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APPLICATION OF SILVER NANOPARTICLES IN EXPERIMENTAL PHYSIOLOGY AND CLINICAL MEDICINE: CURRENT STATUS AND FUTURE PROSPECTS

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Abstract. Silver nanoparticles (AgNPs) are today one of the most commonly used nanomaterials both in fundamental medical sciences and clinical practice. These nanoparticles are also incorporated into many commercial products and widely available to general population. However, recent reports have linked silver nanomaterials to programmed cell death, and increased cytotoxicity in certain conditions. This short review focuses on the recent findings regarding molecular interactions of silver nanoparticles with living cells and tissues. Potential immunomodulatory effects of AgNPs, as well as recent toxicity concerns are also discussed. Finally, we also describe recent public and government efforts to monitor and control the use and availability of silver nanomaterials used as dietary supplements in some countries.

1. INTRODUCTION

Silver nanoparticles (AgNPs) are today one of the most commonly used nanomaterials both in everyday life, and in research laboratories. These nanoparticles are incorporated into many commercial products including clothing/textiles, furniture, household appliances such as refrigerators, cosmetics, and even children toys [1]. This high degree of AgNP commercialization has been achieved due to their significant antimicrobial and antifungal properties, while on the other hand many manufacturers claim that potential AgNP toxicity is minimal or nonexistent . Silver nanoparticles are also commonly used in medical practice as an integral part of both surgical and nonsurgical equipment such as wound dressings, bandages, catheters, etc. [2-7].

Silver nanoparticles indeed have substantial antibacterial and antifungal effects in *in vitro* conditions, however potential toxic effects of both low and high quantity intake of AgNP on living organisms are not entirely understood. Recently, it has been suggested that AgNPs in some circumstances may have substantial genotoxic effects, and some authors indicated that AgNP exposure in some cells may lead to the process of programmed cell death (apoptosis) [8-15]. Potential immunomodulatory effects of silver nanoparticles also remain unknown with only a handful of studies claiming that AgNPs may change the production and release of some cytokines, and impact molecular signaling in immune cells. This lack of data, together with a wide availability of silver nanomaterials to the general public may in the future pose a significant public health risk.

This short review focuses on the recent findings regarding molecular interactions of silver nanoparticles with living cells and tissues. Potential immunomodulatory effects of AgNPs, as well as recent toxicity concerns are also discussed. Finally, we also describe recent public and government efforts to monitor and control the use and avail-

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ability of silver nanomaterials used as dietary supplements in some countries.

2. ANTIMICROBIAL EFFECTS OF SILVER NANOPARTICLES

Antimicrobial properties of silver nanoparticles and silver ions have been known for decades. Silver in both forms not only has suppressive effects on bacterial growth, but I also efficiently destroys existing bacteria. Recent reports have indicated that AgNPs exhibit toxic effects on the most pathogenic bacteria posing a health risk during wound healing, and postoperative recovery. The similar results were observed for the bacteria that are responsible for the most of food and waterborne infections. Bactericide effects were observed in Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Listeria innocua, Salmonella Choleraesuis [16-18]. Silver nanomaterials have also been shown to have antifungal properties, and the most important findings were related to toxic effects toward Candida albicans species [17, 19-22]. It was shown that colloidal silver nanoparticles in very low cincentrations may have substantial antifungal impact *in vitro*.

The mechanisms of antibacterial AgNP action are unfortunately not completely understood. It is supposed that silver inhibits the function of proteins, and DNA replication [23]. Another theory is that silver causes damage to the bacterial membrane by incorporating itself into membrane structure and interacting with the major “building elements” [24]. This structural damage causes changes of membrane permeability to water and various ions, which in the end leads to bacterial death.

Although it is true that silver in high concentrations has strong antimicrobial effects, however so far, for most bacteria, there is no agreement regarding minimal silver NP concentrations that would lethal in *in vitro* conditions. Furthermore, some data suggest that AgNPs, when in very small concentrations could even promote bacterial growth. The explanation is that in some cases, bacteria are capable of developing resistance to silver [25-28]. These cases have already been described in certain bacteria that can lead to complications of wound-healing process. Another concern is that there is a documented link between heavy metal –bacteria interaction and development of resistance towards some commonly used antibiotics [1,29]. The precise molecular mechanism that is the basis of this relationship is not yet completely understood.

3. IMMUNOMODULATORY EFFECTS OF SILVER NANOPARTICLES

Although some AgNP manufacturers, particularly the ones that produce solutions of colloidal NPs, claim that AgNPs enhance immunity toward infections, potential imunomodulatory effects of silver nanoparticles are unknown. There are several reasons for this lack of data. Firstly, today among scientific community, there is no consensus regarding which immunology / molecular biology test should be used as the gold standard for evaluation of immunotoxicity of nanomaterials [30-32]. Some traditional tests such as T-dependent antibody response (TDAR) assay [33] which were in the past relatively popular for assessment of xenobiotic immunotoxicity, are today rarely used compared to ELISA and other similar protocols. Secondly, due to the small size of AgNPs and their proposed, but not yet completely understood ability to pass through membranes, it is possible that during the interaction with immune cells, they may bypass some conventional signal pathways reserved for larger molecules. Furthermore, silver nanoparticles can dramatically vary in size and shape, and data obtained for one AgNP type do not necessarily imply that similar effects are caused by all AgNPs.

Particularly interesting are the potential interactions between silver nanoparticles and peripheral blood mononuclear cells (PBMCs). It has been suggested that AgNPs may modulate interleukin 6 secretion mediated by Toll-like receptor (TLR) signaling in macrophages [34]. Interleukin 6 is known as both pro-inflammatory and anti-inflammatory cytokine and regulation of its secretion is of major importance during infection and wound healing. Another study on has discovered that monocytes may increase release of interleukin 1β as the result of AgNP exposure. Apart from its role in inflammatory response, interleukin 1β influences lymphocyte proliferation and maturation [35]. If these AgNP effects are confirmed in the future, it could open new possibilities in design of modern immunomodulatory medications as well as experimental models for laboratory research.

4. SILVER NANOPARTICLE TOXICITY

Since silver nanomaterials are today included in so many various consumer products, one should never underestimate the possibility of AgNP toxicity as the result of accidental intake or exposure to higher NP concentrations. There are many ways silver nanomaterials may accidentally enter human organ-

ism the most common of which are orally, by inhalation and through the skin. Toxicity of this high-dose AgNP exposure is predominantly the result of detrimental effects of silver ion to the function of several tissues and organs.

Probably much bigger concern today is the toxicity occurring as the result of chronic intake of low-dose AgNPs over the time period ranging from several days to several years. Wound dressings containing AgNPs may facilitate the NP entry directly through the skin lesion into the blood stream. Refrigerators and other household appliances that come into contact with food may be a source of chronic oral AgNP exposure. There has even been some recent reports suggesting that preteen children chewing textiles/clothing containing AgNP (for antimicrobial purposes) may over time ingest significant AgNP quantity with unknown effects [1].

To completely understand the mechanisms of toxicity of silver nanoparticles, it is important to investigate to what extent this toxicity is caused by silver in ionic form. When AgNPs reach specific cell microenvironment, it is possible that certain percentage of NPs may be transformed into ionic form under influence of certain chemical mediators. Even when the AgNP passes through the cell membrane and enters the cell, additional Ag ions may be created and express cytotoxic or genotoxic effects. This is a major problem in modern AgNP toxicology research. The exact probability of this NP-ion transformation inside and outside the cell is unknown, and unfortunately many years will pass until the adequate experimental model, or method is designed that would accurately quantify this process. Also, even if we find the way to efficiently measure the role of Ag ions in AgNP toxicity in cell cultures, that will not automatically imply that the same rules are present in living organisms.

Today, one of the most important sources of chronic AgNP exposure are colloidal silver solutions that are in many countries commercially available to general public as over-the-counter drug products. These products are commonly sold as dietary/nutritional supplements, and therefore do not undergo the same quality and toxicity check procedures required by government agencies for approval of medications. The manufacturers often heavily advertise these products claiming they have antimicrobial effects, and therefore may be used for combating infections, or for prophylactic purposes. Some even state that colloidal silver may have significant antitumor properties, although so far, no relevant study has been done to even partially support these claims.

There were several cases in the past regarding toxic effects of colloidal silver nutritional supplements. Argyria, manifested as silver discoloration of skin as the result of silver deposition in soft tissues, was probably the most common [36-38]. Many manufacturers claim that argyria occurs only when the product, apart from AgNP, contains significant quantity of silver in ionic form. However, one should have in mind that silver in nano form can easily convert into ionic form, not only inside the product itself, but also in extracellular body fluids (plasma and interstitial fluid), and even inside the cell. As mentioned earlier, the probability that this transformation will occur and lead to the toxic effects of ionic silver is unknown.

Because of the lack of valid scientific data regarding the effectiveness and potential toxicity of colloidal silver, US Food and Drug Administration has recently published a rule to misbrand some of these over-the-counter drug products [39]. Indeed, very few research studies have been done to test the effectiveness and toxicity of colloidal silver nanoparticles in living organisms. In most cases where a colloidal silver manufacturer state scientific data related to the product, these results are obtained in *in vitro* conditions, in cell cultures or bacterial colonies, rather than in laboratory animals or human subjects. This lack of data, in combination with worldwide availability of these products via internet and mass media, might in the future pose a significant public health risk.

5. FUTURE OF SILVER NANOPARTICLE RESEARCH

We expect that in the future, significant research efforts will have to be made to investigate the genomodulatory effects of silver nanoparticles. The recent preliminary study carried out by our laboratory showed that AgNPs impact the fractal morphology of the nucleus in isolated buccal cells [40]. Colloidal silver nanoparticles in concentrations present in today's dietary supplements caused time-dependent reduction of nuclear structural complexity in isolated buccal epithelial cells. This could indicate that AgNPs influence nuclear morphology at a much larger scale than previously thought, since most of the recent research on AgNP genotoxicity was done on a molecular level. Future research will have to confirm the proposed genotoxic potential of AgNPs and investigate possible further morphological changes occurring in cell nucleus after AgNP exposure. This toxicity evaluation will be particularly important since it is expected that in the future

more and more medications for various diseases will include nanomaterials either as an active substance or drug carriers [41-44].

Another issue that would have to be addressed is whether AgNPs have similar effects on the living organisms as they have in cell cultures and isolates. So far, published results indicate this is not the case, at least regarding toxicity. It seems that silver nanoparticles, when administrated to laboratory animal, do not exhibit substantial toxic effects on immune system, blood, and liver, nor do they have any acute toxic effects in dermal and mucosal tissues [45,46]. However, one should have in mind that these are only the first results in this relatively new research area. Many years will have to pass until we can say that presently commercially available silver nanomaterials are truly safe for application both in everyday life and medical practice.

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