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Inflammatory MAPK and NF-κB signaling pathways differentiated hepatitis potential of two agglomerated titanium dioxide particles

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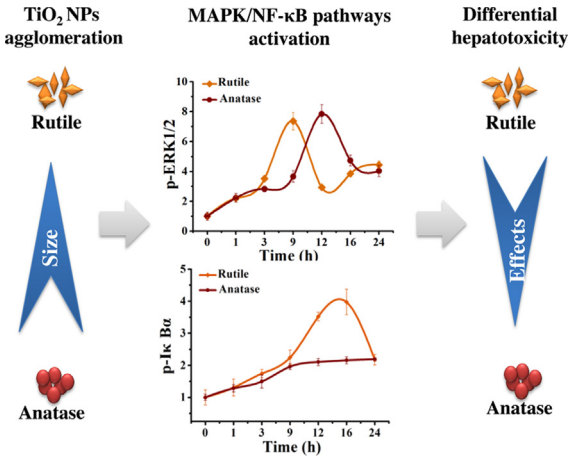
HIGHLIGHTS

- MAPK and NF-κB pathways early differentiated hepatitis potential of TiO_2NPs.
- Signal dysregulation by TiO_2NPs depended on agglomeration shape and size.
- Cell elasticity changes positively correlated with MAPK/NF-κB activation

GRAPHICAL ABSTRACT



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ABSTRACT

TiO_2 nanoparticles (TiO_2NPs) consumption and deposit in liver have possible implications for hepatitis risks. Specific signal dysregulation at early inflammatory responses needs to be characterized in TiO_2NP-induced hepatopathy. MAPK and NF-κB signaling pathways are known to participate in inflammation and respond sensitively to chemical agents, making them preferable biomarkers for hepatitis. In the present study, dynamic activation of MAPK and NF-κB pathways were explored by immunoblotting and NF-κB luciferase reporter assay depending on characterization of TiO_2NP agglomeration in human HepG2 cells. Inflammatory and cytotoxic potential of TiO_2NPs were determined by qRT-PCR and WST-1 assay. AFM and TEM analyses uncovered ultrastructure damages underlying hepatotoxicity of TiO_2NPs. Rod-like rutile agglomerated smaller and induced more pronounced cytotoxicity and immunogenicity than anatase. Correspondingly, though both rutile and anatase significantly activated p38, ERK1/2 and NF-κB pathways, rutile accelerated the maximum phosphorylation of ERK1/2

Abbreviations: TiO_2NPs, titanium dioxide nanoparticles; NF-κB, nuclear factor kappa enhancer binding protein; IκBα, inhibitory protein of NF-κB; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated protein kinase; p38, p38-MAPK; JNK, c-Jun N-terminal kinase; TNFα, tumor necrosis factor α; IL-8, interleukin-8; DLS, dynamic light scattering; qRT-PCR, quantitative reverse transcription polymerase chain reaction; AFM, atomic force microscopy; TEM, transmission electron microscopy.

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and elevated the potency of IκBα phosphorylation to its bell curve shape in comparison with a lower and sigmoid type of IκBα phosphorylation for anatase. Furthermore, cell elasticity indicated by Young's modulus and adhesion force increased accompanied with mitochondria damage, contributing to signal dysregulation and hepatotoxicity. The results suggested that differential activation of MAPK and NF-κB pathways could be early predictors for distinct hepatitis risks of two agglomerated TiO_2NPs.

1. Introduction

The unique health risks of nanoscale materials impel the emerging nanotoxicological research, which necessitates quantitative, mechanistic, and pathway-based toxicity testing to predict the potential intrinsic toxicity from nano-bio interactions [1]. Titanium dioxide nanoparticles (TiO_2NPs) account for 70% pigments worldwide with annually 5000–64,000 metric tons consumed in food, personal care products, paints and adhesives [2]. People expose to TiO_2NPs through skin absorption, ingestion and inhalation at the consumer level of 25–75 μg nano-TiO_2/cm^2 skin, 0.2–2 mg TiO_2/kg body weight per day and 0.061–0.15 μg TiO_2NPs/cm^2 per day, respectively [3–5]. Toxicokinetic results indicated that 94% of the administered TiO_2NPs accumulated in liver making it a major target of toxicity. The elimination of TiO_2NPs in liver was quite slow, which could take up to 90 days post-exposure leading to TiO_2NPs accumulation even at low exposure levels [6,7]. TiO_2NP-induced liver damages have been indicated by changes of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase levels, hydropic degeneration around the central vein and the spotty necrosis of hepatocytes in mouse model [8]. Liver pathogenesis in TiO_2NP-exposed mice could be driven by immune responses, such as decreasing T and B lymphocytes, altering expression of tumor necrosis factor α (TNFα), interleukin-6 (IL-6), and interleukin-1β (IL-1β) [9,10]. Although there is strong evidence on the effect of TiO_2NPs on hepatitis, at present little is understood on the early signaling events of TiO_2NP-related liver injury.

Inflammatory responses are regulated by multiple upstream signaling pathways, predominantly nuclear factor kappa enhancer binding protein (NF-κB) and the mitogen-activated protein kinase (MAPK) signaling pathways, including extracellular signal-regulated protein kinase (ERK), p38-MAPK (p38), and c-Jun N-terminal kinase (JNK) [11]. Analysis of signal dysregulation provides the early surveillance and pharmacological targets to achieve risk prediction and therapeutic specificity in different biological and clinical situations [12]. We previously confirmed that MAPK and NF-κB signaling pathways were involved in immunomodulatory role of microcystin-LR during hepatocytes toxicosis [13]. In nanotoxicity, MAPK and NF-κB signaling pathways regulated CeO_2NP-induced cytotoxicity and AgNP-induced hormesis, and inhibition of MAPK and NF-κB pathways could rescue the pro-inflammatory responses induced by CuONPs [14–16], making MAPK and NF-κB activation a good first step for flagging chemicals that interfere with inflammation state. The ability of TiO_2NPs to interact with MAPK and NF-κB pathways has been implicated in human neutrophils and vascular endothelial cells, which initiated neutrophils activation and expression of vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 (MCP-1) [17,18]. However, the specific activation strength and duration of MAPK and NF-κB pathways responding to TiO_2NPs in hepatocytes remain to be elucidated, which would help understand causal link between early signals and the differential hepatitis risk.

The stimulation of nanoparticles at the particle-membrane interface could propagate to intracellular signal due to membrane or cytoskeleton reorganization, which has caused a surge of interest in the evaluation of nanotoxicity [19]. Particle endocytosis induced cell motility and cell shape adaptations are crucial during inflammation, and inflammatory cells exhibit altered F-actin expression [20]. However, no direct evidence suggests the impact of TiO_2NPs on biomechanical properties of hepatocytes, or the possible involvement of cytoskeleton deformation in inflammatory signaling.

The present study characterized the distinct activation profiles of inflammatory MAPK and NF-κB signaling pathways in response to two crystals of TiO_2NPs, rutile and anatase, in the human hepatoma cell line HepG2, and elucidated how they were engaged by nanomaterials depending on TiO_2NP agglomeration size and shape. Further, inflammatory potential, cytotoxicity and cellular ultrastructure damages were examined to help unravel the predictive molecular events underlying the differential hepatitis risks of TiO_2NPs.

2. Materials and methods

2.1. Reagents

Primary antibodies used in this study were anti-IκBα (inhibitory protein of NF-κB, prepared in the laboratory according to standard methods) [21]; anti-phospho-IκBα (Ser32) (Cell Signaling Technology, Shanghai, China); anti-ERK1/2, phospho-ERK1/2 (Thr202/Thr204) (Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-p38, phospho-p38 (Thr180/Tyr182), JNK, phospho-JNK (Thr183/Tyr185) (Epitomics, Burlingame, CA, USA); and anti-α-tubulin (HuaAn Biotechnology, Hangzhou, China). Anti-rabbit and anti-mouse secondary antibodies were purchased from Promega (Shanghai, China). The pGL2-based NF-κB luciferase reporter plasmid and *renilla* pRL-TK plasmid were obtained from Promega Corporation (Madison, Wisconsin). *Firefly* and *renilla* luciferase substrates were purchased from Sangon Biotech (Shanghai, China) and Promega Corporation, respectively.

2.2. Cell culture

Human hepatocellular carcinoma HepG2 cell line, which displays the morphology and biochemical activities of healthy hepatocytes [22], was obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China) and grown at 37 °C in a humidified atmosphere containing 5% CO_2. Medium used for HepG2 culture was Dulbecco's Modified Eagle Medium (DMEM; Gibco, Shanghai, China) supplemented with 10% fetal bovine serum (FBS) (v/v) (Gibco, Shanghai, China), and penicillin-streptomycin (20 U/ml and 20 μg/ml, respectively) (Invitrogen, Shanghai, China). Cell number was counted by a hemocytometer.

2.3. Nanoparticle characterization

TiO_2NPs, anatase and rutile, were obtaind from Hongsheng Material Technology (Zhejiang, China), and their specifications were provided by the manufacturer. TiO_2NP dispersions were prepared according to the procedure by Taurozzi et al. [23]. Briefly,