Nanotechnology

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Abstract
Nanoparticles are gaining immense popularity in the medical world, specifically in its use in drug delivery systems. The objective of this paper is to study, based on available published literature, how nanoparticles are utilized in drug delivery and more importantly to identify the potential toxic effects of nanoparticles. Based on textual research, it is clear that there are benefits to nanoparticle use, but new studies are showing that there are many potential hazards of nanoparticle-like particles. In order to fully determine the toxicity of the hundreds of types of nanoparticles, a clear method to categorize these particles is needed and more research using empty nano-carriers needs to be done.

Introduction

The U.S. National Nanotechnology Initiative defines Nanomaterial as materials that have at least one dimension in the 1-100 nm range. Despite its size, you can see things with the effects of nanoparticles all around you. They give the sunset its red color, allow birds to navigate, and help geckos stick to trees. All around us, there are nano-sized materials present form volcanoes, forest fires, viral particles, biogenic magnetite and combustion products (Nano.gov). What has taken the scientific world by storm in the last few years is not the naturally existing nanoparticles, but rather, it is the newfound ability to create new materials on a nanoscale. The term "Nanotechnology" by the material science standard refers to the creation of these new particles and its usage. In the physical sciences, nanotechnology is associated with the quantum behavior of subatomic particles in nanoscale structures. In the biomedical sciences, nanotechnology is used in imaging, diagnosing, monitoring diseases, gene delivery, artificial implants, and targeted drug delivery (Nasimi, Haidari 2013). Engineered nanomaterials are useful, specifically in drug delivery, because of their large surface area to mass ratio (Oberdorster 2004). When a drug is encapsulated in a nanoparticle, there is a more accurate delivery to the targeted tissue. Drug permeability will also increase, thereby reducing the dosing frequency. For instance, an intravenously administered hydrophilic drug has poor reabsorption after glomerular filtration, often caused by the rapid renal clearance of the drug; whereas, encapsulating the drug in a nanoparticle reduces renal clearance and allows for better absorption. An orally administered drug has to endure enzymatic degradation in the gastrointestinal tract and a pass through the liver before it enters systemic circulation. By encapsulating the drug in a nanoparticle, the exposures to harsh conditions on the digestive tract are minimized (Kadam, et al 2012).

The key to using nanoparticles in drug delivery systems is ensuring that the drug can be released at the proper time. In that vein, a biodegradable nanoparticle formulation would be needed, as it is the intention to transport and release the drug in order to be effective. However, model studies to the behavior of nanoparticles have largely been conducted with non-degradable particles. Most data concerning the biological behavior and toxicity of particles comes from studies on inhaled nanoparticles. This is part of the unintended release of ultrafine or nanoparticles by combustion-derived processes such as diesel exhaust particles (Oberdörster, Oberdörster et al 2005). Research has demonstrated that exposure to these combustion derived ultrafine particles/nanoparticles is associated with a wide variety of effects, including vascular thrombosis, peripheral thrombosis, increased plasma fibrinogen levels and cardiovascular effects (Radomski, Jurasz et al 2005; Oberdorster, Oberdörster et al 2005). Since the size for both ultrafine and nanoparticles (100 nm) is relatively the same, many use both terms as equivalents. Based on the similarity of character and size between the two, researchers are speculating that the adverse effects of ultrafine particles, as part of environmental pollution, may be similar to the negative effects of engineered nanoparticles.

Methods

The NCBI PubMedCentral database, the Touro College library Database, Proquest, and Google Scholar were search engines used to find information. The following key words were searched to obtain research related to this paper: nanotechnology, nanoparticles, nano-medicine, nanotechnology and medical uses, nanotechnology and drug delivery, toxic effect of nanoparticles, negative effects of engineered nanoparticles, toxicity and nanoparticles, applications of nanoparticles, and hazards of nanotechnology. Further sources were found by using appropriate references cited in various journals and reviews.

Nanoparticles and Drug Delivery

Nanoparticles are formed through natural or human facilitated degeneration of larger structures or by controlled assembly processes. These procedures occur either in the gas phase, in a plasma, in a vacuum phase or in the liquid phase (SCENHIR 2006). Naturally occurring nanoparticles are found in the air in surprisingly high concentrations - approximately 106 to 108 nanoparticles per liter of air depending on conditions. They originate from the oxidation of volatile compounds, diesel and car engines, and photo-oxidation. The most significant concentration of particles and smallest particle size are associated with high-speed road traffic, apparently due to the subtle conditions during concomitant cooling and dilution of the exhaust gases (SCENHIR 2006). Although there are many naturally occurring nanoparticles, the ones that are man-made and biodegradable are the ones that are used in drug delivery systems (Oberdorster 2004).

Preparation of Nanoparticles

Before choosing a method to prepare nanoparticles for use in drug delivery, many factors have to be taken into account. Some of these factors are the size of the nanoparticle required, the inherent properties of the drug (ex. solubility, stability), surface characteristics and the degree of biodegradability, biocompatibility and toxicity (Mohanraj, Chen 2006). The following are the three main methods used to prepare nanoparticles for drug delivery:

Dispersion of preformed polymers: A polymer is dissolved in an organic solvent, which is also used as the solvent for the hydrophobic drug. This mixture is then emulsified in an aqueous solution containing a surfactant. After a stable emulsion is formed, the organic solvent is evaporated. Some of the polymers used in this method include poly- lactic acid, poly(D, L-glycolide), poly(D, L-lactide-co-glycolide) and poly(cyanoacrylate) (Mohanraj, Chen 2006).

Polymerization method: Monomers a polymerized and form nanoparticles in aqueous solution. The drug is encapsulated either by dissolving it in the polymerization medium or by absorption onto the nanoparticles post- polymerization. The nanoparticles are separated from the suspension by ultra-centrifugation (Reis, et al 2006).

Coacervation or ionic gelation method: A significant amount of research has been focused on the preparation of nanoparticles using hydrophilic, biodegradable polymers such as chitosan, gelatin and sodium alginate. A method was developed by P. Calvo, et al. for preparing hydrophilic chitosan nanoparticles by ionic gelation. They mixed two aqueous solutions together- one was the polymer chitosan and the other was the polyanion sodium triphosphate. The positively charged group of the chitosan interacts with the negatively charged triphosphate to form coacervates (an aggregate of colloidal droplets held together by electrostatic attractive forces) with a size in the range of a nanometer (Calvo, et al 1997).

Drug Loading and Release

The ideal nanoparticle for drug delivery should have a high drug loading capacity, thereby reducing the quantity of matrix materials (Mohanraj, Chen 2006). The two main ways to load a drug into a nanoparticle is by incorporating it at the time of nanoparticle production, or absorbing the drug after formation of the nanoparticles by incubating the nanoparticle carrier with a concentrated drug solution. The efficiency in loading the drug is very much dependent on the solubility of the drug in a solid state into the matrix material or polymer. The solubility is related to the polymer composition, molecular weight, the drug-polymer interactions and the type of functional group present (ester or carboxyl) (Govender, et al 1999). Once the drug is loaded, the next step is to ensure an opportune release. In general, the release of the drug is dependent on the solubility of the drug, drug diffusion through the nanoparticle matrix and nanoparticle matrix degradation. (Mohanraj, Chen 2006). Very often, the drug is released by interactions between intracellular chemicals and the nanoparticle matrix. For example, the cationic surface of some nanoparticles allows penetration through the cell membrane and the drug is then released after the nanoparticle matrix is triggered by intracellular glutathione. Another example is the reduction of cadmium sulfate or ferric oxide (which are used to cap silica nanoparticles) by thiols that release the molecules inside the nanoparticle (Nasimi, Haidari 2013). An alternate mechanism for release is the use of pH-responsive nanomaterials. A further achievement in the area of drug release was reached when a method was developed to use multifunctional super-magnetic nanoparticles that can be released remotely (Derfus, et al. 2007).

Passing the Blood Brain Barrier (BBB)

From several standpoints the brain is a challenging organ for drug delivery. First, the occurrence of progressive diseases in the brain will increase with the aging population. Secondly, the blood brain barrier (BBB) is well known as the best gatekeeper in the body toward exogenous substances. Generally pharmaceuticals including most small molecules do not cross the BBB. The BBB is formed by tight junctions between the cerebral endothelial cells, which abolish all aqueous diffusion pathways, and by biochemical systems consisting of enzymes, which specifically metabolize many drugs. However, the barrier properties may be compromised intentionally or unintentionally by allowing the passage of nanoparticles (Kreuter, et al. 2002). Many studies were done that show coating of the nanoparticles with the polysorbate (80) surfactants resulted in transport of drugs across the blood brain barrier (Schroder 1996). It is interesting to note that studies were first done using engineered nanoparticles to cross the BBB; however, with increased research it was discovered and published that natural ultrafine particles can cross the BBB and cause damage.

Toxicological Hazards of Nanoparticles

General concepts

To effectively tap into the potential of Nanotechnology in Nanomedicine, full attention is needed to focus on safety and toxicological issues. For pharmaceuticals, precise drug delivery formulations may be used to increase the so called "therapeutic ratio" which is the margin between the dose needed for clinical efficacy and the dose that would induce adverse side effects (toxicity). Also, for these specific formulations, a toxicological evaluation is needed. The US Food and Drug Administration approval is crucial for clinical applications of nanotechnology, but considerable problems come into account when it comes to

approving nanotechnology-based products. The Food and Drug Administration regulate pharmaceuticals and biological devices differently, and it is not yet clear how emerging nanotherapeutics will be evaluated. It is important to have a safety guide particularly in the applications of nanoparticles for drug delivery. In these applications, particles are brought intentionally into the human body and environment (Buxton, et al 2003). Opinions started to divert when toxicologists claimed that new science, methods and protocols are needed (Nel et al 2006). However, the need for a safety guide is now underlined by several expert reports and more importantly by the following concepts:

1) Nanomaterials are made for their unique surface properties as opposed to the similar properties of bulk materials. Since the surface is the layer that interacts with the body tissue, and an essential factor of particle response, these unique properties need to be investigated from a toxicological perspective. When nanoparticles are used for their distinctive reactive characteristics, it may be probable that these same characteristics also have an impact on the toxicity of such particles. Although existing tests and procedures in drug and device assessment may work to detect many risks related to the use of these nanoparticles, it cannot be presumed that these assays will detect all potential risks. (SCENIHR 2006) The toxicity may differ depending on the type of particles used, i.e., biological versus non-biological origin.

2) Nanoparticles are recognized as having different physical and chemical characteristics from micron-sized particles. This may result in changed body distribution, passage of the blood brain barrier and triggering of blood coagulation pathways. In view of these characteristics, specific emphasis should be on testing and studying the distribution of nanoparticles. What is currently lacking is a basic comprehension of the biological behavior of nanoparticles in relation to the distribution in vivo both at the organ and cellular level.

Using nanoparticles as a drug carrier may reduce the toxicity of the incorporated drug. In general, research focuses on the toxicity of the entire formulation. The results of the nanoparticles itself are not described, so differentiation between drug and nanoparticle toxicity cannot be made. There should be a specific emphasis on the toxicity of the "empty" non-drug loaded particles. This is especially important when slow or non-degradable particles are used for drug delivery. (Oberdorster, Maynard, et al. 2005)

Evidence for Nanoparticle Toxicity

The largest database on the toxicity of nanoparticles comes from the PM10 literature (particulate matter with a size below 10 mm), where studies on inhalation and the 'Nanoparticle hypothesis' have proved to be a powerful drive for research (Oberdörster, Oberdörster et al 2005). Therefore it is relevant to discuss this

Table 1

Particle type	Description
PM10, PM2.5	Particle mass fraction in ambient air with a mean diameter of 10 or 2.5 μm respectively. Basis of current standards for ambient particles in Europe and USA
Coarse particles	The mass fraction of PM10, which is bigger than 2.5 μm
Ultrafine particles (PM0.1)	The fraction of PM10 with a size cut-off at 0.1 μm. Contains primary particles and agglomerates smaller than 100 nm
PSP	Poorly soluble particles with low specific toxicity. Maybe be fine or ultrafine. Terminology used in relation to bulk synthetic particles. Examples TiO2, carbon blacks, Amorphous silica, Iron oxides (Fe2O3), Zinc oxides (ZnO)
CDNP	Combustion derived nanoparticles, such as diesel exhaust particles (DEP)
DEP	Diesel exhaust particles

Various denominations of particles in inhalation toxicology and drug delivery in relation to their source (ambient, bulk, engineered)

evidence with the expectation that it will shed light on the toxicity of engineered nanoparticles. The adverse health effects of particulate matter (PM) are measurable as causes of respiratory disease and deaths as well as hospitalizations and deaths from respiratory and cardiovascular disease. Many laboratory studies have been done to investigate the effects of ultrafine and PM particles. It was found that some nanoparticles might have the extra potential of affecting cardiovascular disease directly. Vascular function was impaired after inhalation of diesel exhaust particles. However, data to date is limited and not all studies of nanoparticles have shown significant translocation from lung to the blood. Understanding clearance kinetics of inhaled ambient air nanoparticles will also be important in understanding the potential for adverse effects. (Oberdorster, Maynard, et al. 2005)

The current standard in particle toxicology is that ultrafine ambient air particles have the potential of affecting cardiovascular disease both indirectly via pulmonary inflammation and directly through particle distribution. Although significant, this property of redistribution has yet to be demonstrated for nanoparticles present in real PM10's. It should be noted that there are several mechanisms whereby nanoparticles could lead to inflammatory effects, as is the case for larger particles. These mechanisms are either based on the large surface area of a particle core or on soluble components released by the nanoparticles (Schins, et al. 2004). Several toxicological studies support the argument that nanoparticles in PM10's could drive inflammatory effects. There are a number of components of PM10's that contribute to the mass but have little toxicity, including salts such as sulfates, chlorides and ammonium salts and nitrates. In fact, within PM10's there are only few components that toxicologists would identify as likely causes of adverse effects – i.e., particle surfaces, organics, metals and endotoxins. A large surface area, organics and metals are all characteristic of combustion–derived particles and so these have attracted considerable toxicological attention (Donaldson, et al. 2005).

Effects of Nanoparticle Toxicity

Many physicochemical factors can influence the potential biological interactions and toxicity of nanoparticles. Therefore, it is important to consider the extent to which the physicochemical properties of nanoparticles have been characterized in any given study. Without sufficient characterization, it is extremely challenging to interpret the results of individual studies and virtually impossible to compare the results of different studies, even in cases where the same nanoparticle has been investigated. As a result, the ability to identify parameters that might influence toxicity is hampered. Although there is not yet a universally accepted standard of parameters that is deemed necessary for nanoparticle characterization, recent reports have highlighted several key physicochemical elements for which it is strongly recommended that data be reported (Oberdörster, Maynard, et al. 2005). These limitations include method of synthesis, size, size distribution, shape, composition, crystal structure, aggregation and agglomeration status, dissolution, purity, surface area, and other surface characteristics. Classification of nanoparticles in the context of the experimental contact media (cell culture media, dosing solution, aerosol, etc.) is also of substantial importance, as some physicochemical parameters are likely to differ depending on whether they are determined in the experimental media or in the bulk (i.e., "as received") state. Unfortunately, the inclusion of all these parameters in publications describing nanoparticle toxicity studies appears to be rare.

Nanoparticles in The Lungs

When nanoparticles enter the respiratory system, they are thought to cause damage that results primarily from lung particle overload. This is due to the inability of alveolar macrophages to recognize and/or clear particles of this size, resulting in a particle build up, chronic inflammation, fibrosis, and tumor-genesis. However, many studies have not shown a correlation between nanoparticles and inflammation (Card, et al, 2008). Many studies also track whether the particles translocate from the pulmonary system into systemic circulation. One of the studies reported to date indicate that inhaled 99mtechnetium-labeled carbon nanoparticles, which are man-made, are not detected outside of the lungs in significant quantities after inhalation. However, as mentioned to by Mills et al., these findings do not indicate that other nanoparticles will behave in the same manner, nor do they rule out the possibility that nanoparticles may interact with and influence the vascular system in the lungs. Moreover, the studies conducted to date have used a single inhalation exposure protocol, and it is possible that repeated exposures may result in greater pulmonary accumulation and transfer of significant quantities of nanoparticles to the circulation (Mills, et al. 2006).

Fibrosis is a condition that many researchers believe is caused by nanoparticle exposure. Many experiments were done using animals to test the effects of primarily carbon nanotubes, carbon black, fullerenes, silica, and metal-based nanoparticles including titanium dioxide, silver, and nickel. Though it is known that the pathogenic mechanisms underlying animal models of lung fibrosis and human lung fibrosis are not necessarily the same, increased collagen deposits and structural deviations to the lungs can result in changed respiratory mechanics that are common features of both. Fibrosis in animal models is defined by increased collagen content and/or histopathological evidence of structural alterations to the lung that are consistent with fibrosis (Card, et al, 2008). It was found that the type of carbon nanotubes, their length, and the way specific fibers interact, all have varying effects on pulmonary inflammation and fibrosis. Studies have found that the longer the nanotube fiber length, the greater the toxicity, and the more likely it is to cause fibrosis and cancer (Donaldson, et al. 2006).

Effects on Blood and Cardiovascular System

In a study by Radomski, et al, (2005) the effects of various nanoparticles on platelet function were studied. In vitro studies were done using human blood samples, and then in vivo studies were done in rats to confirm the effects of platelet- aggregation found in the human blood. Engineered nanoparticles were found to cause activation and aggregation of human platelets. The efficacy of the nanoparticles in blood aggregation in vitro was matched by the same results in the rats. Treatment with nanoparticles caused rat vascular thrombosis. The data shows that not all nanomaterials act similar in this test, and that surface area is not the only factor playing a role here. The data also validates the idea that mainly cationic species have an effect on blood clotting. Interestingly, this was the first study that allows bridging of data, since a PM10 sample (SRM1648) was included in the test-series, in combination with nanoparticles. The PM sample actually showed a lower effect on platelet aggregation compared to the carbon nanotubes (Radomski, Jurasz et al 2005). Another study shows that repeated exposure to PM10's causes a systemic inflammatory response, including bone marrow stimulation, and is related to the progression of atherosclerosis in the coronary arteries and aorta (Suwa, et al. 2002).

Uptake and Effects of Nanoparticles in The Brain

Nanoparticles can gain access to the brain by two different mechanisms. (1) Trans-synaptic transport after inhalation through the olfactory epithelium. (2) Uptake through the blood-brain barrier. The first pathway has been studied primarily with model particles such as carbon, Au and MnO2 in experimental inhalation models in rats (Oberdörster et al 2004; Oberdörster, Oberdörster et al 2005). The pathway via the BBB has been the topic of research for a while, especially for drug delivery. Studies suggest that the physiological barrier may hinder the distribution of some proteins and viral particles after trans-vascular delivery to the brain, suggesting that the healthy BBB contains defense mechanisms protecting it from blood-borne nanoparticle exposure. When nanoparticles with different surface characteristics were evaluated, neutral nanoparticles and low concentrations of anionic nanoparticles were found to have no effect on the BBB, whereas high concentrations of anionic nanoparticles and cationic nanoparticles were toxic for the BBB (Nel et al 2006). Fullerenes and C60 nanoparticles have been shown to induce the production of reactive oxygen species, oxidative stress, and rapid brain lipid peroxidation in marine species. Because of all the negative effects on the brain by nanoparticles, further tests would need to be done before using fullerenes and C60 for human and industrial use (Oberdorster 2004).

Conclusion

Although there is a considerable amount of data on the toxicity of nanoparticles, this data is mainly based on a small sampling and the assumption that a lot of effects by particulate matter are driven by the ultrafine particle fraction in it (Oberdörtster, Oberdörster et al 2005). This small sampling doesn't really give enough information to adequately determine the potential hazards. Although hazard identification is the general approach for safety evaluation of healthcare products, it is recommended to add testing driven by the anticipated application and classification by risk. Some engineered nanoparticles that are airborne will pose inhalation and cardiovascular hazards, while cosmetics with nanoparticles provide dermal exposures. Each nanoparticle formulation should be tested on a case-by-case basis in the requisite ways focusing on their method of entry. In this respect the potential adverse effects of empty particles should also be considered. In developing testing procedures and protocols a number of basic issues need to be considered:

1) It needs to be determined whether noticed effects are caused only by nanoparticles, or are the adverse effects caused by something else and only aggravated by nanoparticles. It is clear from research that both PM and ultrafine particles can cause inflammation, cancer, etc., but these new and smaller nanoparticles may cause different effects.

2) Most of the conclusions drawn about nanoparticles are based on correlations made between the behavior of ultrafine particles and PM's to that of nanoparticles. The question is how much of a correlation is permitted to be drawn, how many assumptions can be made, as manufactured nanoparticles and ultrafine/ PM particles are not identical.

3) The scientific world is dealing with a increasingly growing number of nanomaterials. All have the potential to create a new toxic effect that has never been studied before. The current testing and classification system for nanoparticles does not seem sufficient to fully identify and quantify the toxicological effects of these new nanoparticles.

For years pharmaceutical sciences have been using nanoparticles to reduce toxicity and the side effects of drugs. Up to recently it was not realized that these drug carrier systems themselves may cause risks to the patient. The type of hazards that are introduced by using nanoparticles for drug delivery are beyond what is posed by conventional hazards imposed by chemicals. However, as of current data, the scientific example for the possible toxic and adverse reactivity of nanoparticles is lacking and we have little understanding of the basics of the interaction of nanoparticles with living cells, organs and organisms. A conceptual understanding of biological responses to nanomaterials is needed in order to develop and apply safe nanomaterials in drug delivery in the future. Furthermore, a close partnership between those working in drug delivery and those working in particle toxicology is necessary for

the exchange of concepts, methods and to establish a common system for identifying the potential dangers of nanoparticles.

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