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Genotoxicity evaluation of fullerene C_{60} nanoparticles in a comet assay using lung cells of intratracheally instilled rats

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ABSTRACT

The genotoxicity of fullerene C_{60} nanoparticles was evaluated *in vivo* with comet assays using the lung cells of rats given C_{60} nanoparticles. The C_{60} nanoparticles were intratracheally instilled as a single dose at 0.5 or 2.5 mg/kg or repeated dose at 0.1 or 0.5 mg/kg, once a week for 5 weeks, to male rats. The lungs were obtained 3 or 24 h after a single instillation and 3 h after repeated instillation. Inflammatory responses were observed in the lungs obtained 24 h after a single instillation at 2.5 mg/kg and repeated instillation at 0.5 mg/kg. Histopathological examinations revealed that C_{60} nanoparticles caused slight changes including hemorrhages in alveoli and the cellular infiltration of macrophages and neutrophils in alveoli. In comet assays using rat lung cells, no increase in % Tail DNA was found in any group given C_{60} nanoparticles. These findings indicate that C_{60} nanoparticles had no potential for DNA damage in comet assays using the lungs cells of rats given C_{60} even at doses causing inflammation.

1. Introduction

Nanomaterials are small-scale substances less than 100 nm in at least one dimension (ISO, 2008), which exhibit physical, chemical and/or biological characteristics associated with a nanostructure (Oberdörster et al., 2005a). Humans have been exposed to airborne nanoparticles throughout evolution, but exposure has increased dramatically because of anthropogenic factors including combustion engines, power plants, and other sources of thermodegradation (Oberdörster et al., 2005b). The rapidly developing field of nanotechnology, which is creating materials with size-dependent properties, is likely to become another source of exposure to nanosubstances. These nanosubstances have an increased surface area: mass ratio thereby greatly enhancing their chemical/catalytic reactivity compared to normal-sized forms of the same substance.

C_{60} fullerene is a remarkably stable compound consisting of 60 carbon atoms with a diameter of approximately 0.7 nm and a molecular weight of 720 g/mol, and 30 carbon double bounds are present in the structure, to which free radicals can easily be added (Aschberger et al., 2010). There are a variety of fullerene derivatives available, which stems from the number of carbon atoms used to generate fullerenes, diverse array of moieties that can be attached to the fullerene surface, and different processes utilized to render fullerenes water soluble (Johnston et al., 2010). Surface modifications are often used to make fullerenes dispersible in water, allowing their use in pharmaceutical applications or in cosmetics (Aschberger et al., 2010). Chemically-modified fullerenes are being developed for targeted drug delivery, molecular ball bearings acting as lubricants, and nanoscale chemical sponges (ENRHES, 2009). Widespread production and use have caused the release of increasing amounts of nanomaterials into the environment. Introduction of novel materials into industry requires safety evaluation as well as an understanding of the impact of the nanomaterials on human health, because the unique properties and size of nanomaterials may also result in unique health risks, which are not able to be predicted by the toxicological effects of larger substances of the same composition (Murray et al., 2009). Despite growing concern over the potential risk that nanomaterials pose, there is a lack of information on their potential toxicity. There is a knowledge gap between the increasing development and use of nanomaterials and the prediction of possible health risks. At present, knowledge on the toxicological effects of fullerenes is limited.

A key area governing the risk assessment of chemical substances for human health is genotoxicology due to the fact that classic genotoxic substances lead to carcinogenesis (Singh et al., 2009). Genotoxicity testing, and thus the evaluation of the carcinogenicity and mutagenicity of chemicals, is the most important

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part of the safety assessment of chemical compounds. Genotoxicity studies, including *in vitro* and *in vivo* assays, of fullerenes and their derivatives are available (Ema et al., 2011). Positive results were obtained from *in vitro* comet assays (Dhawan et al., 2006), gene mutation assays using bacteria (Sera et al., 1996) and transgenic mouse embryonic cells (Xu et al., 2009a), and micronucleus assays (Totsuka et al., 2009). Positive results were also reported on *in vivo* comet assays (Totsuka et al., 2009), oxidatively damaged DNA assays (Folkmann et al., 2009), and *gpt* gene mutation assays (Totsuka et al., 2009). Negative results were obtained using *in vitro* comet assays (Jacobsen et al., 2008), bacterial gene mutation assays (Babynin et al., 2002; Shinohara et al., 2009), and mammalian chromosomal aberration assays (Shinohara et al., 2009). Negative results were also reported on *in vivo* comet assays (Jacobsen et al., 2009) and micronucleus assays (Shinohara et al., 2009). As described, there are inconsistencies in the results of reports on the genotoxicity of fullerenes and their derivatives that make it very difficult to draw firm conclusions.

Two principle modes of genotoxic action can be considered for particles, referred to as primary and secondary genotoxicity (Greim et al., 2001; Schins, 2002; Schins and Knaapen, 2007). Primary genotoxicity is defined as genetic damage elicited by particles in the absence of inflammation, whereas secondary genotoxicity is a pathway of genetic damage resulting from oxidative DNA attack by reactive oxygen/nitrogen species (ROS/RNS), generated during particle-elicited inflammation (Greim et al., 2001; Schins, 2002; Schins and Knaapen, 2007). Clarification of the principle modes of genotoxic action is very important for risk assessment, because secondary genotoxicity is considered to involve a threshold. One of the major routes of exposure to fullerenes is inhalation, and the lungs area major target organ of fullerenes. Therefore, we conducted a genotoxicity study of C_{60} nanoparticles with the comet assay using the lung cells of rats intratracheally instilled with C_{60} nanoparticles, at doses that did and did not elicit pulmonary inflammation.

2. Materials and methods

The experiments were performed at the Biosafety Research Center, Foods, Drugs and Pesticides (BSRC, Iwata, Japan) in 2010–2011 according to Guidelines for Animal Experimentation (1987), the Law Concerning the Protection and Control of Animals (1973), and Standards Relating to the Care and Management of Experimental Animals (1980). The study was approved by the Institutional Animal Care and Use Committee of the BSRC and performed in accordance with the ethics criteria contained in the bylaws of the Committee of National Institute of Advanced Industrial Science and Technology (AIST).

2.1. Chemicals

Commercially available fullerene C_{60} nanoparticles (Nanom purple SU, refined by sublimation, purity >99.5%, Frontier Carbon Co., Ltd., Kitakyushu, Japan) were used throughout this study. The specific surface area of the purchased C_{60} before hand-grinding in agate mortar was 0.92 m^2/g, measured with the BET method (Brunauer et al., 1938). Ethyl methanesulfonate (EMS), Dulbecco's phosphate-buffered saline, regular melting agarose, and triton-X were obtained from Sigma–Aldrich Corporation (St. Louis, MO). Low melting agarose (Rockland, Inc., Troy, MI), ethylene diamine tetra acetic acid (EDTA) disodium salt (DOJINDO LABORATORIES, Kumamoto, Japan), Hanks' balanced salt solutions (Life Technologies Corporation, Carlsbad, CA), SYBR® Gold nucleic acid gel stain (Life Technologies Corporation), and TE buffer solution (pH 8.0) (Nippon Gene, Tokyo, Japan) were used. Polyoxyethylene sorbitan monooleate (Tween 80), dimethyl sulfoxide (DMSO), tris hydroxymethyl aminomethane, and sodium *N*-lauroyl sarcosinate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Particle preparation and characterization

Fullerene C_{60} nanoparticles were dispersed in distilled water containing 0.1% Tween 80. The preparation and characterization of the C_{60}nanoparticle suspension were reported by Morimoto et al. (2010). Briefly, bulk fullerene material was dispersed in distilled water containing 0.1% Tween 80 and milled in an agate mortar for 30 min. The milled fullerene material was suspended with 50-μm zirconium particles using a high-performance dispersion machine. The C_{60} nanoparticle suspension was separated by centrifugation at 8000*g* for 60 min. The concentration was determined by high-performance liquid chromatography. The mean diameter based on the volume and mass of fullerenes in the 0.1% Tween 80 aqueous solution was 33 nm.

2.3. Animals and treatment

MaleCrl:CD(SD) rats (7 weeks-old) were purchased from Charles River Laboratories, Japan, Inc. (Yokohama, Japan). Rats were maintained in an air-conditioned animal room at 20–26 °C with a relative humidity of 35–75%, a 12:12-h light/dark cycle, and ventilation with 12 air changes/h. Standard rodent pellet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan)and tap water were given *ad libitum*. After a 7-day acclimation, rats were subjected to treatment.

The C_{60} nanoparticles were intratracheally instilled at a dose of 0.5 or 2.5 mg/kg (single instillation groups), or 0.1 or 0.5 mg/kg once a week for 5 weeks (repeated instillation groups). In the single instillation groups, rats were sacrificed 3 or 24 h after the instillation. In the repeated instillation groups, they were sacrificed 3 h after the last instillation. At higher doses, eight rats were given C_{60} nanoparticles to secure five rats per group for comet assays. At lower doses, five rats per group for each time point were instilled. As a negative control, five rats were given Tween 80 at 1 mg/mL/kg by a single or repeated intratracheal instillation similar to the C_{60} nanoparticles. As a positive control, five rats were orally given a single dose of EMS at 500 mg/kg at 3 h before sacrifice. In five rats of each group, the left lobes of the lungs were used for histopathological examination and the right lobes were used for the comet assay.

Dosage levels were determined based on the results of a preliminary study in which male rats were given a single intratracheal instillation of C_{60} nanoparticles at 0.5 or 2.5 mg/kg. Rales were heard in one of the three rats at 0.5 and 2.5 mg/kg and one rat died immediately after the instillation at 2.5 mg/kg.

2.4. Histopathological examination

The left lobes of the lungs were fixed in 10% neutral buffered formalin for histopathological examination. Tissues were routinely processed, embedded in paraffin, sectioned at 4–6 μm, and stained with hematoxylin and eosin (HE).

2.5. Comet assay

The comet assay was conducted in accordance with the standard protocol "International Validation of the *In Vivo* Rodent Alkaline Comet Assay for the Detection of Genotoxic Carcinogens" issued by the Japanese Center for the Validation of Alternative Methods (JaCVAM). Briefly, the right lobes of the lungs were washed out with homogenizing buffer (Hanks' balanced salt solutions containing 25 mmol/L EDTA-2Na and 10% DMSO) and

homogenized in 5 mL of the homogenizing buffer using a Downs homogenizer. Cell suspensions were chilled on ice for about 5 min and centrifuged at 800 rpm for 5 min. After the supernatant was removed, the cells were re-suspended in homogenizing buffer. Ten microliters of the single cell suspension was mixed with 90 μL of 0.5% low-melting agarose gel, and 90 μL of the mixture was placed on a slide pre-coated with 1.0% agarose gel. Another 90 μL of low melting agarose was added. Two slides were prepared from each rat. The slides were transferred to lysing solution (2.5 mol/L NaCl, 100 mmol/L EDTA-2Na, 10 mmol/L, pH 10 Tris buffer, 10% DMSO and 1% Triton X-100) for at least one night at 4 °C in the dark. The slides were next covered with chilled electrophoresis buffer (pH >13) for 20 min to allow DNA to unwind. Electrophoresis was conducted at a constant voltage of 0.7 V/cm (25 V) (initial current: 300 mA) for 20 min. The slides were transferred into neutralization buffer and held for about 10 min. Subsequently, they were dehydrated with ethanol, and air-dried. The slides were stained with SYBR® Gold nucleic acid gel stain which was diluted 5000-fold with TE buffer solution. Images of DNA migration were examined using a fluorescence microscope (Olympus Corporation, Tokyo, Japan). The final magnification was 200×. The images were analyzed using a Comet assay analyzer (Comet Assay IV system, Perceptive Instruments Ltd., Suffolk, UK). The comet parameter to measure DNA damage in the cells was the percentage of DNA in the tail (% Tail DNA), because% Tail DNA could be considered meaningful and easy to conceptualize (Kumaravel and Jha, 2006). Images of 100 (50 × 2) cells per rat were analyzed. The mean of the % Tail DNA value (mean value of 100 cells) of each group was calculated.

2.6. Statistical analysis

Data for C_{60} nanoparticle-treated groups and negative and positive control groups were analyzed using Dunnett's multiple comparison tests. Data for the positive control was compared to that for the negative control with Aspin–Welch's *t* test.

3. Results

3.1. Single instillation (autopsy at 3 h after instillation)

No changes were observed in clinical signs and body weights of rats given C_{60} nanoparticles at 0.5 and 2.5 mg/kg. At autopsy, brown-patches on the lungs were found in three rats at 0.5 mg/kg and in all rats at 2.5 mg/kg, whereas no brown-patches were noted in the rats given Tween 80 or EMS.

Images of histopathological changes in the lungs are presented in Fig. 1. The degree of all these changes was slight. The histopathological examinations revealed the focal accumulation of macrophages in one rat of the Tween 80-treated control group, the focal accumulation of macrophages in one rat at 0.5 mg/kg, and hemorrhage in one rat at 2.5 mg/kg.

The results of comet assays using the lung cells of rats given C_{60} nanoparticles are shown in Table 1. The average value of percent Tail DNA in the lung cells was 2.42 in the Tween 60-treated control group, and 2.09 and 3.07 in the group instilled with C_{60} nanoparticles at 0.5 and 2.5 mg/kg, respectively. There was no significant difference in % Tail DNA between the Tween 80-treated control and C_{60} nanoparticle-treated groups. The average % Tail DNA value was 16.98 in the EMS-treated positive control group, significantly higher than that in the Tween 80-treated control group.

3.2. Single instillation (autopsy at 24 h after instillation)

There was no change in clinical signs and body weights of rats given C_{60} nanoparticles at 0.5 and 2.5 mg/kg. At autopsy, a single brown-patch was noted in the lungs of one rat given Tween 80. A single brown-patch on the lungs was observed in two rats at 0.5 mg/kg, and multiple brown-patches on the lungs were found in all rats at 2.5 mg/kg.

Images of histopathological changes in the lungs are shown in Fig. 1. The degree of all these changes was slight. The focal accumulation of macrophages and hemorrhage in the alveoli in one rat

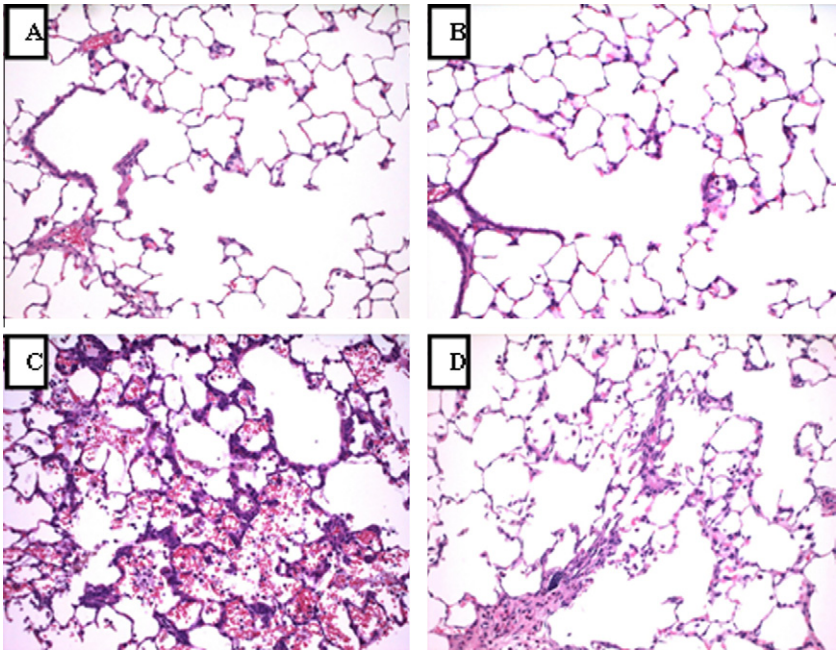

Fig. 1. HE staining of lung histopathology sections (magnification, 20×). (A) negative control: no significant lesions, (B) a single intratracheal instillation at 2.5 mg/kg autopsied 3 after the instillation): no significant lesions, (C) a single intratracheal instillation at 2.5 mg/kg (autopsied 24 h after the instillation): hemorrhage, infiltration of alveolar macrophages and neutrophils in the alveolus, thickening of the alveolar wall, and deposition of the test substances in alveolar macrophages, (D) repeated intratracheal instillation for 5 weeks at 0.5 mg/kg/week (autopsied 3 h after the last instillation): hemorrhage, infiltration of alveolar macrophages, and deposition of the test substances in alveolar macroophages.

Table 1
Effects of C_{60} nanoparticles on % Tail DNA in lung cells following a single or repeated intratracheal instillation in rats.

Treatments	Groups (compounds)	No. of rats	No. of cells analyzed/rat	% Tail DNA[a]
A single intratracheal instillation (autopsy at 3 h after instillation)	Negative control (Tween 80)[b]	5	100	2.42 ± 0.76
	C_{60} (0.5 mg/kg)	5	100	2.09 ± 0.62
	C_{60} (2.5 mg/kg)	5	100	3.07 ± 0.93
	Positive control (EMS)[c]	5	100	16.98 ± 7.08*
A single intratracheal instillation (autopsy at 24 h after instillation)	Negative control (Tween 80)[b]	5	100	3.13 ± 0.76
	C_{60} (0.5 mg/kg)	5	100	2.53 ± 0.62
	C_{60} (2.5 mg/kg)	5	100	3.07 ± 0.93
Repeated intratracheal instillation (autopsy at 3 h after instillation)	Negative control (Tween 80)[b]	5	100	4.65 ± 1.56
	C_{60} (0.1 mg/kg)	5	100	6.80 ± 1.76
	C_{60} (0.5 mg/kg)	5	100	5.08 ± 0.83
	Positive control (EMS)[c]	5	100	16.66 ± 1.94*

[a] Values are given as the mean ± S.D.
[b] Tween 80 was intratracheally instilled at 1 mg/mL/kg.
[c] EMS (ethyl methanesulfinate) was orally administered at 10 mL/kg.
* Significantly different from the negative control group ($p < 0.05$).

each were noted in the Tween 80-treated control group. The focal accumulation of macrophages in the alveoli in two rats was observed at 0.5 mg/kg. Multifocal hemorrhages in the alveoli in four rats, deposition of the test substances in macrophages of the alveoli and cellular infiltration of neutrophils and macrophages in the alveoli in five rats, thickening of the alveolar wall in two rats, and acute pneumonia with focal deposition of the hematoidin crystals in one rat were found at 2.5 mg/kg.

The results of comet assays using the lung cells of rats given C_{60} nanoparticles are summarized in Table 1. The average % Tail DNA value was 3.13 in the Tween 80-treated control group, and 2.53 and 3.07 in the group given C_{60} nanoparticles at 0.5 and 2.5 mg/kg, respectively. There was no significant difference in % Tail DNA between the Tween 80-treated control and C_{60} nanoparticle-treated groups.

3.3. Repeated instillation (autopsy at 3 h after instillation)

No difference was found in clinical signs and body weights between the Tween 80-treated control group and groups given C_{60} nanoparticles at 0.1 and 0.5 mg/kg. At autopsy, no brown-patches were found in the lungs of rats given C_{60} nanoparticles at 0.1 mg/kg or Tween 80. Multiple brown-patches on the lungs were observed in all rats given C_{60} at 0.5 mg/kg.

Images of histopathological changes in the lungs are shown in Fig. 1. The degree of all these changes was slight. No histopathological changes in the lungs were noted in rats of the Tween 80-teated control group. Acute focal pneumonia in two rats and focal hemorrhages in the alveoli in one rat were observed at 0.1 mg/kg. The focal accumulation of macrophages and hemorrhage in the alveoli in one rat each and focal or multifocal deposition of the test substances in the macrophages in the alveoli and cellular infiltration of the macrophages in the alveoli in five rats were found at 2.5 mg/kg.

The results of comet assays of the lung cells are also summarized in Table 1. The average value for % Tail DNA was 4.65 in the Tween 80-treated control group, and 6.80 and 5.08 in the group instilled with C_{60} nanoparticles at 0.5 and 2.5 mg/kg, respectively. There was no significant difference in % Tail DNA between the Tween 80-treated control and C_{60} nanoparticle-treated groups. The average value was 16.66 in the EMS-treated positive control group, significantly higher than in the C_{60}-treated groups.

4. Discussion

In this study, we determined the genotoxicity of fullerene C_{60} nanoparticles in comet assays using the lung cells of rats given C_{60} nanoparticles by intratracheal instillation. Inflammatory changes in the lungs were found 24 h after a single instillation and 3 h after the repeated instillation of C_{60} nanoparticles at high dose. In the lungs obtained 3 h after a single instillation at both doses and 24 h after a single instillation at low dose, and after the repeated instillation at low dose, focal hemorrhage and the accumulation of macrophages in the alveolus and pneumonia were observed. These changes are not thought to be inflammatory responses due to the instillation of C_{60} nanoparticles because they were very slight, also found in the Tween80-treated control groups, and commonly observed in the background control data. These findings indicate that a single instillation at 2.5 mg/kg and repeated instillation at 0.5 mg/kg caused pulmonary inflammation in rats. The most important finding of the present study is that C_{60} nanoparticles did not produce DNA damage in the lungs of rats given C_{60} even at doses causing inflammatory changes. This evidence indicates that C_{60} nanoparticles have no potential for genotoxicity *in vivo*.

Several genotoxicity studies of fullerenes and their derivatives have been performed using lung cells. *In vitro* chromosomal aberration assays were conducted using Chinese hamster lung cells and negative results were reported for C_{60} (Shinohara et al., 2009), a mixture of C_{60} and C_{70} (Mori et al., 2006), and a mixture of water-soluble polyvynylpyrroidone (PVP)-enwrapped C_{60} and C_{70} (Aoshima et al., 2010). In studies *in vitro*, positive results for pure C_{60} were obtained in micronucleus assays using human lung cancer cells (A549) (Totsuka et al., 2009) and comet assays using FE1-MML epithelial cells established from the lungs of a transgenic mouse (Jacobsen et al., 2008). The level of 8-oxoG was increased in the lung tissue of female Fischer rats after a single intragastric administration of pure C_{60} nanoparticles at 0.64 mg/kg (Folkmann et al., 2009). Pure C_{60} increased the frequency of *gtp* mutations and inflammatory changes in the lung tissue of male transgenic *gtp* delta mice intratracheally instilled a single or multiple dose of C_{60} at 0.2 mg/mouse (Totsuka et al., 2009). They also noted that DNA tail moment was increased in the lungs of male C57BL/6J mice given a single intratracheal instillation of C_{60} at 0.2 mg/mouse, but not at 0.05 mg/mouse (Totsuka et al., 2009). As described by the authors, the doses of C_{60} nanoparticles (approximately equivalent to 1.5 and 6 mg/kg) were extremely high compared with human exposure in the workplace. Discrepancy in the findings between this study and our study could be explained by the difference in endpoint, animal strain, and dose of C_{60} used in these experiments. Meanwhile, Jacobsen et al. (2009) reported that pure C_{60} did not significantly increase the values of % Tail DNA in broncho-alveolar lavage (BAL) cells of female apolipoprotein E knockout mice ($ApoE^{-/-}$), a model that may closer resemble humans with elevated cholesterol levels, given a single dose of C_{60} by intratracheal instillation at 0.054 mg/mouse. In these mice, inflammatory

markers, such as mRNA levels of macrophage inflammatory protein-2 (*Mip-2*), interleukin-6 (*Il-6*), and macrophage/monocyte chemoattractant protein-1 (*Mcp-1*), in lung tissue were elevated (Jacobsen et al., 2009). Although comparing the results between previous studies and the present study is difficult because of differences in experimental conditions, including test substances, endpoints, cells, animal species or strain, and doses, the findings of Jacobsen et al. (2009) may support the present finding that C_{60} did not cause DNA damage in the lungs even at doses generating pulmonary inflammation.

A wide range of nanomaterials have been shown to create ROS both *in vitro* and *in vivo* (Allion et al., 2009; Azad et al., 2009; Møller et al., 2010). Although current understanding of fullerene toxicity must recognized that limitation in some initial techniques have led to unintentional erroneous reports of nC_{60} ROS generation and toxicity (Henry et al., 2011), the main molecular mechanism of nanotoxicity is considered to be the induction of oxidative stress resulting from the generation of ROS. Fullerenes and their derivatives generated ROS in various experimental conditions (Sera et al., 1996; Kamat et al., 2000; Sayes et al., 2004, 2005), but their potential for the generation of ROS is weak and lower than that of silica, titanium dioxide, and single-walled carbon nanotubes (Jacobsen et al., 2008; Folkmann et al., 2009; Møller et al., 2010). The generation of ROS in turn provokes inflammatory responses (Azad et al., 2009; Nielsen et al., 2008).

As for pulmonary inflammation due to C_{60} and its derivatives, Sayes et al. (2007) noted that C_{60} nanoparticles and a water-soluble derivative, $C_{60}(OH)_{24}$, caused little or no toxic effects in male Crl:(SD)IGS BR rats given a single intratracheal instillation at 0.2–3.0 mg/kg. Minimum toxicity was observed in the histopathology of the lungs, hematology and serum chemistry, and BAL fluid (BALF) parameters in male Fischer rats that inhaled C_{60} nanoparticles at 2.35 mg/m^3 for 3 h a day, for 10 consecutive days using a nose-only exposure (Baker et al., 2008). Examinations of lung histopathology and BALF parameters revealed that no strong potential effects of fullerenes for the development of neutrophil inflammation were induced in male Wistar rats given well-dispersed C_{60} by a single intratracheal instilation at 0.1 to 1 mg/rat or inhalation for 6 h a day for 5 days a week (Morimoto et al., 2010). Park et al. (2010) reported that C_{60} nanoparticles caused inflammatory responses including increased levels of inflammatory cytokines in BALF and cell infiltration in lung tissue of male ICR mice after a single intratrachel instillation at 0.5–2.0 mg/kg. These inflammatory responses are thought to be slight because the degree of histopathological change was very slight to slight. However, moderate to severe inflammatory changes in the lungs were noted in male SD rats exposed for three days by intratacheally instillation to water-soluble polyhydroxylated fullerenes [$C_{60}(OH)_XX$ = 22, 24] at 5 or 10 mg/rat, but not at 1 mg/rat (Xu et al., 2009b). These doses were extremely high, approximately equivalent to 25–50 mg/kg. These observations indicate that C_{60} nanoparticles, except for high doses of water-soluble fullerene derivatives, have no or very weak potential to cause inflammation. Consideration of these findings suggests that the lack of DNA damage is, at least in part, attributable to the low potential of C_{60} nanoparticles for the generation of ROS and development of inflammatory responses.

In conclusion, the present findings showed that C_{60} nanoparticles did not induce DNA damage in the lung cells of rats intratracheally instilled with C_{60} nanoparticles even at doses that elicited inflammatory responses. These findings suggest thatC_{60} nanoparticles have no potential for genotoxicity *in vivo*.

Conflict of interest statement

The authors declare there are no conflicts of interest.

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