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EFFECTS OF METALLIC NANOPARTICLES ON PHYSIOLOGICAL LIVER FUNCTIONS

Jovana Paunovic[1], Danijela Vucevic[1], Tatjana Radosavljevic[1], Senka Pantic[2], Dubravka Nikolovski[3], Stefan Dugalic[4] and Igor Pantic[5,6]

[1]Institute of Pathological Physiology, School of Medicine, University of Belgrade, Dr Subotica 9, RS-11129, Belgrade, Serbia
[2]Institute of Histology and Embryology, School of Medicine, University of Belgrade Visegradska 26/II, RS-11129, Belgrade, Serbia
[3]Institute of public health Pancevo, Pasterova 2, RS-26000, Pancevo, Serbia
[4]Clinic for Gynecology and Obstetrics, Clinical Center of Serbia, School of Medicine, University of Belgrade, Dr Koste Todorovica 26, RS-11000, Belgrade, Serbia
[5]Laboratory for Cellular Physiology, Institute of Medical Physiology, School of Medicine, University of Belgrade, Visegradska 26/II, RS-11129, Belgrade, Serbia
[6]University of Haifa, 199 Abba Hushi Blvd. Mount Carmel, Haifa IL-3498838, Israel

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Abstract. Today, many issues concerning the interactions between metallic nanoparticles (MNPs) and biological systems (cells and tissues) are unclear. This is particularly the case regarding effects of MNPs on hepatocytes and other liver cells. Although considered safe in certain conditions, numerous studies have shown that some MNPs are capable of inducing severe hepatotoxicity in experimental animal models. Sometimes, MNP-induced changes in liver tissue are visible using conventional histological methods. In other cases, these changes are more discrete and limited only to individual cells, in terms of programmed cell death. Metallic nanoparticles may cause generation of reactive oxygen species with subsequent damage of cell membrane and DNA. This concise article focuses on the recent research on the effects of metallic nanoparticles on liver physiological functions with emphasis on their potential hepatotoxicity in humans and experimental animal models.

1. INTRODUCTION

Nanoparticle (NP) is by definition an object with the diameter of less than 100 nanometers. Because of their small size, large surface area, and specific chemical/physical characteristics, NPs are today widely investigated, and are a focus of many research laboratories across the globe [1,2]. There are many different classifications of NPs, based on their chemical structure, morphology, applicability, synthesis method etc. For example, terms such as nanorods, nanospheres, nanochains and nanoboxes refer to the shape and microscopic morphology of NPs. The nature of nanoparticle can be organic or inorganic, depending on the inclusion of organic compounds (proteins, lipids etc.) in their manufacturing [3,4].

Today, probably best investigated and applied inorganic nanomaterials are the ones which incorporate, or are made from various metals, such as iron, silver, gold or titanium. Metallic nanoparticles (MNPs) are considered as potentially important part of various drug delivery systems applicable in internal medicine, neurology and cancer research [5]. Some MNPs, such as the ones made from silver are known for their antibacterial properties, and are used in industry and engineering. Some MNPs are

Corresponding author: Igor V. Pantic, e-mail: igor.pantic@mfub.bg.ac.rs

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today part of numerous consumer products such as apparel, household appliances, cosmetics, and even dietary supplements [6-8].

Since nanotechnology and nanobiology are relatively new disciplines, many issues regarding the interaction between MNPs and biological systems (cells and tissues) are unclear. This is particularly the case with their potential acute and chronic toxicity in liver, kidneys and central nervous system. Toxic effects of MNPs on liver (hepatotoxicity) are today a major concern in nanomedicine, since liver is often the first organ that comes into contact with MNPs after their ingestion. This concise article focuses on recent research on the effects of metallic nanoparticles on liver physiological functions with emphasis on their potential hepatotoxicity in humans and experimental animal models.

2. PARAMETERS OF LIVER FUNCTION RELEVANT TO MNP RESEARCH

The most important parameters that can be useful in evaluation of MNPs hepatotoxic effects include aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, bilirubin, and gamma glutamyl transpeptidase. These compounds are in most cases measured in plasma or serum, and liver damage usually results in their elevated concentrations.

Elevated transaminases (AST and ALT) may be an indicator of hepatocyte destruction, either due to the direct toxic effects of MNPs or the subsequent immune system activity. AST/ALT ratio may be particularly important indicator for differentiating between different causes of hepatotoxicity (causes other than NPs), although its applicability in MNP toxicity evaluation is unclear. Unfortunately, neither AST nor ALT are specific parameters of liver damage, since diseases that affect various other tissues (i.e. skeletal muscle, heart, thyroid) may also lead to their elevation in blood [9-12].

Alkaline phosphatase (ALP) is also a nonspecific indicator of liver function commonly used both in laboratory research and clinical practice. Factors that affect normal production and secretion of bile may lead to the elevated blood levels of this enzyme. Some of the examples may include cholestasis and bile duct obstruction due to tumors or injuries. Apart from liver, ALP is also present in other tissues such as bone and placenta [9-13].

Bilirubin is a compound that acts as a part of normal catabolism of hemoglobin. It is regularly excreted both in bile and urine and its significant rise in body fluids causes jaundice as a medical symptom. Bilirubin is conjugated in liver by the enzyme glucuronyltransferase which may be inhibited by various substances and medications. Blood concentrations of both conjugated ("direct") and unconjugated ("indirect") bilirubin are important for estimation of liver damage, and its overall capacity to produce and secrete bile [9-12].

The above mentioned indicators of liver function are routinely determined in clinical setting and the biochemical methods for the measurement are relatively simple and inexpensive. In fundamental medicine, in laboratory animals and *in vitro*, additional important parameters worth mentioning, include reactive oxygen species (ROS) such as hydrogen peroxide and superoxide. ROS are generally generated as a product of normal metabolism. However, after cellular damage their concentration in tissue may significantly increase resulting in oxidative stress. These compounds have detrimental effects on fatty acids in cellular membranes, as well as DNA molecule leading to cell death (through apoptosis or necrosis), mutations, or even in some cases malignant transformation [13-15].

Today, most of the MNP drug delivery and other systems are still under development and have not yet been introduced in clinical practice. Some MNP-based compounds have already been tested for acute liver toxicity which yielded promising results. Other MNPs were found to exhibit various cytotoxic and genotoxic properties. It seems that the significant portion of the current MNP hepatotoxicity research is focused on their effects on ROS creation in hepatocytes and other liver cells. Chronic hepatotoxicity and effects of MNPs on liver-related enzyme levels in living organisms is largely uninvestigated.

3. SILVER NANOMATERIALS AND LIVER

Silver nanoparticles (AgNPs) are today probably one the most commonly used nano-sized compounds in industry, manufacturing and biomedicine. Antimicrobial properties of AgNPs and supposed non-toxic nature make them good candidates for integration in numerous commercial products such as refrigerators, air purifiers, air conditioners, washing machines etc. Also, there are numerous, unverified, and sometimes anecdotal reports of beneficial effects of colloidal nanosilver on human health. Many nutritional supplements, available as over-the-counter products today contain colloidal silver. This is why potential toxicity of AgNPs, including hepa-

totoxicity might be a major public health concern in the future [16-18].

Recently, it was suggested that some silver-based nanosystems may induce hepatotoxicity in mice. Ramadi et al. (2016) performed experiments using composite inorganic Ag:Cu:B nanoparticles was associated with increased levels of aspartate transaminase and alanine transaminase. In liver tissue, the authors reported necrosis of hepatocytes, granulomas, abscesses, as well as increased numbers of sinusoidal Kupffer cells [19]. This detrimental effect of NPs on liver may be related to immune system activation, especially mediated by proinflammatory mediators such as interleukin 1 and tumor necrosis factor alpha.

Another important study on potential hepatotoxicity of nanosilver was the one of Heydrnejad et al. (2015). The research was also done on a mouse experimental model (BALB/c mice, oral administration). Similarly to the above mentioned experiment of Ramadi et al. the authors measured significant rise of aspartate transaminase and alanine transaminase as well as changes in liver morphology [20].

Autophagy (autophagocytosis) as a physiological self-degradative process may have a significant role in AgNP-induced hepatotoxicity. In some laboratory animals, administration of AgNPs may lead to increase of autophagic structures in liver cells and changes in expression of major chemical parameters of autophagy such as LC3-II protein [21]. Alterations of autophagy process may be connected to changes in cellular energy state (ATP concentrations) which may increase the probability of programmed cell death (apoptosis).

Silver nanoparticles may induce significant oxidative damage in liver tissue of adult zebrafish. Eom and Choi (2010) described the increase of malondialdehyde (indicator of lipid peroxidation in cells) and total glutathione, as well as reduction of enzymes catalase and glutathione peroxidase. DNA damage in liver was also shown to be significant by measuring γ-H2AX, a specific marker of DNA double-strand break [22].

In experimental model of Sprague-Dawley rats, oxidative stress may also play the major role in overall hepatotoxic effects of silver nanoparticles. For example, in a recent study, Patlolla et al. (2015) indicated that short-term oral intake of relatively high doses of Ag-NPs (50 and 100 mg/kg) increases production of reactive oxygen species [23]. On the same rat model, Ebabe Elle et al. (2013) also showed that dietary exposure to silver nanoparticles (500 mg/d/kg) also increases quantity of free radicals such as superoxide O_2^- anion. Nevertheless, it should be noted that in this study, levels of liver malondialdehyde and superoxide dismutase activity did not significantly change [24].

The data on interactions between silver nanoparticles and intracellular environment remain scarce and unclear. One theory is that AgNPs act as a "Trojan horse", using their specific chemical properties to freely enter the cell (pass through the membrane) after which they are converted to silver ions. This accumulation of silver in its ionic form is toxic both for cell DNA and organelles and may induce various signal pathways leading to ROS creation, apoptosis or necrosis. The other possibility is that AgNPs themselves exhibit cytotoxic and/or genotoxic properties. Also, another possibility that should be considered when investigating AgNP hepatotoxic potential, is the induction of immune system and increased production of chemical mediators (i.e. cytokines) that may indirectly cause liver inflammation and damage.

4. GOLD NANOPARTICLES

There are many forms of gold nanoparticles (AuNPs) today used in fundamental and clinical research. Some of those include gold spherical particles, gold nanorods and nanowires. Colloidal gold was used for hundreds of years as an additional treatment for numerous illnesses. Today, AuNPs are extensively studied as potentially efficient drug carriers that can be functionalized and applied in cancer therapy [25,26]. Specific physical properties of AuNPs enable them to be an integral part of therapeutic cell targeting systems that might reduce toxicity and side effects of anticancer medications. Therefore, any novel insight into modulatory effects of AuNPs on liver functions is potentially valuable in current pharmacology research.

Interaction between these nanoparticles and organs greatly depends on their diameter, shape and surface charge. Some AuNPs accumulate in the liver, while others tend to concentrate in lymphatic organs. For example, gold nanoparticles sized approximately 30 nanometers can decrease liver energy metabolism. When administrated acutely, in rats, they also may increase byproducts of lipid peroxidation such as thiobarbituric acid reactive substances and activity of superoxide dismutase [27]. Particles of other soze, may not have the same properties. Elci et al. (2016) state that positively charged NPs show tendency to accumulate in hepatocytes whereas uncharged NPs have relatively af-

finity to liver Kupffer cells (stellate macrophages, Kupffer-Browicz cells) [28].

Gold nanoparticles, when administrated intravenously, mainly accumulate in liver tissue. This accumulation may be followed by the increase of aspartate transaminase and, interestingly, reduction of alanine transaminase. These changes in AST and ALT levels were demonstrated in recent work of Bednarski et al. (2015), in which the authors also demonstrated that oral administration of AuNPs is followed with relatively low rates of absorption [29].

On the other hand, Rambanapasi et al. (2016) found no evidence of overt hepatotoxicity of AuNPs in Sprague Dawley rats. The study applied colloidal suspensions of 14 nm gold nanoparticles in 7 weeks period, and there were no significant differences in liver-related enzymes as well as in total bilirubin. Histopathological analysis of liver tissue showed no signs of morphological damage [30]. These results are not in accordance with the previously published findings of Sengupta et al. (2013) which demonstrated substantial toxicity visible in various tissues using standard microscopy [31].

It should be noted that many issues regarding potential effects of AuNPs on liver physiology remain unresolved. For example, there is a substantial lack of data on interactions between AuNPs and immune system, particularly locally in the liver tissue. It is unknown if AuNPs can cause alterations in hepatocyte function indirectly via increased or decreased production of certain interleukins. Also, it is unclear if potential toxic effects of AuNPs in other organs (spleen, central nervous system etc.) can cause release of toxic metabolites able to induce liver damage. All these questions need to be answered in the future, before AuNP-based drug delivery systems can become part of conventional therapies in internal medicine and neurology.

5. IRON-BASED NANOMATERIALS

Nanosized iron particles are generally unstable and react with water and oxygen forming iron ions. Iron oxide nanoparticles (IONPs) are composed of magnetite (Fe_3O_4) or maghemite (Fe_2O_3). They possess superparamagnetic properties which give them enormous potential as mediums for diagnosis (i.e. in high-sensitivity biomolecular magnetic resonance imaging, various biosensors) and therapy. Superparamagnetic can be functionalized and associated with numerous organic compounds which is useful for specific targeting of altered pathological cells/tissues and efficient drug delivery. One interesting application of IONPs is inductive hyperthermia in cancer tissue in which the particles are remotely controlled. Unfortunately, potential toxicity of IONPs is their major limiting factor for use in clinical practice.

Iron oxide – based nanomaterials in experimental animal models may elevate concentrations of enzymes associated with liver injury. For example, Kumari et al. (2013) showed that in female Wistar rats, Fe_2O_3 nanoparticles taken orally can increase aspartate aminotransferase and alanine aminotransferase in serum and liver. Activity of another enzyme, lactate dehydrogenase, was also increased [32].

Modified, coated IONPs might also exhibit certain hepatotoxic potential. In a recent research, Rajan et al. (2015) demonstrated that polyethylene glycol-8000 coated ultra-small superparamagnetic iron oxide nanoparticles (PUSPIOs) cause rise of AST activity. It was also shown that PUSPIOs substantially increase the rates of serum and liver lipid peroxidation [33].

There is a possibility that IONPs in some circumstances may cause visible changes in liver tissue structure. For example, Vasili et al. (2015) reported that intratracheal administration of Fe_2O_3 nanoparticles (20 nm size) may cause inflammation, centrilobular sinusoid congestion, ballooning degeneration, and mononuclear cell infiltration. Interestingly, it seems that low intensity aerobic exercise can at least partly attenuate damage caused by IONPs [34].

Finally, it should be mentioned that not all research on IONPs came to the conclusion on their hepatotoxicity. For example, a recent study by Easo and Mohanan (2016) evaluated the hepatotoxic response after intravenous administration dextran stabilized IONPs. The authors did not observe significant changes in lipid peroxidation (despite some induction of redox defenses). Also, the activity of enzymes such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin levels also did not significantly change [35].

These and other discrepancies in the results of IONP hepatotoxicity evaluation indicate that this issue remains unclear and requires additional research. In the future, apart from ROS generation, enzyme activation and histological changes, the ability of IONPs to cause programmed cell death should also be further investigated. Having in mind the numerous potential applications of IONPs in the fields of physiology, pharmacology and oncology, this research should be given high priority.

6. TITANIUM, ZINC, PLATINUM, AND OTHER NANOSYSTEMS

Nanoparticles made of other metals, such as titanium, zinc, platinum, copper, aluminum etc. also play important roles in contemporary physiology, pharmacology and toxicology research. For example, titanium dioxide nanoparticles, apart from the application in drug delivery, might also be used as photocatalysts, as well as in cosmetics industry, ceramics etc. Zinc oxide nanostructures have great potential in production dye-sensitized solar cells and various electrical equipment. In cosmetics and medicine, zinc oxide nanoparticles may be used in sunscreens because of their protective properties against ultraviolet radiation. These nanomaterials also have potentially significant antibacterial activity. Platinum nanoparticles are useful in aging research and in some countries are sold as dietary supplements (colloidal platinum). These properties and applications are just an example of how important various metallic nanoparticles are in the fields of biology, medicine and engineering [36-39].

Titanium, zinc, platinum are all currently under investigation for potential ability to induce creation of reactive oxygen species in both *in vivo* and *in vitro* conditions. Often, reports on their impact on free radical formation are inconclusive and sometimes even conflicting. In the future, it remains to be seen if the amount of generated ROS is sufficient to cause membrane and DNA damage in hepatocytes and other liver cells.

Also, the impact of these MNPs on enzyme parameters of liver function is unclear. Extensive and valid studies on this issue are relatively rare, and are sometimes with numerous methodological limitations. In should be noted that occasional rise in AST and ALT does not necessarily imply liver toxicity, at least not on the level considered unsafe (i.e risk of cirrhosis or hepatic insufficiency). Also, there is a significant lack of data regarding potential chronic hepatotoxic effects of many of these MNPs.

7. CONCLUDING REMARKS

In conclusion, interactions between metallic nanoparticles and biological systems, are not completely understood. This is particularly the case regarding effects of MNPs on hepatocytes and other liver cells. Although considered safe in certain conditions, numerous studies have shown that some MNPs are capable of inducing severe hepatotoxicity in experimental animal models. Sometimes, MNPs-induced changes in liver tissue are visible using conventional histological methods. In other cases, these changes are more discrete and limited only to individual cells, in terms of programmed cell death. MNPs may cause generation of reactive oxygen species with subsequent damage of cell membrane and DNA. In the future, because of the importance of MNPs in various medical and non-medical research areas, additional research will need to be conducted in order to draw definite conclusions on MNP effects on physiological liver functions.

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