Andersson et al., 2011; Wang and Li, 2012; Iavicoli et al., 2011, 2012).

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TiO_2, the most widely used nanoparticle, is a white pigment and has a very high refractive index. Four million tons TiO_2 account for 70% of total production volume of pigments worldwide (Ortlieb, 2010; Baan et al., 2006). TiO_2 nanoparticles are a promising material for many applications and are used in a multitude of consumer products (Valdiglesias et al., 2013; Zhang et al., 2012; Akhavan et al., 2013; Shi et al., 2013). Because of its low production costs, the photostability in solution, general non-toxicity, anticorrosive properties, high stability and redox selectivity, this metal oxide continues to gain further interest for novel applications and industrial use (Gupta and Tripathi, 2011; Riu et al., 2006). Actually TiO_2 is used in sunscreens for UV protection, as a white dye in food and toothpaste or in dental and surgical implants (Lomer et al., 2002; Gélis et al., 2003; Olmedo et al., 2009; Lin et al., 2013; Brown and Clark, 2013; Rossi et al., 2008; Chung et al., 2013). At the interface to the biological medium, TiO_2 corrosion and biodegradation may occur, causing the release of ions to the microenvironment and the possible harmful effects have to be investigated (Olmedo et al., 2009).

There are several studies, which analyzed the different adsorption and uptake routes in the human body. Most of the literature focuses on the respiratory system, which is the primary uptake route of nanoparticles (Shi et al., 2013; Lee et al., 2011; Mühlfeld et al., 2007). After the initial absorption of TiO_2, the particles can be distributed to all organs and tissues in the body. However, the translocation of nanoparticles within the body is not well approved. An explanation can be the interaction with plasma-proteins or coagulation factors, platelets and red or white blood cells (Deng et al., 2009). The gastrointestinal absorption may be also an important route since nanoparticles are present in drug carriers, food products and beverages (Hagens et al., 2007; Lomer et al., 2002). Senzui et al. analyzed skin penetration on intact and injured skin studies and concluded that TiO_2 did not penetrate the human skin (Senzui et al., 2010). There are few *in vivo* studies which investigate the genotoxicity of TiO_2, however, one group found a correlation between nanoparticles and inflammation, which is caused by interleukin-(IL)-1β (Yazdi et al., 2010). Ciu et al. identified that TiO_2 can generate liver injury via activation of toll like receptors (TLR), nuclear factor kappa B (NF-κB) and inflammation outbreak in mice (Cui et al., 2011). Another animal study found an altered mRNA expression of inflammatory cytokines like IL-6, IL-1β, tumor necrosis factor-(TNF)-α or transcription factor NF-κB (Ma et al., 2009).

In human epidermal cells TiO_2 nanoparticles were found to exert genotoxicity via the induction of reactive oxygen species (ROS) (Shukla et al., 2011). Similarly neurotoxicological effects caused by exposure to TiO_2 nanomaterials were detected in mice (Hu et al., 2010) and in human neuronal cells (Valdiglesias et al., 2013). Obviously these compounds are able to gain entry into the body and could exert potential toxic effects at several levels. The US Food and Drug Administration (FDA) established a regulation for TiO_2 nanoparticles as a color additive for food (FDA, 2002). However, thus far effects of nanomaterials to the human immune system were still only rarely described.

During immune activation different types of immune responses can be distinguished by distinct types of T-helper (Th) cells like Th1- and Th2-cells (Jin et al., 2012; Romagnani, 2006). Th1-type cells are characterized by IL-2 and IFN-γ secretion and are primarily observed in the course of viral and microbial infections, malignant tumor diseases and allograft rejections after transplantation. Th2-type cells are predominantly responsible for allergic diseases and asthma (Romagnani, 2004). There exist also other subsets like Th-17, which link innate and adaptive immune responses (Yu and Gaffen, 2008), or regulatory T cells (T_{regs}), which play a role in the development of immunological self-tolerance (Hori et al., 2003).

In the course of a cellular immune response the pro-inflammatory cytokine IFN-γ is released preferentially from T-helper cells type 1. Aside from its immunoregulatory relevance, IFN-γ represents an important trigger for ROS production in human macrophages as part of its cytocidal and antimicrobial repertoire (Nathan et al., 1983). In parallel, IFN-γ induces the expression of the enzymes GTP-cyclohydrolase I (GCH-I) giving raise to the formation of neopterin and of indoleamine 2,3-dioxygenase (IDO). IDO catalyses the initial step in the breakdown of the essential amino acid tryptophan via the kynurenine pathway. A high IDO activity, which is estimated by the kynurenine to tryptophan ratio (Kyn/Trp), results from a strong cellular immune activation (Fuchs et al., 1991). Further, the estimation of neopterin production and tryptophan breakdown have been shown earlier to be robust read outs to monitor and investigate Th1-type immune response *in vitro* (Jenny et al., 2011).

For testing the effects of chemicals and compounds on the cellular immune response, an *in vitro* assay based on freshly isolated human peripheral blood mononuclear cells (PBMC) turned out to be useful (Winkler et al., 2006; Maier et al., 2010; Jenny et al., 2011). In this study applying the PBMC assay, we examined the influence of two different preparations of TiO_2 nanoparticles and of commercial bulk material. Two types of TiO_2 nanoparticles (anatase + rutile) were tested in order to compare effects regarding their different crystalline phase. In culture supernatants the production of neopterin and IFN-γ as well as the breakdown of tryptophan were examined.

2. Materials and methods

2.1. Chemicals

TiO_2 bulk material was purchased from Sigma–Aldrich (Paris, France) and P25 nanomaterial was from Degussa-Evonik (Germany). OCTi60 are laboratory samples from the Service des Photons (Saclay, Gif-sur Yvette, France). Phytohemagglutinin (PHA) was purchased from Sigma–Aldrich (Vienna, Austria) and dissolved in phosphate buffered saline (PBS) and stored at −20 °C until use.

2.2. PBMC isolation and culture

PBMC were isolated from whole blood obtained from healthy donors, which confirmed that their donated blood might be used for scientific purposes in case, when it was not selected for transfusion. Separation of blood cells was performed by density centrifugation (Lymphoprep, Nycomed Pharma AS, Oslo, Norway) as described in more detail earlier (Jenny et al., 2011). After isolation, PBMC were washed three times in phosphate buffered saline containing 1 μmol/L ethylenediamine diacetate (EDTA). Cells were cultivated in RPMI 1640 supplemented with 10% heat-inactivated foetal calf serum (Biochrom, Berlin, Germany), 2 mmol/L L-glutamine (Serva, Heidelberg, Germany) and 50 μg/ml gentamicin (Bio-Whittaker, Walkersville, MD) at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Preparation of nanoparticles

In this study, we used TiO_2 nanopowders from commercial source (P25, Degussa-Evonik, Germany) as well as a laboratory sample (OCTi60, France). These samples are synthesized by gas phase methods, combustion (P25) or laser pyrolysis (OCTi60) (Pignon et al., 2008) and are composed of a major phase of anatase and a minor phase of rutile.

The preparation of suspensions was done using a several steps method detailed elsewhere (Carrière et al., 2013). A stock solution was prepared using a one hundred mg of TiO_2 material manually mixed with 10 ml of sterile filtered water. The suspension was sonicated by a high intensity sonicator for a total time of 1 h at 5 °C (Sonicator Model 750 series autotune watts ref 75043; pulsed 0.1 s on, 0.1 s off). 1.25 ml of resulting suspension was diluted in 3.75 ml foetal calf serum (FCS, =2 mg/ml) and mixed for 1.5–2 h using a magnetic bar. Average hydrodynamic size, size distribution and zeta potential of particles in suspension were determined by Photon Correlation Spectroscopy (PCS) using a Zetasizer (Nano-3000 HS, Malvern Instruments Ltd., Malvern, UK). One ml of the intermediate preparation was diluted in 9 ml of culture medium RPMI 1640 containing 10% FCS and mixed for 1.5–2 h using a magnetic bar. In addition to PCS measurements, the stability of suspensions was explored by turbidimetry using a Turbiscan (Formulaction).