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REVIEW ARTICLE

A Complexity Focus on Nanotoxicology- A Review

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ABSTRACT:
This article will discuss some of the general considerations on the complexity of Nanotoxicology (NT). The real scope of Nanotoxicology involves many processes. Nanotoxicology is an emerging discipline evolving from studies of ultrafine particles called the nano-particles (NP). The small size facilitates uptake into cells and transcytosis across epithelial and endothelial cells into the blood and lymph circulation to reach potentially sensitive target sites such as bone marrow, lymph nodes, spleen, and heart. Access to the central nervous system (CNS) and ganglia via translocation along axons and dendrites of neurons, evidences of mitochondrial distribution and oxidative stress response after NSP endocytosis point to a need for basic research on their interactions with subcellular structures.

KEYWORDS: Nanotoxicology (NT), Nano particles (NP), Nano sized particles (NSP), Central nervous system (CNS).

INTRODUCTION:
Nanomaterials belong to a field that takes a materials science-based approach to nanotechnology. The study materials are with morphological features on the nanoscale, and especially those that have special properties stemming from their nanoscale dimensions. Nanoscale is usually defined as smaller than a one tenth of a micrometer in at least one dimension, though this term is sometimes also used for materials smaller than one micrometer.

Types of Nanomaterials:
Most current nanomaterials can be organized into four types:
1. Carbon Based Materials
2. Metal Based Materials
3. Dendrimers
4. Composites

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Carbon Based Materials:
These nanomaterials are composed mostly of carbon, most commonly taking the form of a hollow spheres, ellipsoids, or tubes. Spherical and ellipsoidal carbon nanomaterials are referred to as Fullerenes, while cylindrical ones are called Nanotubes. These particles have many potential applications, including improved films and coatings, stronger and lighter materials, and applications in electronics.

Metal Based Materials:
These nanomaterials include quantum dots, nanogold, nanosilver and metal oxides, such as titanium dioxide. A quantum dot is a closely packed semiconductor crystal comprised of hundreds or thousands of atoms, and whose size is on the order of a few nanometers to a few hundred nanometers. Changing the size of quantum dots changes their optical properties.

Dendrimers:
These nanomaterials are nanosized polymers built from branched units. The surface of a dendrimer has numerous chain ends, which can be tailored to perform specific chemical functions. This property could also be useful for catalysis. Also, because three-dimensional

dendrimers contain interior cavities into which other molecules could be placed, they may be useful for drug delivery.

Composites:
Composites combine nanoparticles with other nanoparticles or with larger, bulk-type materials. Nanoparticles, such as nanosized clays, are already being added to products ranging from auto parts to packaging materials, to enhance mechanical, thermal, barrier, and flame-retardant properties [(1).]

Nanotoxicology is the study of the toxicity of nanomaterials. Because of quantum size effects and large surface area to volume ratio, nanomaterials have unique properties compared with their larger counterparts. Nanotoxicology is a branch of bio-nanoscience which deals with the study and application of toxicity of nano-materials [(2)]. A nanomaterial is defined as a substance with at least one dimension <100 nm in size and they can take many different forms such as tubes, rods, wires or spheres, with more elaborate structures devised, such as nano-onions and nano-peapods [(3)]. Nano-carrier systems can also be referred to as nanosized materials, or nanosized particles. Even the degree of coating can make a difference in the distance that nanoparticles can reach. When free nanoparticles were injected into the extracellular matrix they remained in the site of injection, whereas polyethylene glycol (PEG)-coated particles try to reach the lymphatic vessels and eventually the circulatory system [(4)].

Nanomaterials, even when made of inert elements like gold, become highly active at nanometer dimensions. Nanotoxicological studies are intended to determine whether and to what extent these properties may pose a threat to the environment and to human beings. For instance, Diesel nanoparticles have been found to damage the cardiovascular system in a mouse model [(5)].

Nanotoxicology is a sub-specialty of particle toxicology. It addresses the toxicology of nanoparticles (particles <100 nm diameter) which appear to have toxicity effects that are unusual and not seen with larger particles. Nanoparticles can be divided into combustion-derived nanoparticles (like diesel soot), manufactured nanoparticles like carbon nanotubes and naturally occurring nanoparticles from volcanic eruptions, atmospheric chemistry etc. Typical nanoparticles that have been studied are titanium dioxide, alumina, zinc oxide, carbon black, and carbon nanotubes, and "nano-C_{60}". Nanoparticles have much larger surface area to unit mass ratios which in some cases may lead to greater pro-inflammatory effects (in, for example, lung tissue). In addition, some nanoparticles seem to be able to translocate from their site of deposition to distant sites such as the blood and the brain. This has resulted in a sea of change in how particle toxicology is viewed- instead of being confined to the lungs; nanoparticle toxicologists study the brain, blood, liver, skin and gut. Nanotoxicology has revolutionized particle toxicology and rejuvenated it.

Nanotoxicology refers to the biokinetic evaluation of engineered nanostructures and nanodevices. Nanoparticles enter cells via endocytotic processes including clathrin-mediated endocytosis, potocytosis, pinocytosis, and patocytosis[(6)]. Smaller size of nanoparticles in addition to their physico-chemical properties may be responsible for adverse biological effects. Among particles of different sizes, it has also been established that ultrafine particles (UFP), which have an aerodynamic size of <100 nm, are potentially most dangerous due to their small size, large surface area, deep penetration and ability to be retained in the lung, and high content of redox-cycling organic chemicals. The main difference between nano particles and UPF is that nanoparticles are anthropogenic and often purposely-engineered materials, whereas ultrafine particles encompass both natural and anthropogenic particles that are not produced in a controlled manner.

A correlation between the size of particles and general health effects has been proposed to exist. Ultrafine particles are described to be more toxic than larger particles with the same chemical make-up due to their large surface area, causing cytotoxicity, allergic response or inflammation. Dosing with both natural and anthropogenic nano-sized particles, in *in-vitro* studies showed pro-inflammatory and oxidative stress related cellular response. Carbon nanotubes (CNTs) have distinct pulmonary effects as compared to carbon black and graphite, which are larger structures of similar chemical make-up (SWCNT's & MWCNT's).

Nanomaterials may have different chemical, optical, magnetic, and structural properties; consequently, differential toxicity profiles. Particle toxicology and the consequent adverse health effects of asbestos fibers and coal dust, serve as a historical reference points to the development of nanotoxicological concepts. CNTs were compared to that of carbon black after intra-tracheal instillation in mice, CNTs proved to be significantly more harmful. Nano-copper was also reported to cause pathological damage to the liver, the kidney, and the spleen. Chronic administration of 70nm- silica nanoparticles caused liver and spleen toxicity.

Due to their small size and high surface area, coupled to other physico-chemical features such as metal contaminants and charged surfaces, nanomaterials may

well have unpredictable genotoxic properties.

Ambit of Nanotoxicity:

Since there is no authority to regulate nanotech-based products, there are many products that could possibly be dangerous to humans. Scientific research has indicated the potential for some nanomaterials to be toxic to humans or the environment. In March 2004 tests conducted by environmental toxicologist Eva Oberdörster, Ph.D. working with Southern Methodist University in Texas, found extensive brain damage to fish exposed to fullerenes for a period of just 48 hours at a relatively moderate dose of 0.5 parts per million (commensurate with levels of other kinds of pollution found in bays). The fish also exhibited changed gene markers in their livers, indicating their entire physiology was affected. In a concurrent test, the fullerenes killed water fleas, an important link in the marine food chain. The extremely small size of fabricated nanomaterials also means that they are much more readily taken up by living tissue than presently known toxins.

Immunogenicity of nanoparticles:

Very little attention has been directed towards the potential immunogenicity of nanostructures. Nanostructures can activate the immune system inducing inflammation, immune responses, allergy, or even affect to the immune cells in a deleterious or beneficial way (immunosuppression in autoimmune diseases, improving immune responses in vaccines). More studies are needed in order to know the potential deleterious or beneficial effects of nanostructures in the immune system. In Comparison to conventional pharmaceutical agents, nanostructures have very large sizes and immune cells, especially phagocytic cells, recognize and try to destroy them.

In the area of medicine, the field of nano-medicine is defined as the monitoring, repair, construction, and control of human biological systems at the molecular level, using engineered nanodevices and nanostructures.

Nanoparticles have biological and medical applications. Iron oxide crystals when injected intravenously, lymph nodes bearing tumors appear dark due to iron oxide crystals accumulated in macrophages, compared to surrounding normal tissues as detected by magnetic resonance imaging. A synthetic analog of integrin αvβ3 has been used to target cationic nanoparticles carrying therapeutic genes to endothelial cells associated with tumors, working as agents to the vasculature of solid tumors [(7)]. After absorption across the lung epithelium nanoparticles can enter the blood and lymph to reach cells in the bone marrow, lymph nodes, spleen, and heart. Nanoparticles can even reach the central nervous system and ganglia following translocation [(8)].

Mechanism:

Due to their small size and high surface area, coupled to other physico-chemical features such as metal contaminants and charged surfaces, nano-materials may well have unpredictable genotoxic properties. They may cause DNA damage indirectly, by promoting oxidative stress and inflammatory responses. Alternatively, if small enough, they may pass through cellular membranes and gain access to the nucleus where they may interact directly with DNA, causing damage. Additionally, if nano-materials were able to accumulate within a cell but not necessarily gain access to the nucleus, they may still come into direct contact with DNA during mitosis when the nuclear membrane breaks down, providing ample opportunity for DNA aberrations to arise. A common mechanism thought to be responsible for the genotoxic effects exerted by nanoparticles involves oxidative stress, which refers to a redox imbalance within cells, usually as a result of intracellular Reactive Oxygen Species (ROS) and decreased antioxidants. When DNA is damaged, a key effector molecule that is activated is p53. If the damage present is extensive, the p53 triggers apoptosis in order to eliminate the individual cell for the benefit if the organism. The toxico-kinetics of nano-materials is still not well understood, but it is becoming increasingly evident that their physicochemical features play a central role in governing their cellular uptake and subsequent physiological consequences.

Commonly used Genotoxicity Assays:

Ames test:

The Ames test, first described in 1972 is used to assess the mutagenic potential of test substances. It uses several strains of the bacteria *Salmonella typhimurium* each of which carries different mutations in various genes, rendering them unable to synthesize the amino acid histidine, thus they require supplemented histidine as a growth supplement. The bacteria are therefore cultured in the presence of the test compound on agar plates lacking histidine and only bacteria that have undergone reverse mutations resulting in the histidine synthesis genes regaining their function (his^+) will survive to grow into colonies. The frequency of colonies formed is proportional to the mutation frequency induced by the test agent at a given dose [(9)].

Chromosome aberration test:

This assay characterizes gross structural and numerical chromosomal alterations induced by the test agent and can be performed on both *in vitro* and *in vivo* basis. In the in vitro chromosome aberration test, cultured mammalian cells are treated with the test material and then exposed to a chemical that arrests the cell cycle at

metaphase, the stage immediately before the replicated chromosomes are separated into two daughter nuclei. The *in vivo* version of the assay involves the treatment of rodents with the test material followed by the metaphase-arresting chemical. Metaphase chromosome preparations are made from harvested bone marrow cells and then they are stained and scored as described for the *in vitro* assay.

Comet assay:

The comet assay, also known as the single-cell gel electrophoresis (SCGE) assay is a versatile, sensitive and rapid method for measuring DNA single- and double-strand breaks at the level of individual cells. The technique can also be adapted for the quantification of alkali-labile sites, oxidative base damage, DNA–DNA or DNA–protein cross-linking and a-basic sites. Individual cells encapsulated in a thin layer of low melting point agarose gel on a microscope slide are lysed and the DNA is electrophoresed. Under the electric charge, intact DNA moves minimally due to its large size, but if present, small DNA fragments are able to migrate much further resulting in a comet shape with an extended tail drawn out towards the anode (containing the damaged DNA). The DNA is detected following staining with ethidium bromide or propidium iodide. Analysis of the length and fluorescence intensity of the comet tail is directly proportional to the amount of DNA damage [10, 11].

Cytokinines blocked micronucleus assay:

This is a rapid and sensitive method for the quantification and classification of chromosomal damage. Cells that have undergone cell division in the presence of a test substance can be easily identified by using cytochalasin B (an actin polymerization inhibitor), which blocks the cell cycle at cytokinesis, resulting in bi-nucleated cells. If the test chemical causes chromosomal fragmentation or loss, then the damaged genetic material lags behind during chromosome segregation and is not included in either of the resulting daughter nuclei. Instead, they are enclosed within a micronucleus and their frequency in bi-nucleated cells gives a measure of genotoxicity induced by the test chemical at a given concentration. To determine whether the micronuclei formed are the result of a clastogenic (chromosome fragmentation) or aneugenic (whole chromosome loss) mode of action, the micronucleus assay is coupled to kinetochore staining [11, 12].

HPRT (hypoxanthine–guanine phosphoribosyltransferase) forward mutation assay:

The HPRT gene is located on the X-chromosome, thus loss of function mutants in mammalian male cells (XY) is not masked by the presence of a competent duplicate copy (i.e. are homozygous) and can be easily identified as they confer resistance to the lethal guanine analogue 6-thioguanine. The enzyme encoded by the HPRT gene is involved in the salvage pathway for the generation of nucleotides where it is required for the phosphoribosylation of hypoxanthine and guanine, resulting in their salvage for nucleic acid (DNA) biosynthesis. When cells are grown in the presence of the poison 6-thioguanine, the HPRT enzyme will also act on this analogue, enabling the incorporation of it into DNA during replication, leading to the death of normal cells. However, if a mutation arises in this gene following exposure to the agent under investigation, the salvage pathway for nucleotide generation will no-longer function, hence the toxic analogue will not be incorporated into the DNA and viable cell colonies will form [13, 14].

γ-H2AX staining:

In higher eukaryotic cells, the histone H2A variant is phosphorylated on serine 139 in response to DNA double strand breaks (DSB) to form γ -H2AX. This phosphorylation is required for DSB signaling and is thought to act as a beacon to recruit and retain DNA repair proteins to the DSB site. The presence of γ -H2AX is therefore a sensitive reporter of DNA damage and these sites can be detected by immunofluorescence microscopy utilizing fluorescently labeled antibodies specific to γ -H2AX [15].

The GSH/GSSG redox pair not only serves as the principal homeostatic regulator of redox balance but also functions as a sensor that triggers these stress responses that, depending on the rate and level of change in this ratio, could be protective or injurious in nature [16].

Effects of nanoparticles in the respiratory tract have been studied through inhalation and instillation studies in rodents and in vitro cell culture systems [17]. In rodents, ultrafine particles cause mild pulmonary inflammatory responses and have effects on extra-pulmonary organs [18,19].

SUMMARY:

Nano-materials, even when made of inert elements like gold, become highly active at nanometer dimensions. Small size makes them easily accessible at the cellular level. Nanoparticles action is highly efficient i.e., they act directly on cells that are aimed as the targets (e.g. Tumour cells). With the rapid expansion in the nanotechnology industry, it is essential that the safety of engineered nano-materials and the factors that influence their associated hazards are understood.

Nanotoxicology is the study of toxicity caused by nanoparticles. Nanoparticles are administered to diagnose, to treat or to cure a disease. Translocation of nanoparticles during the exposure causes toxicity in

tissues other than targeted one. Nano-carriers cause DNA damage, nerve damage, inflammation, production of ROS, liver damage, kidney damage, spleen damage etc. Mechanism behind the toxicity of nanoparticles is based on the material used for the building of nanoparticles, any metals as carriers or very small particles may directly bind to DNA during mitosis and cause apoptosis. Effective Toxico-kinetic studies are not yet designed for the study of nanoparticle's toxicity. Various assay methods are available for the assessment of the extent of toxicity caused and to know the mechanism of action behind the toxicity.

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