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Research Paper

Determination of Genotoxic Effects in vitro of $ZnOTiO_2$ Nanoparticles on Human Peripheral Lymphocytes

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Abstract: The aim of this study was to examine the genotoxic effects of $ZnOTiO_2$ NPs using chromosome aberration (CA) and micronucleus (MN) tests in human peripheral lymphocytes. Cells were treated to $ZnOTiO_2$ NPs concentrations in the range of 12.5 to 125 µg/mL for 24 and 48 hours. In addition, it induced structural CA at all concentrations with $ZnOTiO_2$ NPs treatment. However, nanoparticles did not stimulate CA in a dose-dependent manner for 24 and 48 hours of treatment, and there were no statistically significant differences between both the administration time and the concentrations ($P > 0.05$). The formation of MN induced by $ZnOTiO_2$ NPs increased depending on concentration and dose. In particular, the highest 2 concentrations (100 and 125 µg/ml) were observed to be significantly increased during the 24 and 48-hour treatment period ($P > 0.05$). Again, there is no significant difference in the occurrence of MN depending on gender ($P > 0.05$).

Keywords: *Chromosome aberration, $ZnOTiO_2$ NPs, nanoparticles, nanotoxicology, micronucleus*

Introduction

Nanotechnology is a scientific discipline that discovers nanometer-sized objects and is accepted by the world of science as a science integrated with biology, chemistry, physics and various engineering programs (Lehn, 2002). For this reason, different disciplines define nanotechnology in different ways. It is commonly referred to as fabrication, manipulation, precision placement, measurement, or modeling of scaled materials of 100 nanometers (nm) or small (Meyer & Kuusi, 2000). Nanotechnology applications cause changes in the basic, physical and chemical properties of traditional materials. These changes result from the reduction of the dimensions of traditional materials to nanoscale so that new materials/materials with excellent electrical, optical and mechanical properties are created (McWilliams, 2006). Nanoparticles (NPs) have been used in many industrial applications due to their varied quantum behaviour and increased reactivity (Handy et al., 2008). Today, a wide variety of NPs are being produced and used. In fact, even a single NPs can be produced in many different sizes, even using different synthetic methods. Therefore, toxicological studies of all of the NPs produced in such different numbers and forms are required (Dağlıoğlu and Yılmaz Öztürk, 2016; Yılmaz Öztürk and Dağlıoğlu, 2018; Yılmaz Öztürk et al., 2018). Because up to now, it has been noted that the toxicity of NPs is altered by factors such as nanoparticle size, surface area, shape, crystallinity, synthesis type, solvent type used (Fabrega et al., 2012; Pal et al., 2007; Scown et al., 2010; Zhou et al., 2010; Minhas et al., 2018).

Metal and metal oxide NPs are the most commonly used types of nanoparticles. Metal and metal oxide NPs form a large part of the growing nanotechnology market and are made of many metals such as silver, gold, copper, nickel, cobalt, zinc and titanium (Griffitt et al., 2009). Metal oxide nanoparticles have recently been produced at an industrial level and are used in large areas such as water treatment, medicine, cosmetics and engineering (Pendashte, 2013). Titanium dioxide nanoparticles (TiO_2 NPs) and zinc oxide nanoparticles (ZnO NPs) have broad UV spectral attenuation characteristics (Yılmaz-Ozturk and Daglıoglu, 2018). For this reason, it is used in sunscreen applications to protect human skin against UV radiation (Maier and Korting, 2005). ZnO NPs are the starting material for electronic applications, as transparent UV protective films and chemical sensors (Meulenkamp, 1998). It is also used as UV filter in sun protectors (Serpone et al., 2007). TiO_2 NPs are the most used group of nanomaterials known as catalysts and adsorbents. (Fujishima *et al*., 2000). In addition, as a catalyst in sterilization and as a chemical engineering water-cleaning adsorbent (Meng et al., 205; Pena et al. 2005; Strigul *et al*. 2009), self-cleaning surfaces (Fujishima et al., .2000) and the development of solar cells (Shipway et al.2000)

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is used in the production of cosmetics, paint, food additive and construction materials, as well as a very wide range of products consisting of air, soil and water environmental decontamination (Aitken et al., 2006; Choi et al., 2006; Esterkin et al., 2005). These areas of use can cause unexpected effects on the living organism and environment (Handy et al., 2008; Dağlıoğlu and Yılmaz Öztürk, 2018).

In this study, the $ZnOTiO_2$ NPs composing the most commonly used metal oxide NPs (ZnO and TiO_2) in nanotechnology were evaluated for their genotoxic and cytotoxic potential by *in vitro* micronucleus test and chromosome abnormalities in human lymphocyte culture.

Materials and Methods

In this study, zinc oxide titanium dioxide nanoparticle ($ZnOTiO_2$ NPs) was used as a test substance. Peripheral blood from two men and two females under the age of 30 who did not have any chronic non-smoking disease and did not use drugs were used as the material.

Test materials and preparation of $ZnOTiO_2$ NPs

ZnOTiO2 nanoparticles were used in the experiments. TiO_2 (Titanium dioxide) (99.0% pure) nanoparticle in pure anatase form was obtained from Sigma Aldrich, Germany. $ZnOTiO_2$ (zinc oxide titanium dioxide) nanoparticle in the chemistry laboratory of the Faculty of Science of the was synthesized by Professor. Dr. Münevver SÖKMEN. ZnO metal oxide was added to the TiO_2 photocatalyst by 1%. 10 g of TiO_2 catalyst and 10 ml of water were mixed and slurried. 0.3355 g of ZnO was added to the mixture and calcined at 400 ° C for 6 h. It was then cooled in a desiccator and stored in a dark coloured bottle.

Preparation of aqueous suspensions of $ZnOTiO_2$ NPs

At the desired concentrations, stock solutions were prepared by dissolving the $ZnOTiO_2$ NPs powders, which are the test substances to prepare, in deionized water. This solution was then vortexed for 20 seconds. While preparing stock solutions of $ZnOTiO_2$ NPs, an ultra-sonic water bath (Bandelin, SonoRex) was used to increase water distribution and ensure maximum distribution of $ZnOTiO_2$ NPs in water. The test concentrations determined after all these steps were prepared by stock solution dilution.

Size Distribution and Characterization of $ZnOTiO_2$ NPs

The size distribution and morphology of $ZnOTiO_2$ NPs were characterized by scanning electron microscope (SEM). The dry (powder) dimension measurement of the nanoparticles was determined by XRD. SEM images were also taken to determine the morphology of the nanoparticles (aggregation / aggregation; Figure 1).

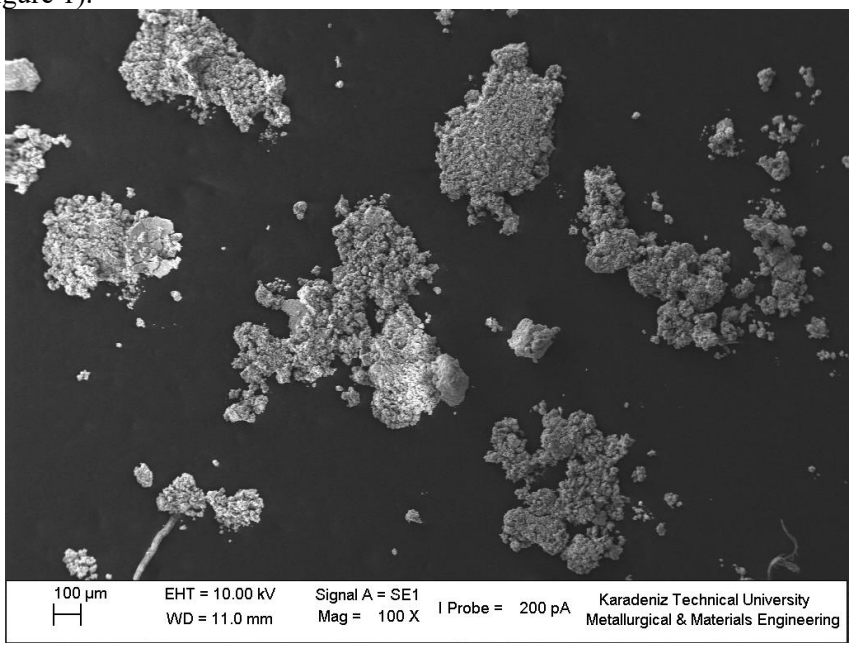

Figure 1. SEM image of a powdered $ZnOTiO_2$ NPs

Micronucleus (MN) Test

In the in vitro micronucleus test, Rothfuss et al. (2000)'s method has been modified and used. Blood samples were collected from two healthy men and two women (< 30). Blood was taken into the syringe containing heparin (0.5 ml). The syringe shaked to allow heparin and blood to mix. Blood samples (300 sterill) were plated in sterile tubes with 2.5 ml chromosome medium. The cell culture was placed in the incubator for incubation at 37 ± 1 °C for 72 hours.

Chromosome aberrations (CA) test

In this study, cell culture was performed to determine CA and preparations were prepared. To determine the chromosome structure and number abnormalities, a total of 100 cells (from a total of 400 cells from four individuals) with well-distributed chromosomes from each person prepared from each person were examined to determine CA.

Results and Discussion

In order to determine the number of micronuclei, a total of 1000 cells with two nuclei were examined in each preparation and the number of micronucleus bearing from these binucleated cells was determined. In addition, the total number of micronuclei and the total number of micronuclei were divided by the number of two nucleated cells in the studied binucleate cells and the number of micronuclei per cell (MN / Cell) and% MN were calculated.

Table 1. Effects of $ZnOTiO_2$ NPs applications on erythrocyte micronucleus frequency

Concentration (µg/ml)	Treatment duration (h)	MN number		Treatment duration (h)	MN number	
		Female	Male		Female	Male
0	24	2	3	48	4	4
12.5		3	4		5	4
25		7	8		7	9
50		18	16		21	17
100		23	24		20	25
125		27*	26*		26*	28*
Mitomycin C (MMC)		29	33		34	36

In this study, micronuclus (MN) s are the non-core structures of the cell. From this, the increase in the number of MNs was evaluated as an indicator of the numerical and structural chromosome irregularities and genomic instability that the cells exposed to $ZnOTiO_2$ NPs. MNs were counted in 1000 erythrocytes randomly counted in each preparation prepared as a male and female under the microscope. The presence and frequency of MN induced by $ZnOTiO_2$ NPs application in erythrocyte cells are given in Table 1. As can be seen from the results, 24 and 48 hours $ZnOTiO_2$ application in the negative control groups (0 µ/ml) in the formation of MN is very rare, while erythrocyte cells $ZnOTiO_2$ NPs depending on the concentration of MN formation was observed. The highest MN formation was observed in the positive control MMC. However, the determined MN numbers were not statistically significant ($P > 0.05$). In the erythrocyte cells, the highest MN formation was observed at a concentration of 125 µ/ml $ZnOTiO_2$ for 48 hours. In this group, a total of 54 MNs were counted, including 26 in female and 28 in male, in erythrocyte cells. It was determined that these increases in MN numbers were statistically significant compared to other concentration groups ($P < 0.05$).

Chromosomal abnormality (CA) test is often used as a biological indicator of chromosomal damage and genomic instability. (El-Zein et al., 2011). In this study, chromosomal abnormalities (CA) are changes in the normal chromosome structure (structural abnormality) or number (numerical abnormality) that occur as a result of exposure to $ZnOTiO_2$ NPs. In addition, the increased frequency of chromosomal abnormalities in lymphocytes is often considered an indicator of cancer risk for those exposed to DNA damaging agents (Hagmar et al., 1998; Yüzbaşıoğlu et al., 2014). A total of 100 cells (from a total of 400 cells from 4 individuals) with well-distributed chromosomes from the preparations prepared from each individual were examined for the purpose of CA detection. Percentage of structural and total chromosomal abnormalities were found in these 100 cells. In this study, abnormalities such as chromatid fracture and single arm union were evaluated as chromatid type abnormality. In addition,

abnormalities such as chromosome fracture, sister union, chromatid exchange, ring chromosome and dicentric chromosome formation were also evaluated as chromosome type abnormalities. Most chromatid type abnormalities, especially chromatid fracture, were observed in human peripheral lymphocytes treated with $ZnOTiO_2$ NPs. Chromosomal abnormalities, in a lesser extent, have been found, in particular, chromosome fractures, sister union chromosomal abnormalities such as sister union and chromatid exchange. Polyploidy was not observed in human peripheral lymphocytes treated with $ZnOTiO_2$ in this study. It was determined that $ZnOTiO_2$ did not increase the number of chromosome number abnormalities.

Table 2. CA number in human lymphocytes treated with $ZnOTiO_2$

			Structural CA		Numerical CA
	Treatment duration (h)	Concentration (µg/mL)	Chromatid	Chromosome	
Control (water)	24	0	-	-	-
$ZnO\text{-}TiO_2$	24	12.5	8	-	-
$ZnO\text{-}TiO_2$	24	25	8	-	-
$ZnO\text{-}TiO_2$	24	50	13	-	-
$ZnO\text{-}TiO_2$	24	100	14	1	1
$ZnO\text{-}TiO_2$	24	125	15		-
MMC	24		87	2	-
Control (water)	48	0	-	-	-
$ZnO\text{-}TiO_2$	48	12.5	8	-	-
$ZnO\text{-}TiO_2$	48	25	11	2	-
$ZnO\text{-}TiO_2$	48	50	13	-	-
$ZnO\text{-}TiO_2$	48	100	14	-	-
$ZnO\text{-}TiO_2$	48	125	16	5	3
MMC	48		45		

In conclusion, $ZnOTiO_2$ NPs administered to human peripheral lymphocytes for 24 and 48 h were found to lead to an increase in CA and MN formation. The increase in MN frequency was determined to be related to the increase in concentration, but there was no increase in the frequency of CA due to concentration increase.

Conclusion

With the rapid advancement of nanotechnology in recent years, various nanotechnology products that are increasing in number of in the our environment, come into our daily life. It is thought that products produced by nanotechnology may be genotoxic. Therefore, the evaluation of genotoxicity of nanoparticles with the potential to have these effects is particularly important. It is concluded that $ZnOTiO_2$ NPs can show genotoxic effects at determined concentrations and it can be dangerous.

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