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Medical Aspects of Nanomaterial Toxicity

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Abstract

Nanosilver is the most popular and most studied nanomaterial, however, a family of nanomaterials is rapidly enlarging. They are used in various branches of industry and everyday life. In medicine new nanomaterials can be used either alone or in combination with other “classical” drugs, e.g. cytostatic drugs or antibiotics. They can be also used as diagnostic agents. A development of nanoparticles has led to a new combination of diagnostic and therapy - theranostic. Size of a particle makes a difference not only between bulk material and nanomaterial, but also in their properties and toxicity. Nanomaterials can have beneficial properties, but can also be toxic. New issues concerning nanomaterials arise - an industrial exposure and environmental pollution. They can enter human body in various ways. Cellular mechanisms of nanomaterial toxicity comprise mainly a generation of reactive oxygen species and genotoxicity. The differences between toxicity of fine particles and nanoparticles have led to an origin of a new branch of science, nanotoxicology.

Keywords: nanomaterials, nanoparticles, medical use, toxicity

1. Introduction

Nanoparticles can be of various origin: natural, incidental, or manufactured. Natural nanoparticles can be met in fumes or smoke (e.g., carbon black). A fast-growing branch of science, nanotechnology has led to a development of a variety of newly engineered nanoparticles. As their use each day becomes broader, the proper evaluation of their toxicity becomes an urgent

must. Properties of nanomaterials change as their size goes down approaching a nanoscale. As properties of a bulk form and nanoparticles differ, so we must know if the toxicity also changes.

2. Nanomaterials used in medicine

When one says “nanoparticle” or “nanomaterial,” the first association usually is nanosilver. It was the first nanomaterial introduced into medicine due to its antibacterial properties. Nanosilver has a broad spectrum of both medical and paramedical applications. It is used as an antibacterial addition to wound dressings and ointments used to protect from infection wounds, burns, ulcers, and pemphigus. Nanosilver is also applied to cover the surface of surgical threads, tools, and catheters introduced into veins. Medical protective clothes, gloves, bed clothes, mattresses, syringes, respiratory tubes, and masks also may contain nanosilver to reduce the risk of infection. Nanosilver is also used in orthopedics to cover the surface of various implants and as a component of bone cement to prevent a development of bacterial infection which in orthopedics may be very dangerous and difficult to treat. Nanosilver is also used in production of a variety of drug and food packings. Zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles also have antibacterial properties and are used as an additive to food packing [1-3].

Magnetic nanoparticles comprise a next group of medical applications. They are a well-known diagnostic tool used as magnetic resonance imaging contrast comprising iron oxide nanoparticles or nanoparticles with iron core. Calcium phosphate nanoparticles doped with a near-infrared dye for optical imaging, fluorophore, have been tested for in vivo diagnostic of human breast cancer.

A development of new nanoparticles has led to a development of a new combination of diagnostic and therapy, theranostic. The simplest explanation of this new term is an identification of diseased tissues or cells and delivery of a medicine or a therapy (e.g., heating) to this very site of pathology. A gold-silica nanoparticle system for optical imaging and photothermal ablation has been developed [4-6].

Superparamagnetic iron oxide nanoparticles (SPIONs) and ultra-small superparamagnetic iron oxide (USPIO) can be used for targeted drug delivery [7]. Another kind of intensively studied nanomaterials are mesoporous silica nanoparticles. They may be used for high drug or imaging agent loading. “Cornell dots” are the first silica-based diagnostic nanoparticles approved for human clinical trials. Gold nanoparticles can have various medical applications such as improving efficacy of high-resolution ultrasound imaging, photothermal therapy, and photothermal release of DNA cargo upon laser irradiation. They have been also shown to pose antibacterial activities. Quantum dots constitute another class of nanoparticles. They can be extremely small, typically having only few nanometers. They are usually composed of heavy metals such as lead or cadmium. Scientists hope to employ them in sophisticated multimodal imaging techniques [8]. Nanogels, dendrimers, and liposomes are the other classes of nano-

materials. They can be used for controlled drug delivery. Cell growth scaffolds may be another application of these classes of nanomaterials [8].

3. Entry portal of nanomaterials into the human body

Various nanomaterials can be used in different ways. Many nanomaterials are used as drug carriers or imaging agents with an intravenous entrance into the human body. This gateway for nanoparticles can be well controlled. This way of introducing nanoparticles into the human body allows for their easy distribution to all organs. The nanoparticles only have to be able to cross the barrier of blood vessel wall.

Absorption through the skin is another way in which nanoparticles can enter the human organism. The skin is the largest organ of the human body, reaching above 10 % body mass. It gives a large area of contact with the external environment, approximately 1,73 m². Skin contact with nanomaterials may be due to various reasons, occupational contact in industry, medical applications, and a still-growing amount of cosmetics containing nanoparticles and clothes with nanoparticles, e.g., sock containing nanosilver to reduce odors. Cosmetics and products for everyday hygiene like soaps, shampoos, gels, creams, and deodorants also may contain nanosilver. This nanoparticle is not the only one the skin may be exposed to. Sunscreens protecting the skin against UV radiation contain titanium dioxide (TiO₂) and ZnO nanoparticles. The nanoform of these two oxides is more often used due to its transparent form which is more acceptable by customers.

Often nanoparticles, mainly nanosilver, are used for treatment of burns, wounds, or ulcers. Although they are intended to be used topically to prevent infections, a damaged skin makes their penetration into the body easier. There are four pathways the chemical compounds can penetrate across the skin: intracellular, transcellular, and two of transappendageal through sweat glands and hair follicles. The way of penetration depends on physicochemical properties of nanoparticles. Many other factors can influence the extent of dermal uptake. They can be roughly divided into two groups, caused by a condition of the skin and by external factors. The anatomical side resulting in differences of epidermis thickness, a skin barrier integrity and the presence of wounds or scratches, and skin diseases like allergic or irritant contact dermatitis, atopic eczema, and psoriasis compose the first group. The external factors include the contaminated skin surface, irritant detergent and chemicals, mechanical flexion, and exposure to heat, infrared, or UV radiation. They can increase the skin absorption of nanoparticles [8].

Metallic nanoparticles smaller than 10 nm have been shown to penetrate epidermal layers [3, 9]. Most dermal exposure studies indicate that TiO₂ does not penetrate the stratum corneum [10]. Carbon nanoparticles comprising single-walled and multiwalled carbon nanotubes and fullerenes have a large variety of applications. Fullerenes have been shown to penetrate flexed but not unflexed skin [10]. Unrefined single-walled carbon nanotubes have been shown to exert negative effects on cultured skin cells, increasing generation of free radicals and causing ultrastructural and morphological changes in keratinocytes. These carbon nanoparticles induced also cellular apoptosis and necrosis response. The surface area of carbon nanotubes is the best predictor of their negative effects [10].

Quantum dots also can penetrate the intact skin. This fact is important in an aspect of occupationally relevant skin contact. A surface coating of quantum dots does not influence their penetration into the skin, but is responsible for a magnitude of toxic effects on skin cells including cytotoxicity and immunotoxicity [10]. Nanosilver which is contained in various dressings may influence both keratinocytes and fibroblasts. The latter are more sensitive to nanosilver than keratinocytes [10]. Gold nanoparticles can penetrate into the skin. This ability is size dependent. The smaller the nanoparticle, the deeper it can penetrate. Citrate/gold nanoparticles can be toxic to human dermal fibroblasts [11]. Iron oxide nanoparticles can penetrate into the skin. They can be rapidly endocytosed by cultured human fibroblasts and disrupt their function [10].

While discussing skin penetration of nanoparticles, another question arises. Can nanoparticles cross the skin barrier and be distributed by blood to other organs? Scientists mainly focus their attention on evaluation of nanoparticle penetration into either usually porcine skin or culture skin keratinocytes or fibroblasts. If some nanoparticles can penetrate into deeper layers of the skin, the abovementioned question requires an urgent answer.

Inhalation constitutes the next entry portal of nanomaterials into the human body. This gateway is very important in a case of occupational exposure to nanoparticles. Lansiedel et al. applied a new method for evaluation of toxicity of inhaled nanoparticles, a short-time inhalation study (STIS) instead of a 90-day rodent inhalation study. Nanoparticles and microscale zinc oxide were evaluated in this study. Among tested materials, only polyacrylate-coated silica and both forms of ZnO were found in extrapulmonary organs. The first was found in the spleen, whereas both forms of ZnO elicited necrosis on the olfactory epithelium. Five materials – coated nano TiO₂, nano-CeO₂, Al-doped nano-CeO₂, and both forms of ZnO – evoked transient, dose-dependent pulmonary inflammation. The results of this study enabled to classify studied materials into three groups. Nano-BaSO₄, nano-SiO₂, four types of surface coated silica – SiO₂-polyacrylate, SiO₂-PEG, SiO₂-phosphate, and SiO₂-amino – nano-ZrO₂, ZrO₂ TOTA, and ZrO₂-acrylate were of low toxic potency. The group of medium toxic potency had only one studied nanoparticle, non-coated amorphous silica (naked silica). The third group of higher toxic potency comprised coated nano-TiO₂, nano-CeO₂, Al-doped nano-CeO₂, microscale ZnO, and coated nano-ZnO [11]. ZnO nanoparticles induced collagen formation 4 weeks after instillation [12].

Titanium dioxide has gained much attention in recent years. It can be used in a form of either fine particles or nanoparticles. TiO₂ fine particles have been considered as poorly soluble, low-toxicity particles. Due to their properties, they have been used as a “negative control” in many toxicological studies. A long-term, 2 years, study with high-dosed TiO₂ revealed that fine particles of TiO₂ might cause lung tumors in rats. The International Agency for Research on Cancer (IARC) has classified TiO₂ as possibly carcinogenic to humans (carcinogen group 2B). With this fact arises a question: Can the results of studies of larger particles be automatically transferred to nanoparticles? Some studies have shown that TiO₂ are more toxic than TiO₂ fine particles. Although TiO₂ nanoparticles were shown not to penetrate through the skin, the effects of inhalation of these nanoparticles seem to require further studies [11].

Gastrointestinal absorption is another important route nanoparticles may enter the human body. This way is important for nanoparticles which are allowed as “food contact substances”

and which may come in contact with drinking water, e.g., nanosilver present in water filters or water purifiers.

The most common way of uptake for both microparticles and nanoparticles in gut is endocytosis by M-cell in Peyer's patches. Once nanoparticle enters the blood stream, they are distributed throughout the body. Nanoparticles can pass through barriers of the body and accumulate in certain organs. Small gold nanoparticles (10 nm) were found to accumulate in the liver, spleen, kidney, lungs, brain, and reproductive system, whereas larger ones (50–100 nm) and 250 nm were found only in the blood, liver, and spleen [13]. The size of nanoparticles is an important factor in determining their blood circulation time and places of deposition in an organism [14]. Nanoparticles entering the gastrointestinal tract are exposed to a wide range of pH. Hydrochloric acid secreted in the stomach has been shown to influence dissolution of silver nanoparticles. In these conditions, silver ions are generated from silver nanoparticles. As the ability of silver particles to cross the gut epithelium is limited, it seems that the main route of silver uptake from gastrointestinal tract is ion transport. Silver ions may be transported by mechanism responsible for transport of sodium and copper ions [1,15].

Biological fluids present a wide spectrum of chemical conditions. They can influence nanoparticles in various ways and can cause their agglomeration, changing their properties, penetration potency, and also toxicity. The fact that some in vivo conditions may change the particle size must be taken into consideration while evaluating the toxicity of nanomaterials. It may lead to a conclusion that in some conditions, not a particle size but a size of agglomerate may determine the properties of nanoparticles and consequently their toxicity [16].

Sometimes nanoparticles can be administered using the intraocular and intranasal routes which have not been widely studied. Nanosilver can be used in some intrauterine devices, creating another plausible gateway for nanoparticles.

Nanomaterials can be used as drug carriers and enhancers. The term "drugs" should be widely understood. They can be of classical chemical nature. Nanoparticles can improve dissolution and solubility of poorly soluble drugs and successful delivery of hydrophobic drugs, increasing their concentration at targeted tissues. Nanoparticles may also be used for delivery of drugs into specific cell compartment. This application of nanomaterials may comprise various routes of administration, not only intravenous.

Nanoscale changes structure-activity relationship of complex nanoparticles. The use of nanoformulations of drugs allows them to pass some biological barriers which for classical drug forms are not transferrable. The blood-brain barrier may be a prominent example. The complex of drug on a nanoporous silica gives a tool for controlling the release of drug at its side of destination and action [17]. As a progress of pharmacology and nanotechnology enables to create nanoparticles carrying various drugs, an important question arises. What is responsible for specific side effects, a carrier or a cargo? This approach creates the novel branch of science – nanotoxicology.

The efficacy of nanoparticles relies on a variety of their modifications and functionalities. This advantage creates a variety of tasks for nanotoxicology to evaluate the safety/toxicity profile of each modification. The study of Landsiedel et al. shows that modifications of nanoparticles

may change their toxicity. Those modifications can alter hydrophobicity and isoelectric points of particles. Various nanoparticles can have different abilities to agglomerate in specific biological conditions that may have an important influence on their toxicity [14,18].

4. Toxicity of nanoparticles

The size of nanoparticles of the same chemical compound or the same chemical element may vary in a wide range from a few nm to about 100 nm. Results of studies concerning nanoparticles of the same chemical nature can give various results depending on the size of used nanoparticles. A kind of exposure resulting in a specific way of possible absorption is another factor influencing the results of toxicological studies. TiO_2 which is thought not to cross the skin barrier in humans may induce lung tumors in rats when inhaled in high doses for a long time. TiO_2 nanoparticles may be absorbed both from the lungs and gastrointestinal tract. However, the rate of absorption appears to be low. Studies with intravenous administration of TiO_2 indicate that these nanoparticles can induce pathological lesions of the liver, spleen, kidney, and brain. These effects may be attributed to very high doses of TiO_2 nanoparticles used in this study [10]. The use of TiO_2 is widespread. It may be used even as a white color food additive. It is difficult to estimate how much of total TiO_2 used is the form of larger, fine particles and how much in the form of nanoparticles. The difference in toxicity of TiO_2 fine particles and nanoparticles is reflected in allowances of occupational exposure. In the USA, the threshold limit value of TiO_2 fine particles (total dust) is assigned as 10 mg/m^3 as time-weighted average (TWA) for an 8-hour daily work time 5 days per week. That value was established by the American Conference of Governmental Industrial Hygienists (ACGIH). The regulation of the Occupational Safety and Health Administration (OSHA) is 15 mg/m^3 . The US National Institute for Occupational Safety and Health (NIOSH) proposed a recommended exposure limit (REL) for TiO_2 nanoparticles of 0.3 mg/m^3 , being 10 times lower than REL for TiO_2 fine particles. In Japan, TWA for TiO_2 nanoparticles is 1.2 mg/m^3 . The fact that the same recommendation in the USA and Japan differs four times indicates that further studies concerning safety limits of exposure to TiO_2 nanoparticles are required [10].

The skin is a site where the first side effect of nanomaterial was observed. Silver and nanosilver used as antibacterial agent when overdosed caused an irreversible pigmentation of skin termed argyria or argyrosis. In these patients, silver deposits form usually in skin regions exposed to light. The silver deposits are usually collocated with sulfur and selenium. Silver is usually bound to protein thiol groups or selenium in the case of selenoproteins. Both sulfides and selenides have a high binding affinity for silver, causing its uptake from biological fluids. Silver bound to these chemical groups is easily exchangeable and has significant biomolecular mobility. If silver complexes with thiol groups are located in the skin or near-skin region, silver can be easily reduced by visible or UV light to metallic nanosilver particles, resulting in an immobilization of silver nanoparticles in the skin. This process puts new light on a pathogenesis of a very old side effect of treatment with various drugs containing silver, argyria. It also explains why this side effect is usually located in skin regions exposed to light [15].

Animal experiments with topical administration of colloid nanosilver showed both acute and chronic dermal toxicity. It was also observed that nanosilver could penetrate from the skin to the blood and accumulate in the liver and spleen, causing their mild damage detected in histopathological examination [19]. The fact that nanosilver can cross the skin barrier makes us look closer and with a greater attention on results of *in vitro* studies showing nanosilver toxicity to various cultured cell lines. Nanosilver has been shown to be toxic to peripheral blood mononuclear cells, human alveolar epithelial cells and alveolar macrophages, rat hepatocytes, and mouse germline cells [17]. These results indicate that the safety of a still-increasing use of nanosilver should be better monitored.

In biological fluids, proteins can associate with nanoparticles. The competing of proteins for the nanoparticle surface leads to a generation of protein “corona” surrounding the nanoparticle. This process can take place in the blood. Not only albumins and fibrinogen can compete to create “corona” but also lipoproteins. Such an association alters nanoparticle characteristics and their biological properties. Some nanoparticles associated with apolipoprotein E can cross the blood-brain barrier. This modification giving some nanomaterials new important biological property has a potential significance for a development of new neurotherapies as well as for neurotoxicity [18,19].

In humans, nanosilver was shown to inhibit the extrinsic coagulation pathway evaluated by activated partial thromboplastin time (APTT) [20]. In animal experimental model, nanosilver decreased platelet activation [21]. Nanosilver can cross the blood-brain barrier and accumulate in the brain, causing necrosis and neuronal degeneration [22]. In a study of Ahamed et al. [23], nanosilver particles elicited genotoxicity on mouse embryonic fibroblasts. This was caused by both polysaccharide-coated and uncoated nanosilver particles. Nanosilver was also shown to accumulate in the testis [24].

Detailed nanotoxicological studies dealing with mammalian reproductive tissues and gametes have yet to be carried out. A very limited group of nanomaterials have been shown as biocompatible with mammalian sperm: magnetic iron nanoparticles, a specific type of CdSe/ZnS quantum dots, halloysite clay nanotubes, and commercial nanopolymer-based transfectants [25]. A group of nanomaterials biocompatible with mammalian embryo is smaller and comprises chitosan, CdSe/ZnS quantum dots, and externally applied polystyrene nanoparticles.

Results of studies of sperm toxicities of nanogold remain highly contradictory [25]. Some of them reported that nanogold elicited spermatotoxicity [18].

Nanocopper was shown to reduce in a dose-dependent manner cell viability of mouse testis Leydig cells. *In vitro* studies of TiO₂ nanoparticles and ZnO nanoparticles demonstrated a dose-dependent sperm DNA damage in human spermatozoa. Various studies evaluating the influence of TiO₂ nanoparticles on mammalian ovarian cells gave contradictory effects [26].

Various nanoparticles can also influence the endocrine system. In animal models, TiO₂ nanoparticles and CdTe/ZnTe quantum dots conjugated with transferrin could alter serum levels of sex hormones and gonadotropins [26].

Nanoparticles can easily cross the placental barrier and enter a fetus. The animal experiments showed that fullerene nanoparticles were traced in embryos and at higher doses caused significant toxicity and death [27]. TiO_2 was shown to transfer from pregnant mice to their offsprings, affecting the central nervous system and genitals [28]. The results of abovementioned studies raise a question about the risk of nanoparticle exposure to pregnant women.

Nanoparticles can influence the immune system. They were shown to induce inflammatory response. ZnO nanoparticles can exert their activity on immune cells through three mechanisms. Promoting antigen uptake by antigen-presenting cells is the first one. Targeting to specific cells like dendritic cells, Langerhans cells, or macrophages is the second mode. The third one is immunopotentiality and modulation of activity of antigen-presenting cells through receptors of innate immune system [29]. ZnO nanoparticles have been found to induce toxicities particularly in the immune cells as these nanoparticles are specifically found to be internalized in these cells [29]. Nanoparticles can also act as haptens and modify protein structures, raising their potential for autoimmune effects [18].

5. Cellular mechanisms of nanoparticles toxicity

In this chapter, the cellular mechanisms of nanomaterials toxicity will be only very briefly mentioned as they are discussed in detail in other chapters of this book.

5.1. Reactive oxygen species production

Reactive oxygen species (ROS) constitute a pool of reactive species of molecular oxygen, previously termed free radicals, including superoxide anion ($\text{O}_2^{\cdot -}$), hydroxyl radical (OH^{\cdot}), hydrogen peroxide (H_2O_2), singlet oxygen ($^1\text{O}_2$), and hypochlorous acid (HOCl). Most of them are produced via electron transport chain in the mitochondria. ROS are generated intrinsically or extrinsically within the cell. ROS production is widely used in cell signaling, regulation, and homeostasis [14,30,31].

Nanoparticles entering the cell interact with mitochondria and other subcellular organelles increasing ROS production. Various nanomaterials of various sizes can disturb mitochondrial function. ZnO nanoparticles can generate Zn^{2+} ions interrupting charge balance in electron transport chain in the mitochondria and triggering ROS formation. Semiconductor nanomaterials can elicit an excited energy state, leading to the generation of $\text{O}_2^{\cdot -}$. This ROS is capable of damaging cellular macromolecules or interrupting cell signaling, leading to cell dysfunction. It also can cause further generation of other ROS. Some nanoparticles can interact between one another. Excited CdSe quantum dots are capable of injecting electrons into TiO_2 [32]. It was observed that photoactivation of TiO_2 could also generate $\text{O}_2^{\cdot -}$ and OH^{\cdot} radicals [31]. This fact seems to be important considering the still-growing use of TiO_2 nanoparticles. An increased generation of ROS causes further intracellular disorders [31].

Nanosilver has been observed to significantly decrease incorporation of selenium into selenoproteins constituting enzymes engaged in antioxidant protection such as glutathione

peroxidase, thioredoxin reductase, or methionine sulfide reductase [1,21]. Nanomaterials via increased ROS generation lead to a glutathione depletion. Glutathione is a main intracellular antioxidant. This depletion may significantly affect cellular metabolism causing mitochondrial dysfunction and ATP depletion [30].

Generation of lipid peroxides is a next effect of increased ROS generation. Various nanomaterials have been reported to generate lipid peroxides and resultant cell membrane damage. Nanosilver can induce both mitochondrial damage and mitochondrial-dependent apoptotic pathway [30].

The destruction of cell membrane can cause a membrane leakage of lactate dehydrogenase. The assay for evaluating the extracellular activity of this enzyme is most commonly used for membrane leakage testing. Monitoring lactate dehydrogenase activity can be a useful tool for evaluation of toxicity of nanomaterials [30].

5.2. Genotoxicity

Genotoxicity is caused by agents interacting with DNA and other compounds controlling the integrity of the genetic material and includes DNA strand breaks, point mutations, adducts, and structural or numerical chromosomal changes. Nanosilver was the first nanomaterial reported to cause DNA damage. This effect was observed in various cells. Ma et al. [33] observed that nanosilver caused DNA damage accompanied by cell cycle arrest in human dermal fibroblasts. Hackenberg et al. [34] demonstrated genotoxic effect of nanosilver in human mesenchymal cells. However, to elucidate these effects, nanosilver had to be used at a significantly higher concentration as compared to antimicrobial effective levels. In mammalian cells, nanosilver caused an increase of p53 protein expression and Rad51 expression. The latter is a double-strand break repair protein. Nanosilver also induced apoptosis. The strength of this genotoxic influence of nanosilver depended on a kind of nanosilver particles used. Polysaccharide-coated nanosilver particles exhibited more severe DNA damage than uncoated [23].

Nanosilver can cause DNA damage mainly by two mechanisms. The augmentation of ROS generation by nanosilver may result in an oxidative damage of both proteins and DNA. Increased oxidation may lead to a transformation of nanosilver to silver ions Ag^+ which can bind to guanine N7 atom. While increasing concentration, they can also bind to adenine N7 atom. Silver ions can also induce G1 phase cell cycle arrest and, at a higher concentration, a complete arrest in the S phase [35]. Nanosilver was also reported to induce formation of micronuclei due to the disruption of genetic material division. Formation of micronuclei is used to measure a potential of genotoxicity due to its sensitive response to various abnormalities in chromosomal segregation [36].

A study of Ivask et al. [36] revealed that nanosilver and graphene oxide nanoparticles shifted a melting point, triggering an early onset of DNA melting. These nanoparticles could also change a DNA hydrodynamic size. These observations may indicate novel mechanisms of nanoparticle genotoxicity. Very thin (1.4 nm) gold nanostructures can intercalate with DNA,

leading to cell death in human cancer cells [37]. SiO₂ nanoparticles can enter into the nucleus and localize in the nucleoplasm. However, they are not considered as carcinogenic [37,38].

Lan et al. observed that TiO₂ nanoparticles and carbon black nanoparticles could damage DNA via oxidative stress in human cells. These authors reported that eukaryotes, especially mammalian cells, were more sensitive to the genotoxicity than prokaryotes [39]. NIOSH has classified TiO₂ as carcinogenic. Interesting results were obtained by Darnes et al. who observed that genotoxicity of carbon nanotubes increased with their width [40].

CdS quantum dots are semiconductor nanocrystals with an increasing use. Their potential toxicity has become a health concern. A study of Munari et al. [41] indicated that this nanomaterial exhibited a concentration-independent genotoxicity in rainbow trout cell line RTG-2. In the same experiment, nanosized Ag₂S showed neither cytotoxicity nor genotoxicity.

While evaluating and comparing results of studies on genotoxicity of nanomaterials, an influence of several factors should be considered. The conflicting results of various studies may be caused by two groups of factors. The first one deals with nanoparticles and comprises variation of size of the nanoparticles, variations of size of distribution, various purities of the nanomaterials with the same average size of nanoparticles, differences of their coatings, differences of crystal structure of the types of nanomaterials, differences of size of aggregates in solution or medium, and different concentrations of nanomaterials used in assay test. The second group comprises testing conditions such as cell number, cell culture plate format and volume of treatment medium on the plate, and differences in assays [42,43].

5.3. Activation of inflammatory pathways

Nanoparticles can influence macrophages and neutrophils eliciting an inflammatory response. These cells try to destroy foreign objects, usually microbial pathogens, inducing enormous ROS generation. Nanoparticles are also treated by macrophages and neutrophils as something foreign and these cells try to get rid of them, increasing production of ROS. It leads to an induction of inflammation [20,31]. An augmented ROS generation can increase the inflammatory response by activation of expression of nuclear factor kappa B (NFκB). It is one of the major transcriptional factors. It plays an important role in cell survival, differentiation, and proliferation. It is also involved in growth and development of the immune system. NFκB is heavily involved in the initiation of inflammatory response [14].

Many nanoparticles have also been reported to induce inflammatory response through activation of expression of tumor necrosis factor alfa (TNF-α). Nanoparticles can also lead to an increased expression of other proinflammatory cytokines such as interleukins 2, 6, 8, and 10 (IL-2, IL-6, IL-8, and IL-10) [14,20].

ROS generated by nanoparticles can be either bound to the nanoparticle surface or generated as free entities in surrounding aqueous suspension. An antioxidant enzyme, glutathione reductase, can reduce metal nanoparticles into intermediates potentiating the ROS generation. In this way, nanoparticles can not only increase the generation of ROS but also interfere with the antioxidant protection mechanisms [31].

Macrophages and neutrophils can internalize nanoparticles. The consequence of this process is an activation of these cells, elucidation of inflammatory response, and even cytotoxic effects of nanoparticles on these cells [20,31].

Pulmonary inflammation seems to be the most prominent inflammatory response generated by nanoparticles. SiO₂ and TiO₂ nanoparticles have been reported to induce this response, whereas occupational exposure to metal nanoparticles such as Fe, Mn, Si, Cr, and Ni present in welding fumes may elicit both inflammation and fibrogenic response [31].

6. Conclusions

A family of nanomaterials is rapidly enlarging. The spectrum of the use of nanomaterials is getting wider, comprising medical, paramedical, and everyday use. In medicine, they can be used either alone or in combination with other “classical” compounds, e.g., cytostatic drugs or antibiotics. The latter situation uses their ability to penetrate to certain cells or intracellular compartments. Nanoparticles are also introduced as valuable tools improving existing diagnostic methods, e.g., MNR.

New issues concerning nanomaterials arise, an industrial exposure and environmental pollution. Nanoparticles are being discovered in already known pollutions like diesel exhaust or welding fumes. These “unwanted” nanoparticles put new light on the toxicological mechanisms of already known pollutions. It also makes us look at pollutions in macroscale, microscale, and nanoscale. A rapidly developing branch of electronics also creates new sources of possible occupational exposure hazard. This situation creates new challenges for both classical toxicology and nanotoxicology. Centuries ago, Paracelsus said, “everything is a poison and nothing is a poison, it is only a matter of a dose.” In a case of nanomaterials, it is a case of both dose and particle size [1].

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