Andreea R Lupu1, 2*, Lidia Cremer2 and Traian Popescu3

1Immunobiology Laboratory, "Victor Babes" National Institute of Pathology, Bucharest, Romania
2Immunomodulation Group, "Cantacuzino" National Institute for Research/Development in Microbiology and Immunology, Bucharest, Romania
3LASDAM Laboratory, National Institute of Materials Physics, Bucharest, Romania

*These authors contributed equally to this work

**Corresponding author: Andreea-Roxana Lupu, Immunobiology Laboratory, "Victor Babes" National Institute of Pathology, Bucharest, Romania; Tel: +40213192732; E-mail: andreea.lupu@vb.ro

www.peertechz.com
ISSN: 2455-3492
Keywords: TiO2 nanoparticles; Hepatocytes; Toxicity; Solvents; Paints

Research Article

Combined In vitro Effects of TiO2 Nanoparticles and Dimethyl Sulfoxide (DMSO) on HepG2 Hepatocytes

Abstract

Introduction: Professional workers that manufacture or use titanium dioxide (TiO2)-based paints are exposed to potentially toxic TiO2 nanomaterials as well as to different paint solvents such as dimethyl sulfoxide (DMSO). In this context, we evaluate the combined cytotoxic effects of TiO2 nanoparticles and DMSO on HepG2 human hepatocytes.

Methods: Three types of TiO2 nanoparticles were used: commercial Degussa P25 and two samples synthesized by a hydrothermal procedure – undoped and Fe3+-doped TiO2. The effects of TiO2 nanoparticles on HepG2 cells exposed to DMSO before, after or together with the TiO2 treatment were investigated by viability and intracellular reactive oxygen species (ROS) determinations, performed using the MTT and DCFH-DA/2’-7’-dichlorofluorescein-diacetate methods respectively.

Results: Results indicated that DMSO made HepG2 cells more susceptible to toxic effects induced by nanosized TiO2. In the absence of DMSO, none of the tested nanoparticles exhibited significant cytotoxic effects. Viability increases were detected after 48 hours of treatment and attributed to possible redox-sensitive proliferation mechanism triggered by the low and moderate amounts of produced ROS. The combined action of TiO2 and DMSO led to a general viability decrease tendency. Significant effects (viability reductions and ROS generation) were observed in the case of cells first treated with Degussa P25 TiO2 and afterwards exposed to DMSO. The hydrothermal materials exhibited reduced in vitro reactivity on HepG2 hepatocytes.

Conclusion: The study reveals the enhancement of nanosized TiO2 toxicity induced by DMSO exposure, its findings having potential to help in the evaluation of professional health risks associated to the combined action of TiO2 nanomaterials and paint solvents.

Introduction

Recent studies regarding the widespread use of paints that contain titanium dioxide (TiO2, titania) nanoparticles for bacterial decontamination and self-cleaning purposes revealed significant advantages of these new technologies, but also potential health risks induced by the release of nanosized TiO2 from painted surfaces [1-4]. If the amount of released TiO2 was shown to be relatively small under the studied conditions [5], significant professional health risks may be associated to workers that manufacture or use TiO2-based paints. These workers are exposed to potentially toxic TiO2 nanomaterials as well as different solvents used during the processing or application of such paints. Among the used solvents, dimethyl sulfoxide (DMSO) was considered to be more convenient due to its relatively reduced toxicity [6,7]. However, DMSO is also known to easily penetrate human skin and, in some cases, to serve as carrier agent, promoting the percutaneous absorption of other compounds (including drugs and toxins) [8,9]. Under these circumstances, DMSO may facilitate the penetration of skin by TiO2 nanoparticles.

To evaluate the implications and hazards involved by the potential exposure to both, TiO2 nanoparticles and DMSO (or other paint solvent), it is essential to possess detailed knowledge regarding their combined effects on vital organs, such as brain, liver, heart, kidney, lung or spleen. Among these, the liver represents one of the most important organs involved in the processing of exogenous compounds (including nanomaterials) and detoxification, a significant amount of published studies concerning the hepatotoxic effects of solvents (including DMSO in several cases) [10-13].

The present study gives an insight into the combined in vitro effects of TiO2 nanoparticles and DMSO (seen as a generic paint solvent).

Citation: Lupu AR, Cremer L, Popescu T (2015) Combined In vitro Effects of TiO2 Nanoparticles and Dimethyl Sulfoxide (DMSO) on HepG2 Hepatocytes. Int J Nanomater Nanotechnol Nanomed 1(1): 002-010. DOI: 10.17352/2455-3492.000002
The experiments have been performed on HepG2 cells (human hepatocarcinoma cells- ATCC HB-8065®), a well characterized cell line, widely used in cytotoxicity studies due to its convenient specific characteristics, such as:

- biosynthetic capabilities similar to those of normal hepatocytes
- retention of cell surface receptors – response capacity similar to normal cells [14,15].

The studied effects concern the cytotoxicity and intracellular reactive oxygen species (ROS) production induced in HepG2 cells under different treatment schemes with nano-TiO2 and DMSO.

We have used three types of TiO2 nanoparticles: the commercial Degussa P25 TiO2 (P25) and other two samples synthesized under hydrothermal conditions in our laboratory – undoped (HT) and Fe3+-doped (FeHT) anatase TiO2. Degussa P25 TiO2 was often tested (and utilized as reference material) in studies concerning intracellular ROS generation [16] and toxicity induced by titania nanomaterials [17-19]. The hydrothermal TiO2 samples have similar structural characteristics (crystal structures, shapes, sizes and specific surface areas [20] – the relevance of these factors being frequently considered in TiO2 nanotoxicology studies [21,22]), but different band gap energies (relative to each other) and colloidal behaviors (compared to Degussa P25) [20].

The obtained results are analyzed with respect to the structural and physicochemical properties of the tested nanomaterials [20], the characteristics of the used cells and the cell penetration and hydroxyl radical scavenger properties of DMSO [23].

Materials and Methods

Materials synthesis

The undoped and iron-doped TiO2 nanoparticles were prepared starting from TiCl4 (solution 15 % in HCl 10 %, from Merck) and FeCl3 (RITVERC 95.44 % 9Fe Isotopic Enrichment) [20].

To obtain the Fe3+ (1 at. %)-doped TiO2, the titanium and iron precursors were processed as follows: TiCl4 was oxidized (by air barbotage) to TiCl3, and FeCl3 was reacted to hydrochloric acid (4N) to form FeCl3. The resulted solutions were involved in a coprecipitation process, NH4OH being drop wise added to their mixture up to pH=8. The obtained precipitate was washed with deionised water, resuspended in double-distilled water and exposed to hydrothermal treatment in a 50 cm3 Teflon-lined autoclave at 200 °C for one hour. The undoped TiO2 was synthesized using the same procedure involving the titanium precursor only.

Material characterization

The structural, morphological, optical and physicochemical characteristics of the used materials have been studied by X-ray diffraction (XRD), transmission electron microscopy (TEM), Energy-dispersive X-ray spectroscopy (EDX), Mössbauer spectroscopy, Brunauer-Emmett-Teller (BET) nitrogen adsorption, UV-Vis reflectance spectroscopy, Dynamic Light Scattering (DLS) and Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) and described in a previous work [20].

Cellular and noncellular experiments regarding the in vitro effects of TiO2 nanoparticles on HepG2 hepatocytes pre-treated, co-treated or post-treated with DMSO

Cell viabilities and intracellular ROS productions in HepG2 hepatocyte cultures exposed to TiO2 nanoparticles and DMSO have been performed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay and the DCFH-DA (2',7’-dichlorofluorescein-diaceitate) test respectively.

The experiments were such designed to elucidate the following aspects:

- effects of the studied TiO2 nanoparticles on hepatocytes that were “already damaged” – the cells were first treated with DMSO and two hours later exposed to the action of nano-TiO2;
- viability and intracellular ROS production in case of HepG2 cells simultaneously treated with DMSO and nano-TiO2;
- response of hepatocytes to the action of DMSO, administered two hours after the cells were exposed to nano-TiO2;

1. The working protocol was established based on the following: LD50 for DMSO after 24 h of exposure – the DMSO dose capable of killing 50% of the cells after 24 h of exposure;
2. the effects of nano-TiO2 alone (without DMSO) on the studied cells;

Cell treatment

The HepG2 cells were seeded in 24-well culture plates at a density of 104 cells/cm2 in volumes of 1 ml of DMEM-F12 culture medium containing 10% FBS. After 24 h required for cell adherence and growth, the culture medium was discarded and replaced with fresh medium (1 ml/well) containing the necessary treatment agents (TiO2 (2.5, 7.5, 15, 25, 50, 75, 100 μg/ml), DMSO (LD50) or both) according to the stimulation scheme presented above. In case of pre-treatment or post-treatment with DMSO, either TiO2 or DMSO, were added two hours after the initial stimulation.

Preliminary experiments have established the LD50 for DMSO to be 5 μl DMSO/1 ml of culture medium.

Cell viability assay

After 24, 48 and 72 h of treatment, the culture medium was removed and MTT solution (1 mg/ml MTT in phosphate buffered saline (PBS)) was added to each well (300μl/well). The obtained samples were incubated for 2 hours, the MTT solution being afterwards discarded. To dissolve the produced formazan crystals, DMSO (Sigma-Aldrich) was added to each well (300μl/well). The optical density of the purple formazan solution was determined at 540 nm using a Thermo Multiskan EX spectrophotometer.

The obtained results were quantified with respect to control samples consisting of untreated cells (in case of experiments in which TiO2 alone was used) or cells treated with DMSO (in case of experiments involving both TiO2 and DMSO). Cell viability was expressed as percents versus control. Standard deviations were...
computed based on three technical replicates corresponding to each sample and three independent biological replicates of each experiment.

The results are represented as average values +/- standard deviations (error bars).

**Determination of intracellular ROS production**

To quantify the intracellular ROS production, the cells were incubated for 24 hours with the treating agents (TiO₂, 2.5, 25, 50, 100 μg/ml), DMSO (LD₅₀) or both. After incubation, the culture medium was removed and replaced with fresh medium containing DCFH-DA (0.2 μl of 2',7'-dichlorofluorescein-diacetate (DCFH-DA) stock solution (25 mg/ml DCFH-DA in TFS) in 1 ml of culture medium). The obtained samples were incubated again for 30 minutes. The DCFH-DA medium was afterwards removed; the cells were detached with trypsin (0.25% trypsin and 0.53 mM EDTA solution), suspended in PBS and centrifuged for 10 min at 1500 rpm and 4°C, the supernatant being discarded. The excess of fluorescein was removed by washing the cells twice in PBS and the cells were resuspended in 500 μl PBS. The homogenized suspensions were transferred to 96-well plates (100μl/well). A Fluoroskan FL (Thermo) equipment (excitation wavelength 485 nm/emission wavelength 530 nm) was used to perform the fluorimetric determinations.

The obtained results were quantified with respect to control samples (described above for cell viability experiments) and expressed as percents versus control. Standard deviations were computed based on three technical replicates corresponding to each sample and three independent biological replicates of each experiment.

The results are represented as average values +/- standard deviations (error bars).

**Data analysis and representation**

Data statistical analysis and representation were performed using the Sigma Plot-11 software package. Depending on data normality, either one-way ANOVA or one-way ANOVA on ranks tests were performed. The Student–Newman–Keuls (SNK) posthoc test was employed in order to complete the analysis. A value of p < 0.05 was considered significant. All samples statistically different from controls were marked on figures with a (*).

**Results**

**Materials characterization**

The detailed structural and physicochemical characterization of the three TiO₂ nanomaterials used in this study was published in a previous work [20]. Briefly, all three types of titania have similar shapes (no acicular shaped particles) and average particle sizes between 10-30 nm. The hydrothermal, HT and FeHT, samples have anatase structure and Degussa P25 TiO₂ is a mixture of anatase and rutile polymorphs with anatase/rutile weight ratio of 85:15(%). BET specific surface areas are 49 m²/g for Degussa P25, 130.62 m²/g for HT and 114.81 m²/g for FeHT. The band gap energies were approximately

![Figure 1: Cell viability (a-c) and intracellular ROS production (d) for HepG2 treated with P25, HT and FeHT; (*) - significant differences with respect to control (p < 0.05 - calculated based on three biological replicates).](image-url)
3 eV for Degussa P25 and HT and 2.848 eV for the iron doped, FeHT, sample. Degussa P25 has considerably higher colloidal stability in aqueous suspensions compared to HT and FeHT. The colloidal stabilization effect of proteins from culture medium was revealed.

**In vitro** effects of undoped and Fe$^{3+}$-doped TiO$_{2}$ nanoparticles on HepG2 hepatocytes

The results obtained in this study are displayed for each of the tested nanomaterials (P25, HT and FeHT) in Figures 1-4.

**Cell viability**

TiO$_{2}$ alone: Cell viabilities obtained in experiments involving TiO$_{2}$ alone (no DMSO) are represented in Figure 1a-1c. No significant viability variations were observed after 24h and 72h of treatment for none of the tested TiO$_{2}$ samples. At 48h after exposure, viability increases (up to 49%) were detected for all nanomaterials, being more pronounced in case of FeHT. The observed increases were proportional to the concentration of TiO$_{2}$ in the studied samples.

To better illustrate the specific features of each experimental case
Figure 3: Cell viability (a-c) and intracellular ROS production (d-f) for HepG2 cells treated with HT nanoparticles and DMSO; (*) - significant differences with respect to control ($p < 0.05$ - calculated based on three biological replicates). The (*) symbols placed above or below particular data points refer to all cases (treatment times) specified in the brackets.

Figure 4: Scatter plots showing the correlation between TiO$_2$ concentration and cell viability for HepG2 cells treated with HT nanoparticles and DMSO.

Lupu et al. (2015)

(pre-, co- or post-treatment with DMSO) and each type of tested TiO$_2$, the results are described and discussed comparatively below.

**TiO$_2$ and DMSO:** While for all the tested materials, the most prominent viability reductions were observed in the case of cells post-treated with DMSO (TiO$_2$-DMSO) (Figures 2a-4a), the highest cell killing effect was induced by Degussa P25 nanoparticles (Figure 2a). The hydrothermal materials (HT and FeHT) induced only weak or insignificant cytotoxic effects.

On the other hand, no significant cellular effects were detected in the case of cells simultaneously exposed to TiO$_2$ and DMSO (TiO$_2$+DMSO) (Figures 2b-4b).

The observed viability variations did not depend on the treatment time (24, 48, 72 hours), for none of the tested TiO$_2$ types.

**Intracellular ROS production**

**TiO$_2$ alone:** Regarding the intracellular ROS production,
significant increases (between 21-39%) were induced by the iron-doped sample (Figure 1d). The commercial P25 TiO₂ induced a significant increase only at its maximum concentration (100 µg/ml). The observed increases were concentration-dependent. No variation was detected in case of HT sample (Figure 1d).

**TiO₂ and DMSO:** Small or moderate increases in the intracellular ROS levels of the treated cells were observed in all experimental cases (Figures 2(d-f)-4(d-f)), being more pronounced in the case of DMSO post-treatment (TiO₂-DMSO) (Figures 2d-4d). Although the highest ROS production was determined for Degussa P25 TiO₂, corresponding to the TiO₂-DMSO case, no clear distinction can generally be made between the pro-oxidative effects of commercial and hydrothermal samples.

The determined ROS production increases were, in most cases, proportional to the concentration of TiO₂.

**Discussion**

To ensure the validity of the obtained results, one should
consider the possible interferences that may occur between the studied nanomaterials and the biochemical methods used through the performed study. Being a known photocatalyst [24-29], TiO$_2$ may interfere with MTT and induce experimental artifacts, as described by Lupu and Popescu [30]. This hypothesis was tested in noncellular experiments for each of the studied TiO$_2$ samples. Only one experimental case (Degussa P25, no DMSO) revealed weak TiO$_2$ (P25)-MITT interferences (data not shown). This effect was considered in data analysis in a manner similar to that described by Lupu and Popescu [30].

The cell viability results obtained in experiments involving TiO$_2$ alone revealed the lack of toxicity of the three tested nanomaterials on HepG2 cells, under the experimental conditions of the present study. The proliferation effects observed after 48 hours of TiO$_2$ treatment suggest the possible involvement of redox-sensitive cell proliferation mechanisms [31-37], triggered by the low and moderate levels of intracellular ROS production induced by the action of nano-TiO$_2$. One such ROS species is H$_2$O$_2$, which is known to either promote cell proliferation or induce cell cycle arrest, as a function of its concentration in the studied system [35,38-45]. In this context, it is important to note that H$_2$O$_2$ is among the characteristic oxygen species detected by the DCFH-DA method [46-48], its formation and proliferative effects being thus likely to occur in our study. Moreover, the DCFH-DA test was performed after 24h of TiO$_2$ treatment, the detected ROS generation being a plausible cause for the cell proliferation effect observed after 48h of treatment. After longer times however, cell viability is reduced by the oxidative environment. The onset of ROS effects (either cell proliferation or toxic effects due to oxidative stress) requires more or less time to occur, depending on the type and amount of generated ROS. Under the experimental conditions of our study, the effects of ROS produced during the first 24h of TiO$_2$ treatment and later became significant after longer times, being determined after 48h and 72h respectively. The increased amounts of ROS generated at later times induced the viability decrease observed after 72h of treatment.

Although the mechanisms by which TiO$_2$ nanoparticles induce intracellular ROS formation are not well understood, in the case of HepG2 cells, ROS overproduction may be favored by their tumor nature [49,50] and/or their role in detoxification [51,52].

Regarding the combined effects of TiO$_2$ and DMSO on HepG2 cells, the results presented above (Figures 2-4) are displayed with respect to control samples containing DMSO (LD50). Thus, the observed variations in viability or intracellular ROS production describe only the effects of TiO$_2$ nanoparticles on cells exposed to LD50 of DMSO. To understand how DMSO exposure influences the action of TiO$_2$ on the studied cells, these results should be analyzed in comparison to those obtained on cells that were not exposed to DMSO (Figure 1). The comparison indicates that DMSO exposure makes HepG2 cells more susceptible to toxic effects induced by nanosized TiO$_2$.

In this view, cells exposed to DMSO show no proliferation effects induced by the tested TiO$_2$. Although a general tendency towards viability reductions can be observed, only Degussa P25 produces consistent (for all treatment times), concentration-dependent toxic effects, mainly visible when cells are first exposed to TiO$_2$ and afterwards treated with DMSO (the TiO$_2$-DMSO case (Figure 2a)). Not only the highest viability reduction but also the highest intracellular ROS production was associated to the TiO$_2$-DMSO experiment, this finding suggesting that the observed effects were dictated by the early action of TiO$_2$ and associated to oxidative stress.

A possible mechanism to account for the enhanced toxicity of TiO$_2$ in the case of DMSO treated cells involves autophagy, which may be induced by both TiO$_2$ [53,54] and DMSO [55]. TiO$_2$ was also reported to induce lysosome membrane permeabilization, which represents a well known cell death mechanism (including lysosomal-iron mediated oxidative stress) [54].

The attenuated cellular effects observed either in the case of co-treatment (TiO$_2$+DMSO) or pre-treatment with DMSO (DSMO-TiO$_2$) may indicate a possible interaction between TiO$_2$ and DMSO, leading to the attenuation of the damaging effects of TiO$_2$ (especially of Degussa P25). This hypothesis is supported by two aspects: the known photocatalytic properties of TiO$_2$ and the known hydroxyl radical scavenger properties of DMSO [56-58]. In principle, by photocatalytic processes, TiO$_2$ nanoparticles may generate hydroxyl radicals on their surface and induce oxidation reactions. Also, in principle, DMSO may act as scavenger for these radicals and reduce their oxidative action. However, it is unfortunately not possible to accurately test, at least not in a straightforward manner, whether these processes can occur under relevant in vitro conditions.

All experiments performed in this study confirm the reduced toxicity of the hydrothermal TiO$_2$ samples (HT and FeHT) to HepG2 hepatocytes. Besides identifying nanomaterials with low toxicity, our study points towards the risks involved by the combined exposure to DMSO and nano-TiO$_2$. Results also reveal the importance of material properties (other than chemical composition) for their biological effects. Regarding the hydrothermal TiO$_2$ samples, although they exhibit larger surface specific area than Degussa P25, their in vitro reactivity appears to be reduced. This may be related to their different surface charge properties in culture media as well as ROS photogeneration capacities and hydrophilicity [20].

To clearly establish the relation between the in vitro reactivity of the tested TiO$_2$ nanomaterials and their complex physicochemical properties, further studies are required.

**Conclusion**

The described study gives an insight into the combined in vitro effects of TiO$_2$ nanoparticles and DMSO (seen as a generic paint solvent) on human hepatocytes. The observed effects were shown to depend on the properties of TiO$_2$ and the characteristics of the exposure. Results indicated that DMSO makes HepG2 cells more susceptible to toxic effects induced by nanosized TiO$_2$. These findings may help in the evaluation of professional health risks associated to workers that manufacture or use TiO$_2$-based paints.

**Acknowledgements**

Author Lupu A.-R. acknowledges support under PN-II-PT-PCCA-2013-4-1386 national grant. Author Popescu T. acknowledges support from Romanian National Authority for Scientific Research, under the Core project PN09-450103.

---

**Citation:** Lupu A.R, Cremer L, Popescu T (2015) Combined In vitro Effects of TiO2 Nanoparticles and Dimethyl Sulfoxide (DMSO) on HepG2 Hepatocytes. Int J Nanomater Nanotechnol Nanomed 1(1): 002-010. DOI: 10.17352/2455-3492.000002
References

1. Kaiser JP, Roesslein M, Diener L, Wick P (2013) Human Risk of Ingested Nanoparticles That Are Added as Multifunctional Agents to Paints: an In vitro study. PLoS ONE 8: e38215. doi:10.1371/journal.pone.0038215.

2. Occupational Exposure to Titanium Dioxide. Current Intelligence Bulletin 63. Department of Health and Human Services. Center for Disease Control and Prevention. National Institute for Occupational Safety and Health. 2011.

3. Koponen IK, Jensen KA, Schneider T (2009) Sanding dust from nanoparticle-containing paints: physical characterization. Journal of Physics. Conference Series 151: 012048.

4. Wang J, Fan Y (2014) Lung Injury Induced by TiO2 Nanoparticles Depends on Their Structural Features: Size, Shape, Crystal Phases and Surface Coating. Int J Mol Sci 15: 22258-22278.

5. Al-Kattan A, Wicher A, Vorbank R, Brunner S, Ulrich A, et al. (2013) Release of TiO2 from paints containing pigment-TiO2, organo-TiO2 by weathering. Environ Sci Process Impacts 15: 2186-93. doi:10.1039/c3em00331k.

6. Marti M, Molina L, Alemán C, Armelin E (2013) Novel Epoxy Coating Based on DMSO as a Green Solvent, Reducing Drastically the Volatile Organic Compound Content and Using Conducotory Polymers As A Nontoxic Anticorrosive Pigment. ACS Sustainable Chem Eng 1: 1609-1618.

7. Wu G (2010) Assay Development. Fundamentals and Practices. Chapter 13. High-Throughput Screening Assay Development. Wiley ISBN 978-0-470-19115-6, page 328.

8. Capriotti K, Capriotti JA (2012) Dimethyl Sulfoxide. History, Chemistry and Clinical Utility in Dermatology. J Clin Aesthet Dermatol 5: 24-26.

9. Botana LM (Ed) (2007) Phytotoxins: Chemistry and Biochemistry. Blackwell Publishing ISBN-13: 978-0-8138-2700-1, page 41.

10. Malagaquerna G, Catadella E, Giordano M, Nunnari G, Chisari G, et al. (2012) Toxic hepatitis in occupational exposure to solvents. World J Gastroenterol 18: 2756-2766.

11. Baratl Y, Charles AL, Wolff V, Tahar LB, Smiri L, et al. (2014) Age Modulates Fe3O4 Nanoparticles Liver Toxicity: Dose-Dependent Decrease in Mitochondrial Respiratory Chain Complexes Activities and Coupling in Middle-Aged As Compared to Young Rats. Bio-Med Research International 474081: 10.

12. Kishinsagar A, Vetal Y, Ashok P, Bhosle P, Ingawale D (2008) Drug Induced Hepatotoxicity: A Comprehensive Review. The Internet Journal of Pharmacology.

13. Moharramnadi S, Mehrparvar A, Labbafinejad Y, Attarchi MS (2010) The effect of exposure to a mixture of organic solvents on liver enzymes in an auto manufacturing plant. J Public Health DOI:10.1007/s10389-010-0340-z.

14. Pinti M, Towano L, Nasi M, Ferraresi R, Dobrucci J, et al. (2003) Hepatoma HepG2 cells as a model for in vitro studies in mitochondrial toxicity of antiviral drugs: which correlation with the patient? J Biol Regul Homeost Agents 17: 166-71.

15. Dehn P, White C, Conners D, Shipkey G, Cumbo T (2004) Characterization of the human hepatocellular carcinoma (HePG2) cell line as an in vitro model for cadmium toxicity studies. In Vitro Cellular & Developmental Biology – Animal 40: 172-182.

16. Long TC, Tajuba J, Sama P, Saleh N, Swartz C, et al. (2007) Nanosize titanium dioxide stimulates reactive oxygen species in brainmicroglia and damages neurons in vitro. Environ Health Perspect 115: 1631-1637.

17. Sayes CM, Wahi JP, Kuriyan J, Liu Y, West JL, et al. (2006) Correlating nanoscaleinteractions with toxicity: a cytotoxicity and inflammatoryresponse study with human dermal fibroblasts and human lung epithelial cells. Toxicol Sci 92: 174-185.

18. Han W, Wang YD, Zheng YF (2008) In vitro biocompatibility study of nano TiO2 materials. Adv Mater Res 47-50: 1438-1441.

19. Wadhwa S, Rea C, O’Hare P, Mathur A, Roy SS, et al. (2011) Comparative in vitro cytotoxicity study of carbon nanotubes and titania nanostructures on human lung epithelial cells. J Hazard Mater 191: 56-61.

20. Popescu T, Popescu T, Darmanescu L, Tarabasanu-Mihaila D, Teodorescu VS, et al. (2013) Effects of TiO2 nanoparticles on the NO2- levels in cell culture media analysed by Griescolitometry methods. J Nanopart Res 15: 1449-1450.

21. Jin C, Tang Y, Yang FG, Li XL, Xu S, et al. (2011) Cellular toxicity of TiO2 nanoparticles inanatas and rutile crystal phase. Bio Trace Elem Eum 141: 3-15.

22. Hsiao IL, Huang YJ (2011) Effects of various physicochemical characteristics on the toxicities of ZnO and TiO2 nanoparticles toward human lung epithelial cells. Sci Total Environ 409: 1219-1228.

23. Mokudai T, Nakamura K, Kanno T, Niwano Y (2012) Presence of Hydrogen Peroxide, a Source of Hydroxyl Radicals, in Acid Electrolyzed Water. PLoS ONE 7: e46392. doi:10.1371/journal.pone.0046392.

24. Linsebigler AL, Lu G, Yates JT Jr. (1995) Photocatalysis on TiO2 surfaces: principles, mechanisms, and selected results. Chemical Reviews 95: 735-758.

25. Carp O, Huisman CL, Reiter A (2004) Photoinduced reactivity of titanium dioxide. Progress in Solid State Chemistry 32: 33-177.

26. Chen X, Mao SS (2007) Titanium dioxide nanomaterials: synthesis, properties, modifications, and applications. Chemical Reviews 107: 2891-2959.

27. Fujishima A, Rao TN, Tryk DA (2000) Titanium dioxide photocatalysis. Journal of Photochemistry and Photobiology C: Photochemistry Reviews 1: 1-21.

28. Hashimoto K, Irie H, Fujishima A (2005) TiO2 photocatalysis: a historical overview and future prospects. Japanese Journal of Applied Physics 44: 8269-8285.

29. Kumar SG, Devi LG (2011) Review on modified TiO2 photocatalysis under UV/visible light: selected results and related mechanisms on interfacial chargecarrier transfer dynamics. Journal of Physical Chemistry A 115: 13211-13241.

30. Lupu AR, Popescu T (2013) The noncellular reduction of MTT tetrazolium salt by TiO2 nanoparticles and its implications for cytotoxicity assay. Toxicology In Vitro 27: 1445-1450.

31. Alforda AA, Sallam RM (2012) Reactive oxygen species in health and disease. J Biomedicine Biotechnol 2012: 936486.

32. Greene EL, Velarde V, Jaffa AA (2000) Role of reactive oxygen species intradkyrin-induced mitogen-activated protein kinase and c-fos induction in vascular cells. Hypertension 35: 942-947.

33. Ruiz-Gines JA, Lopez-Onigl S, Gonzalez-Rubio M, Gonzalez-Santiago L, Rodriguez-Puyol M, et al. (2000) Reactive oxygen speciesinduce proliferation of bovine aortic endothelial cells. J Cardiovasco Pharmacol 35: 109-113.

34. Sainz RM, Lombro F, Mayo JC (2012) Radical decisions in cancer: redox control of cell growth and death. Cancers 4: 442-474.

35. Sarsour EH, Kumar MG, Chaudhuri L, Kalen AL, Goswami PC (2009) Redoxcontrol of the cell cycle in health and disease. Antioxid Redox Signal 11: 2985-3011.

36. Sauer H, Wartenberg M, Hescheler J (2001) Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cell Physiol Biochem 11: 173-186.

37. Trachootham D, Lu W, Ogawara MA, Rivera-Del Valle N, Huang P (2008) Redox regulation of cell survival. Antioxid Redox Signal 10: 1343-1374.

38. Barnoun K, Dubuisslon ML, Child ES, Fernandes de Mattos S, Glassford J, et al. (2002) H2O2 induces a transient multiphase cell cycle arrest in mouse fibroblasts through modulating cyclin D andp21Cip1 expression. J Biol Chem 277: 13761-13770.
39. Burdon RH (1995) Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radic Biol Med 18: 775-794.

40. Burdon RH, Gill V (1993) Cellulary generated active oxygen species and HeLa cell proliferation. Free Radic Commun Res 19: 203-213.

41. Duque I, Rodriguez-Puyol M, Ruiz P, Gonzalez-Rubio M, Diez-Marques ML, et al. (1993) Calcium channel blockers inhibit hydrogen peroxide-induced proliferation of cultured rat mesangial cells. J Pharmacol Exp Ther 267: 612-616.

42. Herbert JM, Bono F, Savi P (1996) The mitogenic effect of H2O2 for vascular smooth muscle cells is mediated by an increase of the afffinity of basic fibroblast growth factor for its receptor. FEBS Lett 395: 43-47.

43. Li M, Zhao L, Liu J, Liu AL, Zeng WS, et al. (2009) Hydrogen peroxide induces G2 cell cycle arrest and inhibits cell proliferation in osteoblasts. Anat Rec 292: 1107-1113.

44. Los M, Droge W, Stricker K, Baeruefe PA, Schulze-Osthoff K (1995) Hydrogenperoxide as a potent activator of T lymphocyte functions. Eur J Immunol 25: 159-165.

45. Timblin CR, Janssen YW, Mossman BT (1995) Transcriptional activation of the proto-oncogene c-jun by asbestos and H2O2 is directly related to increased proliferation and transformation of tracheal epithelial cells. Cancer Res 55: 2723-2726.

46. McNeil SE (Ed.) (2011) Characterization of Nanoparticles Intended for Drug Delivery. Series: Methods in Molecular Biology 699: 173-179.

47. Poncin S, Colin IM, Gerard AC (2009) Minimal oxidative load: a prerequisite for thyroid cell function. J Endocrinol 201: 161-167.

48. Sitharaman B (2011) Nanobiomaterials Handbook. CRC Press 30-38.

49. Afanas’ev I (2011) Reactive oxygen species signalling in cancer: comparison with aging. Aging Dis 2: 219-230.

50. Massaoka MH, Matsuo AL, Figueiredo CR, Farias CF, Girola N, et al. (2012) Jacaranone induces apoptosis in melanoma cells via ROS mediated down regulation of Akt and p38 MAPK activation and displays antitumor activity in vivo, PLoS One 7: e38698. doi: 10.1371/journal.pone.0038698.

51. Liska DJ (1998) The detoxification enzyme systems. Altem Med Rev 3: 187-98.

52. Xu C, Li C, Kong, AT (2005) Induction of Phase I, II and III drug metabolism / transport by xenobiotics. Arch Pharm Res 28: 249-268.

53. Kanazoui BH, Bernasconi CC, Guney-Ayya S, Juillerat-Jeanneret L (2012) Induction of oxidative stress, lysosome activation and autophagy by nanoparticles in human brain-derived endothelial cells. Biochem J 441: 813-821.

54. Stern ST, Adiseshaiah PP, Crist RM (2012) Autophagy and lysosomal dysfunction as emerging mechanisms of nanomaterial toxicity; Particle and Fibre Toxicology 9: 20.

55. Song YM, Song SO, Jung YK, Kang ES, Cha BS, et al. Dimethyl sulfoxide reduces hepatocellular lipid accumulation through autophagy induction. Autophagy 8: 1085-1097.

56. Panganamala RV, Sharma HM, Heikkila RE (1976) Role of hydroxyl radical scavengers, dimethyl sulfoxide, alcohols, and methional in the inhibition of prostaglandin synthesis. Prostaglandins 11: 599-607.

57. Del Maestro R, Thaw HH, Bjork J, Planker M, Arfors KE (1980) Free radicals as mediators of tissue injury. Acta Physiol Scand Suppl 492: 91-119.

58. Ashwood-Smith MJ (1967) Current concepts concerning radio protective and cryo protetive properties or dimethyl sulfoxide in cellular systems. Ann NY Acad Sci 141: 41-62.

Copyright: © 2015 Lupu AR, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.