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ORIGINAL RESEARCH

In vitro genotoxic effects of ZnO nanomaterials in human peripheral lymphocytes

Duygu Gümüş · Ahmet Ali Berber · Kezban Ada · Hüseyin Aksoy

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Abstract In this study, possible genotoxic effects of zinc oxide (ZnO) nanoparticles were investigated in cultured human peripheral lymphocytes by using chromosome aberrations and micronucleus assays (MN). For this purpose, the cells were treated with ZnO (1, 2, 5, 10, 15 and 20 μg/mL) for 24 and 48 h. In this research, four types of chromosome aberrations were observed as chromatid and chromosome breaks, fragment and dicentric chromosomes. ZnO induced significant increase of the ratio of chromosomal aberrations as well as percentage of abnormal cells at concentrations of 1, 5, 10 and 20 μg/mL in 24 h treatments. In 48 h treatments, while ZnO nanomaterials induced significant increase of the percentage of abnormal cells only at a concentration of 10 μg/mL, and of chromosome aberration per cell in comparison to the control at concentrations of 5 and 10 μg/mL. On the other hand, this material significantly increased the micronuclei frequency (MN) at concentrations of 10 and 15 μg/mL in comparison to the control. Cytokinesis-block proliferation index was not affected by ZnO treatments. It also decreased the mitotic index in all concentrations at 24 h but not at 48 h. The present results indicate that ZnO nanoparticles are clastogenic, mutagenic and cytotoxic to human lymphocytes in vitro at specific concentrations and time periods.

Keywords ZnO · Nanomaterial · Chromosome aberration · Micronuclei · Human peripheral lymphocytes

D. Gümüş · A. A. Berber · H. Aksoy (✉)
Department of Biology, Faculty of Arts and Sciences, Sakarya University, 54187 Sakarya, Turkey
e-mail: haksoy@sakarya.edu.tr

K. Ada
Department of Chemistry, Faculty of Arts and Sciences, Kırıkkale University, 71100 Kirikkale, Turkey

Introduction

Nanomaterials are increasingly used in many commercial products and industrial practices. They are also found in plastic wares, textiles, cosmetics, sunscreens, electrical appliances and even food products. Their applications also extend into the biomedical field and healthcare, particularly in medical imaging systems and diagnosis, pharmaceuticals, drug delivery and therapy (Nowack and Bucheli 2007; Ng et al. 2010; Maier and Korting 2005).

Research in the nanoparticle (NP) and nanotechnology field is growing at a breathtaking pace. The reason is simple: the unique properties of NP will allow the development of products with unprecedented characteristics and opportunities in every field of human activity, and with tremendous economic impacts (Ostiguy et al. 2008).

As of March 2011, the nanotechnology consumer products inventory contains 1,317 products or product

lines. The inventory has grown by nearly 521 % (from 212 to 1,317 products) since March 2006. The inventory now includes products from 30 different countries. The United States have most of the products, with a total of 587, followed by companies in Europe (367), East Asia (261), and elsewhere around the world (73). Two products have no country designation. The most common material mentioned in the product descriptions is now silver (313 products). Carbon, which includes fullerenes, is the second most referenced (91), followed by titanium (including titanium dioxide) (59), silica (43), zinc (including zinc oxide) (31), and gold (28) (NCPI 2012). Estimation of the worldwide investment in nanotechnology previews that $3 trillion will be attained in 2014 (Wardak et al. 2008).

In the US, sunscreen agents are issued by the Food and Drug Administration (FDA) as non-prescription products. The latest sunscreen monograph of the FDA includes 16 agents, fourteen of which are organic UV-absorbing filters. The only inorganic filters are TiO_2 and ZnO (Kullavanijaya and Lim 2005). The European directive has recently declared that among 26 sunscreens, TiO_2 is the only mineral filter. ZnO has also been approved in Europe since there are not any restrictions on the level of concentration. The majority of commercial products contain one of these filters (Couteau et al. 2008). The production rate of nano metal oxides for cosmetics is estimated to be 103 tonnes/year (Clausen et al. 2010). Worldwide production of nano zinc oxide is stated to be 528 tonnes/year (Zhang and Saebfar 2010). The zinc oxide industry is a fragmented industry with over 300 companies around the world producing in excess of 1.2 million tonnes of ZnO per year (IZA 2007).

Nanoparticles have higher chemical and physical activity, such as ion release, adsorption ability, and reactive oxygen species production, compared with fine particles. These properties of nanoparticles also induce biological influences including toxic activity.

Zinc oxide (ZnO), one of the most common metal oxides, has been traditionally used in paint formulation and ceramic manufacture. Recently, ZnO nanoparticles (ZnO-NPs) have been used in protective dental composites, and dermal ointments, fabrics and also UV absorbent (Sevinc and Hanley 2010; Cross et al. 2007; Moorer and Genet 1982; Matsunaga et al. 1985; Becheri et al. 2008; Clausen et al. 2010). One of the largest applications of insoluble NP (diameter typically 50–200 nm) is their use in sunscreens (Nohynek et al. 2008). Modern cosmetics often contain nano-sized components, such as nano-emulsions, nanocapsules, nanosomes, niosomes or liposomes, which are microscopic vesicles (range: 50–5,000 nm) consisting of traditional cosmetic materials. ZnO nanoparticles are also used in the form of particles at a size of 30–200 nm in sunscreen cosmetics since it captures the rays due to nano size of the particles. The surface of these particles is frequently treated with inert coating materials, such as aluminium oxide or silicon oils, in order to improve their dispersion in sunscreen formulations. On the other hand, microfine ZnO has become more popular because its protection including skin ageing, herpes as well as skin and lip cancers at a wide UVA range (320–400 nm) has been demonstrated. It is photostable and does not react with other organic sunscreens under irradiation (Sharma et al. 2009; Mitchnick et al. 1999; Gélis et al. 2003; WHO 1998).

Nanomaterials may be respirable in humans and have the potential, based upon their geometry, composition, size, and transport or durability in the body, to cause adverse effects on human health, especially if they are inhaled at high concentrations (Hillegass et al. 2010).

Besides the use of nano products in different fields, their potential adverse effects on human health or environment have not been revealed yet. Although widespread application of ZnO NPs and nanotechnological products are placed on the market, sufficient knowledge on the associated toxicological risks is still lacking (Meyer et al. 2011; Logotheidis 2006).

At nanosize range, the properties of materials may differ substantially from respective bulk materials. As by today there is increasing scientific evidence that these physical and chemical properties of manufactured NPs lead to an increase of bioavailability and toxicity (Nel et al. 2006). NPs can cross the strongest biological barriers such as the blood–brain barrier (Lockman et al. 2003).

Oberdörster et al. (2005) have outlined three key elements of nanoparticle toxicity screening strategies: physicochemical characterization, in vitro assays (cellular and sub-cellular) and in vivo studies. Because in vivo experiments are expensive, slow and ethically questionable there is a strong demand for low-cost high throughput in vitro assays without reducing the efficiency and reliability of the risk assessment (Luther et al. 2004). Indeed, SCENIHR (2007) has

also stated that the short-term in vitro testing of nanoparticles has the potential to play an important role in screening procedures and mechanistic studies on nanoparticle toxicology. Characterization of chemical and physical properties individual nanoparticles is also essential for the evaluation of their biological effect (Horie and Fujita 2011).

Fed and intraperitoneally injected ZnO-NPs (2.5 g/kg) were absorbed into circulation (within 30 min post-dosing), then biodistributed to the liver, spleen and kidney. In both groups, serum zinc levels peaked within 6 h but gradually declined to the baseline in the orally ingested group, whereas the serum zinc levels were sustained in equilibratory level over 72 h in the intraperitoneally injected group (Li et al. 2012). Accordingly, intraperitoneally injected ZnO-NPs could more effectively spread to the heart, lung and testes. However, the authors suggested that the divergence in clearance revealed that a large proportion of fed ZnO-NPs was eliminated directly through fast clearance by defecation.

The main mechanism of toxicity of NPs is thought to be via oxidative stress (OS) (Kohen and Nyska 2002; Fahmy and Cormier 2009). Many investigations about toxicology of nanoparticles have been reported. In vitro studies showed that the some nanoparticles induce apoptosis, production of cytokines, cell death and oxidative stress (Horie and Fujita 2011) that damages lipids, carbohydrates, proteins and DNA (Kelly et al. 1998). Zn ions released from NPs can convert cellular oxygen metabolic products such as H_2O_2 and superoxide anions into hydroxyl radicals, a primary DNA damaging species (Singh et al. 2009).

Lipid peroxidation is considered most dangerous as leading to alterations in cell membrane properties which in turn disrupt vital cellular functions (Rikans and Hornbrook 1997). The propensity of nanoscale substances to get adsorbed, penetrated, and internalised within biological systems may pose an extraordinary hazard to humans (Klaine et al. 2008; Stone and Donaldson 2006).

Lin et al. (2009) conclude that exposure of human cell lines to both sizes of ZnO particles leads to dose- and time-dependent cytotoxicity reflected in oxidative stress, lipid peroxidation, cell membrane damage, and oxidative DNA damage. ZnO particles exhibit a much steeper dose–response pattern unseen in other metal oxides. Neither free Zn^{2+} nor metal impurity in the ZnO particle samples is the cause of cytotoxicity.

Cytotoxic effects were observed in human T cells beginning only at 5 mM (over 400 μg/mL) (Reddy et al. 2007). In addition, cancer T cells were demonstrated to have around 30 times higher sensitivity than normal T cells to ZnO NP toxicity (Hanley et al. 2008).

Brunner et al. (2006) have reported on the cytotoxic effects of ZnO in various mammalian cell lines. After a 72 h exposure to 15 μg/mL of 19 nm particles, nearly complete cell death was observed in human mesothelioma and rodent fibroblasts. In neuroblastoma culture, exposure to 50 nm ZnO particles at 100 μg/mL resulted in nearly 50 % cell death (Jeng and Swanson 2006).

ZnO nanoparticles caused a marked increase in intracellular reactive oxygen species (ROS) level, reduction in glutathione (GSH) and superoxide dismutase (SOD) level, and increase in lipid peroxides in primary mouse embryo fibroblasts, resulting in cell death (Yang et al. 2009). Bergeron and Archambault (2005) reported the results of two studies in which it was shown that the TiO_2 and ZnO contained in sunscreens damaged DNA by free radical production in skin cells.

A slight inflammation of the stomach and the intestine was observed in mice exposed to zinc nanoparticles and microparticles in the study by Wang et al. (2005). No significant pathological change was observed in the other organs.

Oral administration of ZnO-NPs or ZnO-MPs (5 g/kg) in mice did not cause any obvious adverse effects in a 14-day acute toxicity study (Li et al. 2012).

Phytotoxicity of ZnO NPs to *Arabidopsis* (member of mustard plant) was stronger, than solutions containing same concentration of soluble zinc (Lee et al. 2010). Rye grass (*Lolium perenne*) roots showed morphological changes with high concentration of ZnO NPs, i.e. root tips shrank and root epidermal and cortical cells collapsed (Lin and Xing 2008). ZnO NPs repressed seed germination of rye grass and corn (Lin and Xing 2007). ZnO NPs inhibited root growth of radish and rape, when incubated in a suspension of ZnO NPs (Nair et al. 2010). Kumari et al. (2011) demonstrated that exposure of *Allium cepa* roots to ZnO NPs causes cytotoxicity and genotoxicity.

Kasemets et al. (2009) reported that nano ZnO as well as bulk ZnO both showed concentration dependent effects on yeast growth and about 0 % inhibition of the growth was observed at 250 mg ZnO/L level for both types of ZnO formulations. There was no

difference in toxicity due to particle size as the EC_{50} values of nano and bulk ZnO was not statistically different for exponentially growing cells at the 8th hour of growth (121 and 134 mg ZnO/L, respectively) as well as for stationary phase cells after 24 h of growth (131 and 158 mg ZnO/L, respectively). Thus, throughout the experiment, nano and bulk ZnO showed analogous toxicity profiles.

This study aims to research genotoxic effects of ZnO nanoparticles on human lymphocytes in vitro via chromosome abnormalities (CA) and micronucleus (MN) assay, and to shed light on other research on the issue.

Materials and methods

Chemicals

The test substance ZnO NPs was prepared and characterized by Ada et al. (2008) and was obtained from Kirikkale University (Kirikkale, Turkey), Department of Chemistry. The particle diameter of ZnO was 45 nm, and its fragment size was 450 nm. Chromosome Medium B (CAS no.: F 5023) was obtained from Biochrom (Berlin, Germany), Mitomycin-C (CAS no.: 50-07-7), Colchicine (CAS no.: 9754), Cytocalsin B (CAS no: 14930-96-2) were obtained from Sigma (St. Louis, MO, USA).

Chromosomal aberrations, mitotic index and micronucleus analysis

Peripheral venous blood was obtained from two healthy donors (nonsmokers, aged 20–30 years) not exposed to any drug therapy or known mutagenic agent over the past 2 years, not exposed to ionizing radiation within the previous 6 months, and with no history of chromosome fragility or recent viral infection.

For CA assay, heparinized wholeblood sample (0.2 mL) was added to 2.5 mL Chromosome Medium B (with phytohemagglutinin L). Heparinized peripheral blood samples were incubated at 37 °C for 72 h and treated with concentrations of ZnO of 1, 2, 5, 10, 15, 20 μg/mL. A negative and a positive control (Mitomycin-C; MMC, 0.2 μg/mL) were also collected in every experiment. Colchicine (final concentration, 0.06 μg/mL) was added to each culture 2 h before harvesting. For micronuclei analysis, whole heparinized blood was added to 2.5 mL Chromosome Medium B. Blood was incubated at 37 °C for 72 h. MMC (0.20 μg/mL) was used as positive control. After 24 h, ZnO was added to the lymphocyte cultures at concentrations of 1, 2, 5, 10, 15, 20 μg/mL. To block cytokinesis, cytochalasin-B (5.2 μg/mL) was added 44 h after initiation of culture. Other procedures, slide evaluation and statistical analysis were carried out as described in Aksoy et al. (2006) for all cytogenetic tests.

CAs were scored with one hundred well-spread metaphases per donor (total, 200 metaphases per concentration). The Mitotic Index (MI) was also determined by scoring 3,000 cells from each donor (total, 6,000 cells per concentration). Micronuclei were scored from 1,000 binucleated cells (BN) per donor (total 2,000 binucleated cells per concentration). Cell proliferation was evaluated using the cytokinesis-block proliferation index (CBPI). 500 lymphocytes (total 1,000 lymphocytes) were scored to evaluate the percentage of cells with 1, 2, 3 and 4 nuclei. CBPI was calculated according to Surrales et al. (1995) as follows; $[1 \times N1] + [2 \times N2] + [3 \times [N3 + N4]]/N$ where N1–N4 represent the number of cells with 1–4 nuclei, respectively, and N is the total number of cells scored.

Results

Chromosomal aberrations and mitotic index

The results of the CA analysis and MI are shown in Table 1. Zinc oxide induced a significant increase in the frequency of abnormal cells in all concentrations (except 2 and 15 μg/mL) and in the CA/cell in all concentrations (except 2 and 15 μg/mL) for the treatment period of 24 h as compared to the negative control. For the 48 h treatment period, ZnO induced a non-significant increase in the frequency of abnormal cells except for the 10 μg/mL concentration and also induced an increase in the CA/cell (not significant except for the 5 and 10 μg/mL concentrations). While these increases in the frequency of abnormal cells were slightly dose-dependent, there was no dose dependence in the CA/cell for the 24 h treatment ($r = 0.43$ and $r = -0.08$, respectively). Also there were no dose dependent differences for the 48 h treatment ($r = 0.40$ and $r = 0.07$, respectively). ZnO

Table 1 The chromosomal aberrations and mitotic index in cultured human lymphocytes treated with ZnO Nanomaterials

Test substance	Treatment		Aberrations						Abnormal Cell ± SH (%)	CA/Cell ± SH	MI ± SH (%)
	Period (h)	Dose (μg/mL)	B′	B″	F	DC	SU	CE			
Control	24	0	2	2	2	–	–	–	2.50 ± 1.10	0.030 ± 0.012	5.83 ± 0.30
MMC	24	0.20	22	12	7	3	10	8	24.50 ± 1.69	0.310 ± 0.032	3.43 ± 0.35
ZnO	24	1	10	3	4	–	–	–	8.50 ± 1.97**	0.085 ± 0.020*	3.03 ± 0.22***
		2	6	2	5	–	–	–	6.00 ± 1.68	0.065 ± 0.017	3.75 ± 0.25***
		5	7	4	4	–	–	–	7.50 ± 1.86*	0.075 ± 0.019*	3.68 ± 0.24***
		10	11	5	1	–	–	–	7.50 ± 1.86*	0.085 ± 0.020*	3.11 ± 0.22***
		15	5	5	1	–	–	–	5.50 ± 1.61	0.055 ± 0.016	4.15 ± 0.26***
		20	13	2	3	–	–	–	9.00 ± 2.02**	0.090 ± 0.020*	2.76 ± 0.21***
Control	48	0	2	3	2	–	–	–	3.50 ± 1.30	0.035 ± 0.013	5.55 ± 0.30
MMC	48	0.20	30	14	10	5	13	10	32.50 ± 2.26	0.410 ± 0.034	4.15 ± 0.45
ZnO	48	1	5	5	4	–	–	–	7.00 ± 1.80	0.070 ± 0.018	6.15 ± 0.31
		2	6	4	3	1	–	–	6.50 ± 1.74	0.070 ± 0.018	5.92 ± 0.31
		5	9	4	3	1	–	–	8.00 ± 1.92	0.085 ± 0.020*	5.81 ± 0.30
		10	15	8	2	–	–	–	11.50 ± 2.26**	0.125 ± 0.023**	6.38 ± 0.32
		15	4	1	3	–	–	–	4.00 ± 1.39	0.040 ± 0.014	6.03 ± 0.31
		20	3	5	2	–	–	–	5.00 ± 1.54	0.050 ± 0.015	5.75 ± 0.30

Totally 200 cells were scored for each treatment in Abnormal Cell and CA/cell, and 3000 cells were scored for each treatment in MI

B′ chromatid break, *B″* chromosome break, *F* fragment, *DC* dicentric chromosome, *SU* sister union, *CE* chromatid exchange, *MI* mitotic index

* Significant from the control $P < 0.05$ (z test)

** Significant from the control $P < 0.01$ (z test)

*** Significant from the control $P < 0.001$ (z test)

caused four types of structural aberrations: chromatid and chromosome breaks, fragments and dicentric chromosomes. Chromatid breaks were the most common aberrations in ZnO treated cells, followed by the chromosome breaks. ZnO dose dependently ($r = -0.45$) and significantly decreased the MI at all concentrations in 24 h treatments. However, MI was not affected by the 48 h treatments ($r = 0.11$).

Micronucleus assay

ZnO increased the frequency of binucleate cells with micronucleus in all treatment groups as compared to the control. However, this increase was significant only for the 10 and 15 μg/mL concentrations. This increase was dose dependent ($r = 0.51$). Most of the cells observed had just one micronucleus, but five of them had two micronuclei and one of them had three micronuclei. Cytokinesis block proliferation index was not affected by the ZnO treatment (Table 2).

Discussion

Zinc oxide (ZnO) is being used worldwide in consumer products and industrial applications. As humans are being directly exposed to ZnO nanoparticles (NPs) through different routes, it is likely that the NPs would gain access to the liver (Sharma et al. 2011).

Cross et al. (2007) concluded that less than 0.03 % of the applied zinc oxide nanoparticle used in sunscreen formulations penetrated the human epidermis after 24 h of exposure. Sunscreens contain TiO_2 or ZnO nanoparticles (NP), which are efficient UV filters. A number of studies suggest that insoluble NP do not penetrate into or through human skin (Nohynek et al. 2008).

Table 2 Micronucleus frequency and cytokinesis-block proliferation index in human lymphocytes treated with ZnO nanomaterials

Test substance	Treatment		BN cells scored	Distribution of BN cells according to the no. of MN			MN ± SH (%)	CBPI ± SH
	Period (h)	Dose (μg/mL)		(1)	(2)	(3)		
Control	48	0	2,000	6	–	–	0.30 ± 0.12	2.02 ± 0.044
MMC	48	0.20	2,000	68	8	4	0.48 ± 0.01	1.10 ± 0.035
ZnO	48	1	2,000	9	1	–	0.55 ± 0.17	2.11 ± 0.048
		2	2,000	11	1	–	0.65 ± 0.18	1.63 ± 0.032
		5	2,000	9	2	–	0.65 ± 0.18	2.05 ± 0.046
		10	2,000	16	1	1	1.05 ± 0.23**	2.05 ± 0.046
		15	2,000	16	–	–	0.80 ± 0.20*	2.05 ± 0.046
		20	2,000	13	–	–	0.65 ± 0.18	2.08 ± 0.045

(1), (2), (3) BN cells with one, two, three MN, respectively

BN binucleate, *MN* micronucleus, *CBPI* cytokinesis-block proliferation index

* Significant from the control $P < 0.05$ (z test)

** Significant from the control $P < 0.001$ (z test)

The genotoxic activity of ZnO in vitro was investigated for cytogenetic endpoints including CA and MN. These endpoints were selected as they are frequently used and provide sensitive assays to measure mutagenicity, clastogenicity, and potential carcinogenicity of chemical exposures (Surrales et al. 1995; Yilmaz et al. 2008). These assays are the most frequently used and well-established cytogenetic markers for determination of the genotoxicity of compounds (Carrano and Natarajan 1988). Although we defined that ZnO NPs are used in many areas in our daily lives, studies with respect to the genotoxicity of ZnO NPs are restricted. Therefore, this study was performed to investigate the genotoxic potential of ZnO NPs in human peripheral lymphocytes.

In this study we observed that ZnO significantly increased the frequency of CAs at many concentrations and MN in a few treatment groups as compared to their controls. ZnO NP induced four types of structural aberrations in lymphocytes in vitro. These are chromatid and chromosome breaks, fragments and dicentric chromosomes. Chromatid breaks were the most common aberrations in ZnO NP treated cells, following chromosome breaks. Chromatid breaks resulting from DNA double-strand breaks (Bryant 1998) were the first common abnormality. Chromosome breaks resulting from similar mechanisms were the second common abnormality. The third common aberration were fragments. These aberrations usually produce micronuclei and then genetic material is lost. The forth common aberration were dicentric chromosomes which are well known to have serious biological consequence.

MN assay detects both clastogenicity (chromosome/chromatid breakage) and aneugenicity (chromosome lagging due to dysfunction of the mitotic apparatus). Ahmad and Yasmin (1992) reported that micronuclei may originate from lagging chromosomes and fragments occurred in the mitotic stage. MN can be formed from acentric fragments or whole chromosomes/chromatids during mitotic division. They did not attach properly with the spindle during the segregation process in anaphase (Fenech 2007; Fenech and Bonassi 2011). Therefore, both clastogenic and aneugenic effects can be determined with the MN assay which may reflect genomic instability (Inoue et al. 1997; Albertini et al. 2000; Kirsch-Volders et al. 2011). In our study ZnO NPs also increased the MN frequency.

In this study, while these increases in the frequencies of abnormal cells and MN were slight dose-dependent, there were no dose dependence in the CA/cell. In this research, paradoxically, high doses of ZnO NPs led to less abnormalities at 48 h. We suggest that this could be due to an increased aggregation of ZnO NPs at high concentrations in lymphocyte culture. Therefore the addition of increasing concentrations/doses of ZnO NPs to lymphocyte cultures may not be paralleled by an equivalent increase in the genotoxic effects of this nanomaterial.

Studies on the point of the genotoxicity of ZnO are limited. There are only some researches about certain applications of ZnO NPs. Therefore, the mechanisms operating in ZnO-mediated mutation in human lymphocytes is currently unknown. However, Sharma et al. (2009) reported decreases in the GSH level, SOD and catalase activity in human epidermal cells which were exposed with ZnO nanoparticles. On the other hands, they observed a significant increase in the lactate dehydrogenase (LDH) and hydrogen peroxide (H_2O_2) levels. Researchers used the comet technique and they revealed the primer DNA damaging caused by different doses of ZnO NPs (0.8 and 5 μg/mL). They also observed significant increases in the DNA tail moment and tail intensity. After this research, however, authors determined that ZnO caused cytotoxicity and oxidative stress. Yang et al. (2009) studied primary mouse fibroblast cells and they reported a significant increase in the ROS and LDH levels, however, they also reported a significant reduction in the GSH and SOD level. Malondialdehyde (MDA) level was increased by ZnO nanoparticles. In their comet research, they observed a significant increase in the DNA tail length, tail moment and tail intensity. They suggested that ZnO nanoparticles caused cytotoxicity and oxidative damage. It is well known that ROS can interact with biomolecules including DNA and causes DNA single or double strand breaks and it is also well known that MDA causes free radicals. Some studies have reported similar results (Jeng and Swanson 2006; Musarrat et al. 2009; Lin et al. 2009; Gerloff et al. 2009; Zhu et al. 2009; Kim et al. 2010). Our findings also clearly demonstrate that ZnO is clastogenic in human lymphocytes in vitro.

Someya et al. (2008) studied human dental pulp. They reported an increase in the percentage of abnormal metaphase 30 h after administration of ZnO (μm size) as compared to the control. In their study, chromatid gaps and chromatid breaks were observed. Hidaka et al. (2006) studied the damage of ZnO and various nanoparticles exposed to UV on plasmid DNA via gel electrophoresis method, and demonstrated that ZnO caused faster and larger DNA damage. Dufour et al. (2006) studied the effects of ZnO on Chinese hamsters ovary (CHO) cells. They revealed increases in chromosome abnormalities, excluding gaps. Our study did not consider gaps as abnormalities, and observed chromatid and chromosome breaks, fragments, dicentric chromosomes and micronucleus.

Zheng et al. (2009) reported that ZnO nanomaterial distorts division activities of L 929 (mice fibroplast cells) and HeLa cells. Their study agrees with ours. In our study, mitotic activity was decreased by ZnO nanoparticle for the 24 h treatment.

Yoshida et al. (2009) concluded that, using the ames test, the mutagenic activity of tetramethylammoniumhydroxide-coated ZnO nanoparticles is negative.

As the size of the material is decreased to nanometers, it may have very different properties and behavior than the same material at a greater size. Nel et al. (2006) explained that these properties of nanomaterial may differ substantially from respective bulk materials. They also pointed out that physical and chemical properties of NPs lead to an increase of bioavailability and toxicity. On the other hand, NPs can cross the strongest biological barriers such as blood–brain barrier (Lockman et al. 2003). Additional to effects of NPs, there are some researches about the action mechanism of NPs on cells. But, the action mechanism of NPs has not been fully revealed.

There is need for further investigation to explain the dosage and potential damages of these particles because the toxicity tests on the long-term effects of nanoparticle administration on living things are limited. As observed in our study, chromatid breaks, chromosome breaks and fragments lead to the micronuclei, which lead us to suggest that it may result from the clastogenic effect of ZnO. Considering the results of other studies and our results, they show that ZnO NPs induce DNA breaks. In addition to our study, more in vitro and in vivo studies should be conducted to demonstrate whether ZnO nanoparticles have genotoxic risks.

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