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Assessment of pulmonary toxicity of gold nanorods following intra-tracheal instillation in rats

Harikiran Lingabathula, Narsimhareddy Yellu*

Department of Pharmacology and Toxicology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, 506009, India

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ABSTRACT

The present investigation was aimed to evaluate the pulmonary toxicity of 10 and 25 nm gold nanorods (GNRs) following intra-tracheal instillation in rats using bronchoalveolar lavage (BAL) fluid and lung histopathological analysis. The GNRs displayed that the dose-dependent toxicity via elevated lactate dehydrogenase leakage, alkaline phosphatase, lipid peroxidation and total microprotein levels in BAL fluids after 1 day, 1 week and 1 month post exposure periods. All the parameters were returned to normal values after 3 months post exposure period may be due to recovery. The rat lung histopathology displayed that accumulation of macrophages, inflammatory response and tissue thickening for both sizes of GNRs. 10 nm GNRs increased all BAL fluid parameters significantly following 1 day, 1 week and 1 month post exposure periods whereas 25 nm GNRs have shown similar effects but less extent. These investigations proposed that the dose and size dependent pulmonary toxicity of GNRs.

1. Introduction

Nanoparticles (NPs), especially gold nanoparticles (GNPs) hold great promises for future applications. GNPs are particularly exploited in organisms because of their biocompatibility (Bhattacharya and Mukherjee, 2008). Recent investigations have centered on functionalizing the GNPs as phothermal agents for hyperthermically killing pathogens (Norman, 2008; Simon, 2008). The effectiveness of the antibacterial activity of GNPs can be enhanced by adding antibiotics (Burygin, 2009). GNPs generate holes in the cell wall, resulting in the cell contents leakage and cell death. The cell death is also possible that GNPs bind to the DNA of bacteria and inhibit the uncoiling and transcription of DNA (Rai et al., 2010). The GNPs can be used to coat a wide variety of surfaces for instance, fabrics for treatment of wounds, implants and surfaces of glass to maintain hygienic conditions in the home and hospitals (Das, 2009).

GNPs can be manufactured into a variety of shapes including gold nanospheres, nanoprisms, nanobelts, nanorods, nanocages and nanostars. The chemical, optical and electromagnetic properties of GNPs are powerfully influenced by their size and shape (Yang and Cui, 2008). Gold nanorods (GNRs) are having 20 times more optical absorption efficiencies than the GNPs of the same volume (Copland et al., 2004). The scattering coefficients of GNRs are also an order of magnitude higher when compared to those of gold nanoshells and nanospheres (Jain et al., 2006). GNRs of high aspect ratio tender higher fluorescence intensity and this property will encourage the development of techniques using GNRs in fluorescent probe micro array assays and optical biosensor applications (Li et al., 2005). Additionally, GNRs have a strong binding affinity to thiol groups permitting them to be competently conjugated with several biomolecules (Liao and Hafner, 2005).

Because of these enhanced properties of the rod shaped GNPs, the GNRs are finding various industrial and commercial applications. GNRs are useful materials for photothermal therapy, drug delivery, sensing and biomedical imaging due to unique surface plasmon resonance ranging from visible to near infrared (NIR) region, facile synthesis and easy functionalization (Stone et al., 2011; ZhiYa et al., 2013). GNRs could be used as optical sensors for Raman-based intracellular biosensing useful for cancer diagnosis and other diagnostic biomedical applications (Oyelere et al., 2007). GNRs conjugated with photo sensitizers can kill Methicillin-resistant *Staphylococcus aureus* by NIR photothermal radiation (Kuo, 2009; Pissuwan et al., 2009).

In spite of the huge applications of GNRs, the occupational exposure for these nanorods also increased enormously. Recently, some of the studies were investigated the toxicities of GNRs using different human cells and reported in vitro cytotoxicity induced by GNRs (Harikiran and Narsimhareddy, 2016; Harikiran et al., 2015; Ying et al., 2015), but there is lack of availability of pulmonary toxicity effects of GNRs in vivo. The present study was aimed to assess the potential pulmonary toxicity of rod shaped, poly ethyleneglycol (PEG) coated, 10 and 25 nm GNRs following intra-tracheal instillation in rats.

* Corresponding author.
E-mail addresses: ynrucpsc@gmail.com, ynrkuc@gmail.com (N. Yellu).

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2. Materials and methods

2.1. Chemicals

Quartz particles (QTZ; 58–68 μm) of 99.95% purity were obtained from Berkely Springs, Morgan County, WV. The phosphate buffer saline and PEG were acquired from Himedia, Mumbai, India. All the biochemical assay kits were obtained from Raybiotech, New Delhi, India. The rest of the chemicals were purchased from Himedia, Mumbai, India.

2.2. Particle types

The 10 nm GNRs (GNR 10) and 25 nm GNRs (GNR 25) were procured from Sigma-Aldrich, St. Louis, MO. The GNRs were prepared as suspensions in phosphate buffer saline (PBS) with 1% PEG as stabilizer and were first ultrasonicated at 25 °C to achieve optimal dispersion. The average particle size and shape of the GNRs suspensions were determined using transmission electron microscopy (TEM; TECNAI 20, Philips, USA). At least 250 particles were used to determine the average size of nanorods.

2.3. Animals

The six weeks old male wistar rats were procured from National Institute of Nutrition, Hyderabad, India. The rats were kept one week for acclimatization before starting the experiments. The rats were housed in polypropylene cages in a room; water and feed were available ad libitum with controlled temperature (25 ± 2 °C), humidity (55 ± 5%) and a 12 h light/dark cycle during the acclimatization and experimental periods. The animals, weighed approximately 200–220 g were picked and randomly divided into different groups.

2.4. Experimental design

The test GNRs were prepared as suspensions by using a nontoxic dispersion vehicle (Warheit et al., 2005). The test GNRs and QTZ suspensions were prepared in PBS with 1% PEG by briefly shearing and subsequently sonicating (Soltec, Italy) the GNRs samples. The rats were divided into different groups (n = 6) and instilled with a single dose of 1 mg/kg and 5 mg/kg b.w. GNRs, QTZ and control (PBS + 1% PEG) by intra-tracheal instillation method, as this method of exposure was a reliable qualitative screen for estimating the pulmonary toxicity of inhaled particles (Yasuo et al., 2016). The different groups were Group 1: Control, Group 2: 1 mg/kg GNR 10, Group 3: 5 mg/kg GNR 10, Group 4: 1 mg/kg GNR 25, Group 5: 5 mg/kg GNR 25, Group 6: 1 mg/kg QTZ and Group 7: 5 mg/kg QTZ. The experiment was approved by the Institutional Animal Ethics Committee, UCPSC, Kakatiya University (File No. IAEC/05/UCPSC/KU/2016).

2.5. Intra-tracheal instillation

The rats were anesthetized with isoflurane in a small chamber and individual rats were secured on an inclined plastic platform and anesthetization continued via a small nose cone. A small hole was made in the trachea close to the larynx. A 24 gauge plastic catheter was inserted through the hole to the distal end of the trachea and the blunted needle was then inserted inside the plastic catheter. A 1 mL syringe already filled with 200 μL of air and 50 μL of PBS was then connected to the free end of silicone tubing to rapidly propel the test sample from the tubing and needle into the rat lungs. The incision was then sutured, swabbed with povidone iodine and anesthetized with a drop of lidocaine. The rats recovered and were active within 10–15 min after removal of the inhalation anesthetic. The incision was healed within 3–4 days and the rats were observed daily until their scheduled termination (Lam et al., 2004).

2.6. Collection of bronchoalveolar lavage fluid

The BAL fluid (15–20 mL) was collected from all the above control and test nanorod exposed rats at 1 day, 1 week, 1 month and 3 months post instillation periods (Reddy et al., 2012). The lungs of control, QTZ and test nanorod exposed rats were lavaged with a warmed PBS solution as per the procedure already depicted previously (Warheit et al., 2007). Briefly, the lungs were removed from the thoracic cavity and lavaged with a PBS solution that had been heated to 37 °C. A 10 mL syringe was used to fill the lungs with 8 mL of PBS per wash. The rat lungs were gently manipulated after incorporation of the PBS and during the withdrawal of BAL fluid. The first recovered 12 mL of lavaged fluids was used for BAL fluid analysis.

2.7. Biochemical analysis

All biochemical assays were performed on BAL fluids of 1 day, 1 week, 1 month and 3 months post instillation periods in rats for the estimation of lactate dehydrogenase (LDH), alkaline phosphatase (ALP), extent of lipid peroxidation and total microprotein (MTP) using respective diagnostic kit methods as per the manufacturer's protocol.

2.8. Lactate dehydrogenase leakage assay

LDH leakage into the BAL fluid can be used as a sign of cell membrane integrity and therefore used as a measurement of cytotoxicity (Ran et al., 2009). This assay measures cell death in response to environmental pollutants or chemical compounds using a coupled two step reaction. In the first step, LDH catalyzes the reduction of NAD^+ to produce NADH and H^+ by oxidation of lactate to pyruvate. In the second step, diaphorase uses the newly produced NADH and H^+ to catalyze the reduction of a tetrazolium salt to colored formazan which absorbs strongly at 490 nm. The quantity of formazan generated is proportional to the amount of LDH leaked into BAL fluid as a result of cytotoxicity.

2.9. Alkaline phosphatase assay

ALP activity measured to determine the Type II alveolar epithelial cell secretory activity and increased ALP levels in BAL fluids is considered to be an indicator of Type II cell toxicity (Ran et al., 2009). At pH 10.3, ALP catalyzes the hydrolysis of colourless *p*-nitrophenylphosphate (*p*NPP) to yellow coloured *p*-nitrophenol and phosphate. Change in absorbance due to yellow colour formation is measured kinetically at 405 nm and is proportional to ALP activity in the sample.

2.10. Lipid peroxidation assay

Malondialdehyde (MDA) is a naturally occurring by product of lipid peroxidation. The MDA content was estimated in the form of thiobarbituric acid reactive substances (TBARS) and it is a well-established method for screening and monitoring of lipid peroxidation (Yagi, 1998). The MDA-TBA adducts produced by the reaction of MDA and TBA under high temperature (90–100 °C) and acidic conditions is measured colorimetrically at 530–540 nm.

2.11. Total microprotein assay

Raises in BAL fluid protein concentrations generally were consistent with increased permeability of vascular proteins into alveolar regions, indicating a collapse in the integrity of the alveolar capillary barrier (Warheit et al., 2007). This protein determination assay is a microplate based colorimetric method for rapid total protein quantification. It is based on the well-known Bradford method (Bradford, 1976) and it takes advantage of the colour change of dye in acidic medium when it