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Chapter 15

THE POTENTIAL PROTECTIVE EFFECT OF ANTIOXIDANTS ON NANOPARTICLE TOXICITY

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ABSTRACT

Since the advances in nanotechnology of a few decades ago, numerous nanoparticles (NPs) have been manufactured and used throughout the world. NPs have varied technological applications in electronics, photonics, medical, garments, sporting goods, agricultural products and clean energy. Because the amount of NPs to which humans are exposed is likely increasing, the health effects of NP exposure and their underlying mechanism are being investigated. In order to utilize NPs more safely, the means by which their negative effects are reduced must be discovered.

Previous studies have reported that various types of NPs, such as carbon black, carbon nanotubes, silver, nickel, silica, silicon, and various metal oxides induce reactive oxygen species (ROS), which cause oxidative stress and cell damage, to all life forms (plants to mammals) regardless of species. For example, when single-wall carbon nanotubes are intratracheally administered to rats (0.2 and 2.0 mg/kg/day, once every three days, a total of 14 instillations), ROS in the lung are increased and the exacerbation of allergic asthma is induced. Intravenous injection of single-wall carbon nanotube suspension (6.25 and 12.5 mg/kg/day for 9 consecutive days) induces oxidative stress in the brain and decreases locomotor activity in mice. These reports demonstrate that the generation of ROS is one of the most important factors in the mechanism of NP health effects.

These studies also showed the possibility of protective effects of antioxidants such as vitamin C (ascorbic acid) and vitamin E (tocopherol) on NP-induced oxidative stress. The depletion of antioxidants exacerbated the inflammatory response to NP exposure.

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Because antioxidants reduce the effects of NPs via ROS elimination, these studies will contribute to the safe and practical use of NPs. Some evidence suggests that oxidative stress may be a starting point for the main mechanism underlying the NP toxicity. According to the hierarchical oxidative stress hypothesis, the lowest level of oxidative stress is associated with cytoprotective responses, such as the induction of antioxidant and detoxification enzymes. If the level of protection fails, the oxidative stress will lead to proinflammatory effects. Further escalation will trigger disturbances in mitochondrial function, resulting in cellular apoptosis or necrosis. Both endogenous and dietary antioxidants can be considered as a first line of defense against the generation of ROS due to NP exposure. Accordingly, it is highly possible that antioxidants may act as a protective agent against damage induced by various types of NPs through their downregulation of the oxidative stress level.

Keywords: nanoparticle, oxidative stress, antioxidant, ascorbic acid, α-tocopherol, omega-3 polyunsaturated fatty acid

INTRODUCTION

Nanoparticles (NPs) are generally defined as matter of 1-100 nm in scale in at least one dimension (NSET 2010; Feynman, 1970; Taniguchi, 1974). Because the specific surface area per mass of NPs is larger in comparison to bulk materials, they have physicochemically unique properties and higher activity than micro-sized materials. NPs are important materials for nanotechnology, which is widely expected to be one of the next steps in science: integrating engineering with biology, chemistry and physics (Ray et al., 2009). Nanotechnology has progressed from being an exotic research pursuit to being utilized in hundreds of mainstream consumer products. Annually, the nanomaterials market is worth exceeding billions of dollars (Kovacic et al., 2013; Grainger 2009). Actually, NPs have been manufactured and used worldwide since advances in nanotechnology a few decades ago. NPs have varied technological applications in photonics, electronics, sporting goods, garments, agricultural products and clean energy. Numerous medical innovations involving NPs, including imaging, drug delivery, nanotherapeutics, and nanophase formulations are in clinical trials. It is estimated that, at the time of writing, over 1,600 (a number that increases by three or four per week) manufacturer-identified nanoparticle-based products are publicly available and marketed (PEN 2014; Chen et al., 2013; Li et al., 2014).

NPs are expected provide enormous benefits to human life; however, as more NPs are manufactured, more NPs come onto the market, from where they may then be emitted in the environment. Thus, it is possible that the amount of NPs to which humans may be exposed is increasing. The potential for NP exposure to cause adverse health effects and various diseases is a matter of concern. It is therefore necessary to assess the effects of NP exposure, in order to elucidate their toxicity mechanism and to discover a methodology for reducing their negative effects and facilitate their safer use. The present article introduces the usefulness of antioxidant supplementation and discusses the protective effects of antioxidants on NP toxicity with a view to using NPs more safely.

THE MECHANISM OF NEGATIVE EFFECTS OF NANOPARTICLES THROUGH THE INDUCTION OF EXCESSIVE REACTIVE OXYGEN SPECIES

The health effects of exposure to NPs and their underlying mechanism are being investigated. The negative effects of particulate matter, containing NPs, have been explained by a number of mechanisms, such as inflammation, endotoxins, stimulation of capsaicin/irritant receptors, autonomic nervous system activity, procoagulant effects, covalent modification of cellular components, and reactive oxygen species (ROS) production (Mazzoli-Rocha et al., 2010; Nel et al., 2006; Li et al., 2008). A current comprehensive review of the toxicity of various NPs focused on excessive ROS induction and oxidative stress (Kovacic et al., 2013; Manke et al., 2013). It is well recognized that NPs generally produce ROS, which may be a key factor in their toxicological effects, both inside and outside the cells.

There is a great deal of experimental evidence supporting that the generation of ROS by NPs is one of the most important factors in the mechanism of their health effects (Kovacic et al., 2013; Manke et al., 2013). Firstly, various species, from mammals to plants, have been shown to be damaged by NP-induced oxidative stress, e.g., rodent respiratory tract and bronchoalveolar system (Lin et al., 2013; Srinivas et al. 2012), rat serum (Al-Rasheed et al., 2013; Reddy et al., 2011), mouse kidney (Al-Rasheed et al., 2013), mouse liver (Shrivastava et al., 2014), central nervous system of mice (Shrivastava et al., 2014), cardiovascular system of mice (Sheng et al., 2013) (Table 1), human bronchial epithelial (BEAS-2B) cells (Park et al., 2008), human lung epithelial (A549) cells (Ahamed, 2011; Chen et al., 2011; Müller et al., 2010), human monocyte-derived macrophages (MDM) and dendritic cells (MDDC) (Müller et al., 2010), human umbilical vein endothelial (ECV304) cells (Lee et al., 2011), human embryonic kidney (HEK293) cells (Reddy et al., 2010), human kidney epithelial (HK-2) cells (Pujalté et al., 2011), murine macrophage (RAW264.7) cells (Chen et al., 2011), rat aortic smooth muscle (A-10) cells (Lee et al., 2011), rat macrophages (NR8383) (Bhattacharjee et al., 2010) (Table 2), *Drosophila melanogaster* (Ahamed et al., 2010), *Caenorhabditis elegans* (Rui et al., 2013), and plant shoots and roots (Rico et al., 2013) (Table 1). Secondly, various types of NPs, e.g., carbon black (Lee et al., 2011), multi-walled carbon nanotubes (Reddy et al., 2010 and 2011; Chen et al., 2011), single-walled carbon nanotubes (Lin et al., 2013; Müller et al., 2010), titanium dioxide (Al-Rasheed et al., 2013; Shrivastava et al., 2014; Sheng et al., 2013; Rui et al., 2013; Park et al., 2008; Müller et al., 2010; Pujalté et al., 2011), zinc oxide (Shrivastava et al., 2014; Pujalté et al., 2011), erroso-ferric oxide (Lin et al., 2013; Srinivas et al., 2012), silica (Lin et al., 2013), aluminium oxide (Shrivastava et al., 2014), silver (Ahamed et al., 2010), cerium oxide (Rico et al., 2013), nickel (Ahamed, 2011), cadmium sulfide (Pujalté et al., 2011) and silicon (Bhattacharjee et al., 2010) induce ROS, cause oxidative stress, inflammation, cell damage and the reduction of endogenous antioxidants (Table 1, 2). ROS are also produced by combustion-derived ultrafine particles (Müller et al., 2010) and diesel exhaust particulate containing nano-sized particle matter (Nemmar et al., 2010) (Table 1, 2).

Table 1. Nanoparticle-induced oxidative stress response and reduction of endogenous antioxidants *in vivo*

Reference	Particle[*1]	Dose	Exposure route	Species	Organ[*2]	Main endpoints	Major findings in relation to oxidative stress and inflammation[*3]
Lin et al., 2013	SWCNT, SiO_2, Fe_3O_4	2 and 10 mg/kg body weight/day once every 2 days for 35 days	Intratracheal instillation	Rat	BALF, Lung	Oxidative stress markers in BALF. Proinflammatory cytokines in BALF. Comparative proteomics analysis of lung. mRNA and protein expression of Transgelin 2.	Increase in MDA and LDH by the three NPs (10 mg/kg). Decrease in T-AOC and SOD by the three NPs (2 and 10 mg/kg). Increase in IL-6 by the three NPs (2 and 10 mg/kg). Increase in TNF-α by the SWCNT and SiO_2 (10 mg/kg).
Srinivas et al., 2012	Fe_3O_4	640 mg/m^3	Inhalational exposure	Rat	BALF, Lung	Proinflammatory cytokines in BALF and blood. Oxidative stress markers in BALF and lung. Histopathology of lung. Cell population in BALF.	Increase in LDH and alkaline phosphatase of BALF. Increase in MDA of lung. Decrease in GSH and T-AOC of lung. Increase in leukocyte and neutrophils in BALF.
Al-Rasheed et al., 2013	TiO_2	0.6 and 1 g/kg body weight/day for 5 days	Oral administration	Rat	Kidney, Serum	Kidney function biomarkers of serum. Proinflammatory cytokines in serum.	Increase in TNF-α, IL-6, CRP, IgG, VEGF, and NO of serum. Decrease in GSH of kidney.
Reddy et al., 2011	MWCNT	0.2, 1 and 5 mg/kg body weight	Intratracheal instillation	Rat	Serum	Endogenous antioxidants.	Dose-dependent decrease in T-AOC, GSH, SOD, and CAT. Dose-dependent increased in lipid peroxidation product.
Nemmar et al., 2010	DEP	20 μg/kg, (time-course 6 , 18 , 48 and 168 h)	systemic administration	Rat	Whole body	Systemic inflammation markers. Oxidative stress markers. Histopathology of lung, heart, liver, kidney.	Increase in IL-6 of plasma at 6 h. Increase in SOD activity of plasma at 6 and 18 h. Time-dependent accumulation of inflammatory cells in lung.
Shrivastava et al., 2014	TiO_2, ZnO, Al_2O_3	500 mg/kg body weight for 21 days.	Oral administration	Mouse	Liver, Brain	Oxidative stress markers in brain and liver. Ultrastructure of brain and liver Dopamine and norepinephrine in brain.	Decrease in SOD of brain and liver. Decrease in dopamine and norepinephrine of cerebral cortex.
Sheng et al., 2013	TiO_2	2.5, 5, and 10 mg/kg body weight for 90 days.	Intragastric administration	Mouse	Heart	Oxidative stress markers. Endogenous antioxidants. Antioxidative enzymes.	Dose-dependent increase in O_2^- ,H_2O_2, MDA, and 8-OHdG. Dose-dependent decrease in GSH, vitamin C, and thiol. Dose-dependent decrease in SOD, CAT, APx, GR, and GST.
Ahamed et al., 2010	Ag	50 and 100 μg/mL for 24 and 48 h	Oral intake (mixed food)	*Drosophila melanogaster*	-	Heat shock protein 70. Oxidative stress markers. DNA damage and apoptosis.	Decrease in GSH, SOD and CAT. Increase in MDA. Increase in caspase-3, and caspase-9 activities.
Rui et al., 2013	TiO_2	20 μg/L or 25 mg/L	Oral intake (mixed food)	*Caenorhabditis elegans*	-	Antioxidant gene expression.	Induction of genes of sod-2, sod-3, mtl-2, and hsp-16.48.
Rico et al., 2013	CeO_2	62.5, 125, 250, and 500 mg/L for 10 days	Root intake (mixed water)	Rice	Shoot, Root	Oxidative stress markers in roots. Antioxidant activity of roots and shoots. Germination rate and seeding growth.	Dose-dependent increase DHAR of roots and shoots. Increase in SOD activity of roots and shoots.

Abbreviations: *1 Al_2O_3, aluminum oxide; Ag, silver, CeO_2, Cerium oxide; DEP, diesel exhaust particle; Fe_3O_4, ferroso-ferric oxide; MWCNT, multi-walled carbon nanotube; SiO_2, silica; SWCNT, single-walled carbon nanotube; TiO_2, titanium dioxide; ZnO, zinc oxide. *2 BALF, bronch oalveolar lavage fluid. *3 APx, ascorbate peroxidase; CAT, catalase; CRP, C-reactive protein; DHAR, dehydroascorbic acid; GR, glutathione reductase; GSH, glutathione; GST, glutathione-S-transferase; H_2O_2, hydrogen peroxide; IgG, immunoglobin G; IL-6, interleukin-6; LDH, lactate dehydrogenase; MDA, malondialdehyde; NO, nitric oxide; O_2^-,seperoxide radicals; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TNF-α tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

Table 2. Nanoparticle-induced oxidative stress response and reduction of endogenous antioxidants in cultured cell

Reference	Particle[*1]	Dose	Cultured cells	Main endpoints[*2]	Major findings in relation to oxidative stress and inflammation[*3]
Park et al., 2008	TiO_2	5, 10, 20 and 40 μg/ml	BEAS-2B: Human bronchial epithelial cells	Oxidative stress markers. Oxidative stress-related genes. Inflammation-related genes. Activity of apoptotic enzyme.	Decrease in GSH. Induction of HO-1, TrxR, GST, CAT, and HIF gene expression. Activation of cytosolic caspase-3. Elevation of IL-1, IL-6, IL-8, TNF-a, and CXCL2 gene expression.
Ahamed, 2011	Ni	0, 1, 2, 5, 10 and 25 μg/ml for 24 and 48 h	A549: Human lung epithelium cells	Mitochondrial function. Cytotoxic markers. Oxidative stress markers. Activity of apoptotic enzyme.	Dose-dependent reduction in mitochondrial function. Dose-dependent decrease in GSH. Dose-dependent increase in ROS and LPO. Dose-dependent increase in caspase-3 activity.
Chen et al., 2011	MWCNT	0, 2.5, 10, 25, and 100 μg/mL for 24 h	A549: Human lung epithelium cells RAW264.7: Murine macrophages	Cell viability. Oxidative stress markers in cells. Cytotoxic markers in culture fluids. Total ROS in cells (DCFH-DA assay).	Dose-dependent decrease in cell viability of both cell types. Dose-dependent increase in MDA, H_2O_2, TP, LDH, and NO of both cell types. Dose-dependent decrease in SOD and GSH of both cell types. Dose-dependent increase in ROS generation of both cell types.
Müller et al., 2010	SWCNT, DEP, TiO_2	SWCNT: 30 μg/ml DEP: 125 μg/ml TiO_2: 2.5 μg/ml	A549: Human lung epithelium cells MDM: Human monocyte-derived macrophages MDDC: Human monocyte-derived dendritic cells	Total ROS in cells (DCFH-DA assay). Endogenous antioxidants (T-AOC). Inflammatory cytokines and chemokine.	Induction in ROS of A549, MDM, and triple co-culture by all particle types.
Lee et al., 2011	CB (2 types: small and large)	10, 25, 50, 100 and 200 μg/ml	ECV304: Human unbilical vein endothelial cells A-10: Rat aortic smooth muscle cells	Cytotoxic markers in culture fluids. Total ROS in cells (DCFH-DA assay). Effects of antioxidant supplementation.	Increase in LDH of ECV304 by small size CB. Increase in LDH and H_2O_2 of A-10 by large size CB. Suppression of cell cytotoxicity and ROS generation by antioxidants
Reddy et al., 2010	MWCNT (2 types: short and long)	3 - 300mug/ml for 48 h	HEK293: Human embryonic kidney cells	Oxidative stress markers in cells. Inflammatory cytokines. Mitochondrial function. Cytotoxic markers in culture fluids.	Dose-dependent decrease in GSH by both particle types. Dose-dependent increase in MDA, IL-8, and LDH by both particle types.
Pujalté et al., 2011	TiO_2, ZnO, CdS	TiO2: 0.625 - 160 μg/cm^2, ZnO: 0.1 - 20 μg/cm^2, CdS: 0.7 - 6.33 μg/cm^2	HK-2: Human kidney epithelial cells IP15: Human glomerular mesangial cells	Cell viability. Total ROS in cells (DCFH-DA assay). Oxidative stress markers in cells. NF-κB translocation.	Dose-dependent decrease in cell viability by ZnO and CdS. Decrease in GSH and GSH/GSSG ratio by ZnO and CdS. Dose-dependent increase in ROS generation by all particle types. Activation of NF-κB by ZnO and CdS.
Bhattacharjee et al., 2010	Si ($SiNH_2$, SiN_3, SiCOOH)	$SiNH_2$: 0.1 - 370 ng/mL, SiN_3: 0.1 - 2200 ng/mL, SiCOOH: 0.1 - 3000 ng/mL	NR8383: Rat macrophages, Ceco-2: Human colonic adenocarcinoma cells	Cytotoxic markers in culture fluids. Total ROS in cells (DCFH-DA assay). Effects of antioxidant supplementation. Mitochondrial function.	Dose-dependent decrease in mitochondrial function by $SiNH_2$ and SiN_3. Dose-dependent increase in ROS by $SiNH_2$ and SiN_3. Suppression of $SiNH_2$-induced mitochondrial dysfunction by vitamin E. Suppression of SiN_3-induced mitochondrial dysfunction by vitamin C.

Abbreviations: *1 CB, carbon black; CdS, cadmium sulfide; DEP, diesel exhaust particle (combustion-derived nanoparticle); MWCNT, multi-walled carbon nanotube; Ni, nickel; SWCNT, single-walled carbon nanotube; Si, silicon; TiO2, titanium dioxide; ZnO, zinc oxide. *2 DCFH-DA, 2',7'-dichlorofluorescin diacetate; NF-kB, nuclear factor-kappa B; ROS, reactive oxygen species; T-AOC, total antioxidant caoacity. *3 CAT, catalase; CXCL2, C-X-C motif ligand 2; GSH, glutathione; GSSG, glutathione oxidized form; H_2O_2, hydrogen peroxide; HIF, hypoxia inducible factor; HO, heme oxygenase; IL-1, interleukin-1; IL-6, interleukin-6; IL-8, interleukin-8; LDH, lactate dehydrogenase; LPO, lipid peroxidation; MDA, malondialdehyde; NO, nitric oxide; SOD, superoxide dismutase; TNF, tumor necrosis factor, TP, total protein; TrxR, thioredoxin reductase.

The above-mentioned literature shows that oxidative stress induction caused by the increase of ROS is the essence of the main mechanism underlying NP toxicity, regardless of biological species or NP type, and suggests that the reduction of ROS could possibly the mitigate adverse effects of NPs.

THE PROTECTIVE EFFECT OF ANTIOXIDANT SUPPLEMENTATION ON NANOPARTICLE TOXICITY

According to the hierarchical oxidative stress hypothesis, the lowest level of oxidative stress is associated with cytoprotective responses such as the induction of antioxidants and detoxification enzymes (Xiao et al., 2003, Liu et al., 2014). If the level of antioxidative protection fails, the oxidative stress induces inflammation. Further escalation of the stress triggers disturbances in mitochondrial function, resulting in cellular apoptosis or necrosis. Both endogenous and dietary antioxidants are the first line of defense against the generation of ROS by NP exposure (Contestabile, 2001). Hence, it is highly possible that supplied antioxidants act as a protective agent against damage induced by various types of NPs through the downregulation of the oxidative stress level. Actually, a number of studies have shown the protective effects of dietary antioxidant supplementation on NP-induced oxidative stress.

1. Vitamin C

Vitamin C, also called ascorbic acid, is one of the most typical antioxidants. Although vitamin C is readily absorbed by organisms, it is one of the safest nutrients because it is water-soluble. Numerous studies have reported that vitamin C offers a variety of health benefits. However, although many animals can make vitamin C in their own bodies, human beings cannot. Humans must obtain the vitamin from food and other sources. Consequently, in spite of the fact that fresh fruits and vegetables, especially citrus fruits, are good sources of vitamin C, many people, the world over, intentionally ingest vitamin C supplements. Nowadays, vitamin C is one of the most familiar antioxidants.

The oldest study to show that vitamin C was an important antioxidant agent against the oxidative effect of particulate matter was reported in 1999 (Zielinski et al., 1999). The study investigated whether the consumption of vitamin C, which was added in lung epithelial lining fluid, was concentration-dependently increased by particles. There was a strong inverse correlation between the level of vitamin C consumption and particle size, and a positive correlation with particle surface area. Subsequently, there have been few studies to investigate the antioxidative effects of vitamin C on NP-induced damage; however, there has been a gradual increase in the number of studies since 2010.

In October 2014, the highest impact study was reported. The study demonstrated that the supplementation of vitamin C significantly mitigated the damage caused by single-walled carbon nanotubes to the central nervous system of mice (Liu et al., 2014). After intravenous injection of single-walled carbon nanotube suspension (6.25 and 12.50 mg/kg/day for 9 days), neurobehavior (Morris water maze and open-field test), brain histopathology, oxidative stress,

inflammation, and apoptosis were analyzed in the mouse brain. The injection of single-walled carbon nanotube induced cognitive deficits and decreased locomotor activity, damaged the hippocampus CA1 region and induced Nissl substance loss in pyramidal cells, reduced endogenous antioxidant (glutathione), and increased the expression levels of oxidative stress markers (ROS and malondialdehyde), inflammation markers (nuclear factor κB, tumor necrosis factor α, and interleukin-1β), and activation of cysteine-aspartic acid protease 3 (an apoptosis marker in the brain). Subsequently, vitamin C (100 mg/kg/day for 9 days) was concurrently and intraperitoneally administered with the doses of single-walled carbon nanotubes, to investigate the vitamin's protective effects. The results showed that in mice, the administration of vitamin C improved cognitive abilities and locomotor activity, attenuated the levels of oxidative stress in the brain, and suppressed brain cell damage, inflammation, and apoptosis. The downregulation of oxidative stress, inflammation, and apoptosis were proposed to explain the neuroprotective effects of vitamin C.

Some in vitro studies have also investigated the protective effects of vitamin C on NP exposure. In human lung epithelial (A549) cells, vitamin C mitigated the ROS generation and glutathione depletion induced by nickel ferrite NP treatment (Ahamed et al., 2011). Vitamin C also inhibited lipid peroxidation, which was correlated with the ROS production due to talc NPs (Akhtar et al., 2010). Although bare Ag NPs did not produce ROS, citrate-capped Ag NPs generated ROS, which were successfully scavenged by vitamin C and N-acetylcysteine (another antioxidant) in endothelial-like umbilical (ECV304) cells (Oh et al., 2014). Not only engineered NPs but also diesel exhaust particles, an unintentionally produced environmental nanoparticle, increased ROS and induced oxidative damage. Exposure to carbon black and diesel exhaust particles in cultured human ECV304 cells induced oxidative stress and the expression of cell surface adhesion molecules. These effects were also partly attenuated by vitamin C. Furthermore, the anti-oxidative effects of vitamin C on NPs were also valid against other animal species, including *Drosophila melanogaster* (Posgai et al., 2011) and *Caenorhabditis elegans* (Wu et al., 2012).

These findings suggested that it is highly possible that vitamin C acts as a protective agent to various organs, especially the central nervous system, which is one of the organs that is most sensitive to ROS and inflammation, against oxidative damage induced by several NPs through the reduction of the oxidative stress level. Further investigations associated with the protective effects of vitamin C on NP-induced cellular damage or toxicity are required to promote safer applications of NPs. Moreover, in order to facilitate the safer use of NPs, other suitable protective agents (in addition to vitamin C) against NP-induced oxidative damage should be the subject of future research.

2. Vitamin E

Vitamin E is a generic term for all tocopherol and tocotrienol derivatives (Yoshida et al., 2007). There are four major isoforms of vitamin E: α-, γ-, β- and δ-tocopherol. In the diet and tissues, α-tocopherol and γ-tocopherol are the most abundant isoforms (Marchese et al., 2014); they differ by only one methyl group and have a relatively similar capacity to scavenge ROS at equal molar concentrations (Nishio et al., 2013). Hence, vitamin E supplementation is expected to protect from NP-induced oxidative damage.

Interestingly, there are two studies in which antithetical animal experiments were performed. One showed that vitamin E supplementation suppressed single-walled carbon nanotube-induced exacerbation of allergic asthma (Li et al., 2014). The other demonstrated that depletion of vitamin E exacerbated pulmonary inflammatory response and oxidative stress induction by single-walled carbon nanotubes (Shvedova et al., 2007). In more detail, the first study confirmed that single-walled carbon nanotubes, intratracheally administered (0.2 and 2.0 mg/kg/day, once every three days, a total of 14 instillations), aggravated allergic asthma of ovalbumin-sensitized asthma model rats with an increase of asthma-related Th2 response cytokine (interleukin-4 and interleukin-4/interferon-γ ratio) and serum immunoglobulin (IgE and IgG1) concentrations, lung immune cellular profiles (total cell, lymphocyte, neutrophil, and eosinophil), histopathological changes of lung tissue (bronchial remodeling, lung tissue cell infiltration, subepithelial collagen deposition, and mucus hypersecretion), and airway hyperresponsiveness. Subsequently, vitamin E was orally administrated (100 mg/kg) 14 times at 3 hours after every administration of single-walled carbon nanotubes. The protective effects of vitamin E on single-walled carbon nanotubes effects were detected by comparing the asthma symptoms of the vitamin E treated group with the non-treated group. Based on these results, the mechanism for the protective effects of vitamin E was proposed, involving ROS elimination, downregulation of Th2 responses, reduced Ig production, and the relief of allergic asthma symptoms. In the second study, mice were maintained on a vitamin E-deficient diet (containing less than 10 IU/kg of diet) or basal diet (50 IU/kg of diet) for a total of 24 weeks, and then treated with an instillation of single-walled carbon nanotubes into the lung at a dose of 0 or 40 μg/mouse with phosphate buffered saline. At post-treatment days 1, 7 and 28, the vitamin E-deficient mice showed significant accumulation of lipid peroxidation products (malondialdehyde), acute inflammation (total number of inflammatory cells, number of polymorphonuclear leukocytes, released LDH, total protein content, and levels of pro-inflammatory cytokines, TNF-α and IL-6) and enhanced profibrotic responses (elevation of TGF-β and collagen deposition). This was followed by a decrease in the content of antioxidants, namely α-tocopherol, glutathione and vitamin C, in the lung tissue. These results indicate that the depletion of antioxidants like vitamin E may exacerbate the respiratory damage caused by NP induced-oxidative stress.

The protective effects of vitamin E against NP toxicity were also elucidated an *in vitro* study (Wang et al., 2012). The neuroprotective effect of vitamin E on single-walled carbon nanotube-induced neurotoxicity was shown in cultured PC12 cells, an *in vitro* model of neuronal cells. The presence of vitamin E (0.01-2 mM) inhibited the formation of ROS, decreased the level of lipid peroxide and elevated the activity level of several antioxidants in cultured cells. Additionally, vitamin E blocked the reduction in mitochondrial membrane potential and the activation of caspase-3, and the vitamin prevented apoptotic cell death of PC12 induced by single-walled carbon nanotube.

In conclusion, oxidative stress may be an important mechanism of single-walled carbon nanotube-induced aggravating effects. These studies suggested that vitamin E supplementation protects people from the adverse effects (especially with regard to respiratory diseases such as allergic asthma), of single-walled carbon nanotubes and other nanomaterials. Because the most common exposure route of NPs is transtracheal aspiration, vitamin E may possibly be the most often-used antioxidant to decrease the risk that NPs pose to our health.

Importantly, attention should be paid to vitamin E isoforms. Previous studies have shown that α-tocopherol and γ-tocopherol have opposing effects against respiratory function and diseases such as asthma (Marchese et al., 2014; Berdnikovs et al., 2009). α-tocopherol protected lung inflammation and airway hyperresponsiveness. In contrast, γ-tocopherol promoted the symptoms and inhibited the protective effects of α-tocopherol. Therefore, the protective effect against respiratory damage induced by NP inhalation exposure may only be achieved by the α-tocopherol isoform. Furthermore, despite α-tocopherol and γ-tocopherol both having antioxidative functions, these vitamin E isoforms have opposing effects. This fact suggests that the adverse effects of NP inhalation exposure on respiratory organs might be regulated by a mechanism other than the increase of oxidative stress. Additional research, which considers the vitamin E isoforms, is required.

3. Omega-3 Polyunsaturated Fatty Acid (n-3 PUFA)

Omega-3 polyunsaturated fatty acid (n-3 PUFA) has cardioprotective benefits including an anti-inflammatory effect with ROS reduction (Lee et al. 2009). Eicosapentaenoic acid and docosahexaenoic acid, typical n-3 PUFA, can suppress the risk of cardiovascular events (O'Keefe et al. 2006; Saravanan et al. 2010).

Although there is no evidence on the impact of n-3 PUFA, as an antioxidant supplement, on the health effects of NP exposure (Weichenthal et la., 2013), the protective effects of n-3 PUFA against particulate matter of 2.5 μm or less ($PM_{2.5}$), including nanosized-particles, on cardiac effects have been shown. In a study of 50 nursing home residents (>60 years of age) in Mexico City, randomized to receive treatment with either fish oil (2 g/day) or soy oil (2 g/day) with 6 months of follow-up (1 month before supplementation and 5 months after), it was demonstrated that fish oil supplementation, as a source of n-3 PUFA, prevented the reduction in heart rate variability associated with exposure to indoor $PM_{2.5}$ (8 $\mu g/m^3$) (Romieu et al., 2005). In contrast, soy oil supplementation was associated with marginal, non-significant protection. A short exposure to concentrated ambient fine and ultrafine particulate matter (mean mass concentration 278 ± 19 $\mu g/m^3$) for 2 hours induced acute changes in heart rate variability, blood neutrophils, very low-density lipoproteins, and triglycerides. The effects of particle matter were attenuated in healthy middle-aged adults by supplementation with fish oil (3 g/day) containing rich eicosapentaenoic acid for 4 weeks before particle exposure, but not in those supplemented with olive oil (Tong et al., 2012). These findings are also referenced by a current review, which focused on the association between $PM_{2.5}$ and cardiovascular events (Weichenthal et al., 2013).

In general, chronically decreased heart rate variability has been used to predict an increased risk of cardiovascular morbidity and mortality (Kleiger et al. 1987; Lahiri et al. 2008; Tsuji et al. 1996). These reports suggested that supplementation of n-3 PUFA protect against the adverse effects of $PM_{2.5}$, which includes a large number of nano-sized particles, and air pollution on cardiac autonomic function. In the near future, interesting findings may be obtained from research into the protective effects of n-3 PUFA on the adverse effects caused by NP exposure.

CONCLUSION

A number of *in vivo* and *in vitro* studies have shown that antioxidants downregulate oxidative stress and act as protective agents against NP-induced adverse effects. This evidence also suggests that ROS production is a key factor in NP-induced toxicological effects. More research will be actively carried out and the protective effects of dietary antioxidants will be proven as a useful defense agent against the NP-induced oxidative stress associated with various toxicological effects. Antioxidant supplementation may be one of the answers for the safer use of many NPs.

REFERENCES

Ahamed, M., Posgai, R., Gorey, T. J., Nielsen, M., Hussain, S. M., Rowe, J. J., (2010): Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in *Drosophila melanogaster*. *Toxicol. Appl. Pharmacol.* 242, 263-269.

Ahamed, M., (2011): Toxic response of nickel nanoparticles in human lung epithelial A549 cells. *Toxicol. In Vitro* 25, 930-936.

Ahamed, M., Akhtar, M. J., Siddiqui, M. A., Ahmad, J., Musarrat, J., Al-Khedhairy, A. A., AlSalhi, M. S., Alrokayan, S. A., (2011): Oxidative stress mediated apoptosis induced by nickel ferrite nanoparticles in cultured A549 cells. *Toxicology* 283, 101-108.

Akhtar, M. J., Kumar, S., Murthy, R. C., Ashquin, M., Khan, M. I., Patil, G., Ahmad, I., (2010): The primary role of iron-mediated lipid peroxidation in the differential cytotoxicity caused by two varieties of talc nanoparticles on A549 cells and lipid peroxidation inhibitory effect exerted by ascorbic acid. *Toxicol. In Vitro* 24, 1139-1147.

Al-Rasheed, N. M., Faddah, L. M., Mohamed, A. M., Abdel Baky, N. A., Al-Rasheed, N. M., Mohammad, R. A., (2013): Potential impact of quercetin and idebenone against immuno-inflammatory and oxidative renal damage induced in rats by titanium dioxide nanoparticles toxicity. *J. Oleo. Sci.* 62, 961-971.

Berdnikovs, S., Abdala-Valencia, H., McCary C., Somand M., Cole, R., Garcia, A., Bryce, P., Cook-Mills, J. M., (2009): Isoforms of vitamin E have opposing immunoregulatory functions during inflammation by regulating leukocyte recruitment. *J. Immunol.* 182, 4395-4405.

Bhattacharjee, S., de Haan, L. H., Evers, N. M., Jiang, X., Marcelis, A. T., Zuilhof, H., Rietjens, I. M., Alink, G. M., (2010): Role of surface charge and oxidative stress in cytotoxicity of organic monolayer-coated silicon nanoparticles towards macrophage NR8383 cells. *Part. Fibre Toxicol.* 7, 25.

Chen, B., Liu, Y., Song, W. M., Hayashi, Y., Ding, X. C., Li, W. H., (2011): *In vitro* evaluation of cytotoxicity and oxidative stress induced by multiwalled carbon nanotubes in murine RAW 264.7 macrophages and human A549 lung cells. *Biomed. Environ. Sci.* 24, 593-601.

Chen, H., Wang, B., Gao, D., Guan, M., Zheng, L., Ouyang, H., Chai, Z., Zhao, Y., Feng, W., (2013): Broad-spectrum antibacterial activity of carbon nanotubes to human gut bacteria. *Small* 9, 2735-2746.

Contestabile, A., (2001): Oxidative stress in neurodegeneration: mechanisms and therapeutic perspectives. *Curr. Top. Med. Chem.* 1, 553-568.

Feynman, R. P., (1970): There's plenty of room at the bottom. *Engineering Science* 23, 22-36.

Grainger, D. W., (2009): Nanotoxicity assessment: all small talk? *Adv. Drug Deliv. Rev.* 61, 419-421.

Kleiger, R. E., Miller, J. P., Bigger, J. T. Jr, Moss, A. J., (1987): Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am. J. Cardiol.* 59, 256-262.

Kovacic, P., Somanathan, R., (2013): Nanoparticles: toxicity, radicals, electron transfer, and antioxidants. *Methods Mol. Biol.* 1028, 15-35.

Lahiri, M. K., Kannankeril, P. J., Goldberger, J. J., (2008): Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. *J. Am. Coll. Cardiol.* 51, 1725-1733.

Lee, J. G., Noh, W. J., Kim, H., Lee, M. Y., (2011): Generation of reactive oxygen species contributes to the development of carbon black cytotoxicity to vascular cells. *Toxicol. Res.* 27, 161-166.

Lee, J. H., O'Keefe, J. H., Lavie, C. J., Harris, W. S., (2009): Omega-3 fatty acids: cardiovascular benefits, sources and sustainability. *Nat. Rev. Cardiol.* 6: 753-758.

Li, J., Chen, H., Chang, Q., Liu, X., Wu, Y., Wei, C., Li, R., Kwan, J. K., Yeung, K. L., Xi, Z., Lu, Z., Yang, X., (2014): Application of vitamin E to antagonize SWCNTs-induced exacerbation of allergic asthma. *Sci. Rep.* 4, 4275.

Li, N., Xia, T., Nel, A. E., (2008): The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles. *Free Radic. Biol. Med.* 44, 1689-1699.

Lin, Z., Ma, L., X, Z. G., Zhang, H., Lin, B., (2013): A comparative study of lung toxicity in rats induced by three types of nanomaterials. *Nanoscale Res. Lett.* 8, 521.

Liu, X., Zhang, Y., Li, J., Wang, D., Wu, Y., Li, Y., Lu, Z., Yu, S. C., Li, R., Yang, X., (2014): Cognitive deficits and decreased locomotor activity induced by single-walled carbon nanotubes and neuroprotective effects of ascorbic acid. *Int. J. Nanomedicine* 9, 823-839.

Manke, A., Wang, L., Rojanasakul, Y., (2013): Mechanisms of nanoparticle-induced oxidative stress and toxicity. *Biomed. Res. Int.* 2013, 942916.

Marchese, M. E., Kumar, R., Colangelo, L. A., Avila, P. C., Jacobs, D. R. Jr, Gross, M., Sood, A., Liu, K., Cook-Mills, J. M., (2014): The vitamin E isoforms α-tocopherol and γ-tocopherol have opposite associations with spirometric parameters: the CARDIA study. *Respir. Res.* 15, 31.

Mazzoli-Rocha, F., Fernandes, S., Einicker-Lamas, M., Zin, W. A., (2010): Roles of oxidative stress in signaling and inflammation induced by particulate matter. *Cell Biol. Toxicol.* 26, 481-498.

Müller, L., Riediker, M., Wick, P., Mohr, M., Gehr, P., Rothen-Rutishauser, B., (2010): Oxidative stress and inflammation response after nanoparticle exposure: differences between human lung cell monocultures and an advanced three-dimensional model of the human epithelial airways. *J. R. Soc. Interface* 7 (Suppl. 1), S27-S40.

Nel, A., Xia, T., Mädler, L., Li, N., (2006): Toxic potential of materials at the nanolevel. *Science* 311, 622-627.

Nemmar, A., Al-Salam, S., Zia, S., Dhanasekaran, S., Shudadevi, M., Ali, B. H., (2010): Time-course effects of systemically administered diesel exhaust particles in rats. *Toxicol. Lett.* 194, 58-65.

Nishio, M. H., Yoko, A., Mototada, S., Hitoshi, I., Yoshihisa Hagihara, Y. Y., Etsuo, N., (2013): Attenuation of lipopolysaccharide (LPS)-induced cytotoxicity by tocopherols and tocotrienols. *Redox Biol.* 1, 97-103.

NSET, (2010): The National Nanotechnology Initiative. Research and Development Leading to a Revolution in Technology and Industry Supplement to the President's FY 2011 Budget. Washington, DC: Subcommittee on Nanoscale Science, Engineering and Technology, Committee on Technology, National Science and Technology Council.

Oh, S. J., Kim, H., Liu, Y., Han, H. K., Kwon, K., Chang, K. H., Park, K., Kim, Y., Shim, K., An, S. S., Lee, M. Y., (2014): Incompatibility of silver nanoparticles with lactate dehydrogenase leakage assay for cellular viability test is attributed to protein binding and reactive oxygen species generation. *Toxicol. Lett.* 225, 422-432.

O'Keefe, J. H. Jr, Abuissa, H., Sastre, A., Steinhaus, D. M., Harris, W. S., (2006): Effects of omega-3 fatty acids on resting heart rate, heart rate recovery after exercise, and heart rate variability in men with healed myocardial infarctions and depressed ejection fractions. *Am. J. Cardiol.* 97, 1127-1130.

Park, E. J., Yi, J., Chung, K. H., Ryu, D. Y., Choi, J., Park, K., (2008): Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells. *Toxicol. Lett.* 180: 222-229.

PEN, (2014): Woodrow Wilson International Centre for Scholars. Project on Emerging Nanotechnologies. *Consumer Products Inventory of Nanotechnology Products.* Available from: http://www.nanotechproject.org/inventories/consumer/. Accessed: December 10, 2014.

Posgai, R., Cipolla-McCulloch, C. B., Murphy, K. R., Hussain, S. M., Rowe, J. J., Nielsen, M. G., (2011): Differential toxicity of silver and titanium dioxide nanoparticles on *Drosophila melanogaster* development, reproductive effort, and viability: size, coatings and antioxidants matter. *Chemosphere* 85, 34-42.

Pujalté, I., Passagne, I., Brouillaud, B., Tréguer, M., Durand, E., Ohayon-Courtès, C., L'Azou, B., (2011): Cytotoxicity and oxidative stress induced by different metallic nanoparticles on human kidney cells. *Part. Fibre Toxicol.* 8, 10.

Ray, P. C., Yu, H., Fu, P. P., (2009): Toxicity and environmental risks of nanomaterials: challenges and future needs. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev.* 27, 1-35.

Reddy, A. R., Rao, M. V., Krishna, D. R., Himabindu, V., Reddy, Y. N., (2011): Evaluation of oxidative stress and anti-oxidant status in rat serum following exposure of carbon nanotubes. *Regul. Toxicol. Pharmacol.* 59, 251-257.

Reddy, A. R., Reddy, Y. N., Krishna, D. R., Himabindu, V., (2010): Multi wall carbon nanotubes induce oxidative stress and cytotoxicity in human embryonic kidney (HEK293) cells. *Toxicology* 272, 11-16.

Rico, C. M., Hong, J., Morales, M. I., Zhao, L., Barrios, A. C., Zhang, J. Y., Peralta-Videa, J. R., Gardea-Torresdey, J. L., (2013): Effect of cerium oxide nanoparticles on rice: a study involving the antioxidant defense system and *in vivo* fluorescence imaging. *Environ. Sci. Technol.* 47, 5635-5642.

Romieu, I., Téllez-Rojo, M. M., Lazo, M., Manzano-Patiño, A., Cortez-Lugo, M., Julien, P., Bélanger, M. C., Hernandez-Avila, M., Holguin, F., (2005): Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. *Am. J. Respir. Crit. Care Med.* 172, 1534-1540.

Rui, Q., Zhao, Y., Wu, Q., Tang, M., Wang, D., (2013): Biosafety assessment of titanium dioxide nanoparticles in acutely exposed nematode *Caenorhabditis elegans* with mutations of genes required for oxidative stress or stress response. *Chemosphere* 93, 2289-2296.

Saravanan, P., Davidson, N. C., Schmidt, E. B., Calder, P. C., (2010): Cardiovascular effects of marine omega-3 fatty acids. *Lancet* 376, 540-550.

Sheng, L., Wang, X., Sang, X., Ze, Y., Zhao, X., Liu, D., Gui, S., Sun, Q., Cheng, J., Cheng, Z., Hu, R., Wang, L., Hong, F., (2013): Cardiac oxidative damage in mice following exposure to nanoparticulate titanium dioxide. *J. Biomed. Mater. Res. A* 101, 3238-3246.

Shrivastava, R., Raza, S., Yadav, A., Kushwaha, P., Flora, S. J., (2014): Effects of sub-acute exposure to TiO_2, ZnO and Al_2O_3 nanoparticles on oxidative stress and histological changes in mouse liver and brain. *Drug Chem. Toxicol.* 37, 336-347.

Shvedova, A. A., Kisin, E. R., Murray, A. R., Gorelik, O., Arepalli, S., Castranova, V., Young, S. H., Gao, F., Tyurina, Y. Y., Oury, T. D., Kagan, V. E., (2007): Vitamin E deficiency enhances pulmonary inflammatory response and oxidative stress induced by single-walled carbon nanotubes in C57BL/6 mice. *Toxicol. Appl. Pharmacol.* 221, 339-348.

Srinivas, A., Rao, P. J., Selvam, G., Goparaju, A., Murthy, P. B., Reddy, P. N., (2012): Oxidative stress and inflammatory responses of rat following acute inhalation exposure to iron oxide nanoparticles. *Hum. Exp. Toxicol.* 31:1113-1131.

Taniguchi, N., (1974): On the basic concept of "Nano-Technology." 1974 *Proceedings of International Conference in Production Engineering, Japan Society of Precision Engineering*, Tokyo, Japan, Part II, vol. 18.

Tong, H., Rappold, A. G., Diaz-Sanchez, D., Steck, S. E., Berntsen, J., Cascio, W. E., Devlin, R. B., Samet, J. M., (2012): Omega-3 fatty acid supplementation appears to attenuate particulate air pollution-induced cardiac effects and lipid changes in healthy middle-aged adults. *Environ. Health Perspect.* 120, 952-957.

Tsuji, H., Larson, M. G., Venditti, F. J. Jr, Manders, E. S., Evans, J. C., Feldman, C. L., Levy, D., (1996): Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation* 94, 2850-2855.

Wang, J., Sun, P., Bao, Y., Dou, B., Song, D., Li, Y., (2012): Vitamin E renders protection to PC12 cells against oxidative damage and apoptosis induced by single-walled carbon nanotubes. *Toxicol. In Vitro* 26, 32-41.

Weichenthal, S. A., Godri-Pollitt, K., Villeneuve, P. J., (2013): PM2.5, oxidant defence and cardiorespiratory health: a review. *Environ. Health* 12:40.

Wu, Q., Wang, W., Li, Y., Li, Y., Ye, B., Tang, M., Wang, D., (2012): Small sizes of TiO2-NPs exhibit adverse effects at predicted environmental relevant concentrations on nematodes in a modified chronic toxicity assay system. *J. Hazard. Mater.* 243, 161-168.

Yoshida, Y., Saito, Y., Jones, L. S., Shigeri, Y., (2007): Chemical reactivities and physical effects in comparison between tocopherols and tocotrienols: physiological significance and prospects as antioxidants. *J. Biosci. Bioeng.* 104, 439-445.

Xiao, G. G., Wang, M. Y., Li, N., Loo, J. A., Nel, A. E. (2003): Use of proteomics to demonstrate a hierarchical oxidative stress response to diesel exhaust particle chemicals in a macrophage cell line. *J. Biol. Chem.* 278, 50781-50790.

Zielinski, H., Mudway, I. S., Bérubé, K. A., Murphy, S., Richards, R., Kelly, F. J., (1999): Modeling the interactions of particulates with epithelial lining fluid antioxidants. *Am. J. Physiol.* 277, L719-L726.