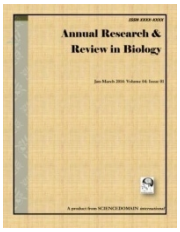

Annual Research & Review in Biology
4(8): 1346-1352, 2014

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The Toxicity of Gold, Silver, and Zinc Oxide Nanoparticles on LDH Enzyme in Male Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author MN designed the study, wrote the protocol and managed the literature searches, author MA performed the statistical analysis, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 17th June 2013
Accepted 13th December 2013
Published 8th January 2014

ABSTRACT

Aims: Nanoparticles have the potential to be used in medical imaging, disease diagnosis, cancer treatment and other procedures. These nanoparticles accumulate in the body tissues and result in oxidative stress with the generation of reactive oxygen species. This study investigates the effects of gold, silver, and zinc oxide nanoparticles on the LDH enzyme in male mice.
Methodology: Adult Wistar strain albino mice (60) weighing 20-38 g were used for this study. The mice were randomly assigned to 3 classes in a way that in each class there were four groups of which one group was control and the other three groups were fed by zinc-oxide nanoparticles **(**ZnONPs), gold nanoparticles (AuNPs), and silver nanoparticles (AgNPs) at 100, 50, 25 ppm concentrations, respectively, for 15 days and the heart blood was taken to measure LDH enzyme activity at the end of treatment.
Results: There was a significant difference ($p<0.05$) in the LDH level with use of a moderate concentration of gold nanoparticles (50mg/kg) and in moderate and low concentrations of silver nanoparticles (50 and 25mg/kg) and in all concentration of zinc oxide nanoparticles (100, 50, and 25mg/kg) as compared to the control group.
Conclusion: Our results show that a moderate concentration in each of the three

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nanoparticles leads to an increase in LDH enzyme activity as compared with the control group.

Keywords: Gold; LDH enzyme; nanoparticles; Silver; Zinc oxide.

1. INTRODUCTION

The oxidoreductase, lactate dehydrogenase (LDH), is an enzyme released by various tissues, e.g. heart, skeletal muscle, and liver, as well as cerebrospinal fluid, that converts lactate to pyruvate and requires the coenzyme NAD^+ which accepts a hydride ion from lactate. LDH consists of two different subunits, M and H that forms five tetrameric isozymes in different organs. LDH deficiency results in exercise intolerance and episodes of myoglobinuria (acute muscle breakdown leading to rust-colored urine), with skin rash being common. The disease is caused by a genetic defect in the LDH enzyme, which normally recycles the byproducts of carbohydrate metabolism [1,2]. The release of LDH into the circulation indicates tissue damage, and by measuring the effects of nanoparticles (NPs) on the body we found that gold, silver and zinc oxide NPs elevate circulating LDH levels, indicating effects on various tissues.

Presently, the vast application of these NPs in the delivery of drugs, toothpastes, toiletries, hygienic cosmetics, medical equipment, and clothing results in damage to the body. Inorganic nano structures applied in the nanometer (nm) range are made from tiny crystals with extended application in medical research, diagnosis of diseases and drug delivery to cells for treatment. Nano-sized particles have been introduced for a wide range of applications with high efficiency in medicine, prevention and treatment of diseases, and drug development [3,4]. With regard to the effects of size, shape, bioavailability, uptake, and sub cellular distribution of NPs, our study focuses on these issues. A recent report addresses the issue of uptake and cytotoxicity of NPs [5]. The study of NP toxicity on the body and in pollution has been implicated in many studies [6-8]. Thus, the current work was performed to study the effects of three kinds of NPs on LDH enzyme activity with the intent to better understand and control human safety.

Today, nano has continued to embed most of science and research is as including human health and the environment [9-12]. In the biomedical field, one of the most important attributes of these NPs is their effect on many enzymes [13]. Silver nanoparticles (SNPs) are categorized as the most commercialized nanoparticles worldwide. According to the biological data received from different studies in terms of the great variety in sizes, coatings or shapes of the particles, SNPs are the most common nanomaterial added to commercially available products. Consequently, understanding how the size influences toxicity is vital for the safe use of these new products. Today, SNPs are used in the clothing industry, sterile gauze manufacturing, and for healing burns [14].The structures and properties of gold nanoparticles (AuNPs) make them useful for a wide variety of biological applications. Many questions arise about the risk and the impact on human health following exposure to NPs because of their toxicity. It has already been demonstrated that inhaled NPs can be rapidly delivered to other organs, such as liver and kidney [15]. Colloidal gold is used in the treatment of arthritis [16]. Zinc oxide nanoparticles (ZnONPs) have also been used as a model material to investigate the nano-specific toxicity by comparing cytotoxicity. NPs of ZnO, due to their unique characteristics, can be used in a variety of colors from cosmetic, ceramics, water and wastewater treatment, and many other industries [17]. Hypotheses

have been raised about the possible damages caused by the threatening attitude which will hinder growth and development of nanotechnology. Unless, the information becomes accurate and impartial about how to avoid the hazards which are found and released, human safety could be compromised [18,19]. It is suggested that ZnNPs should be applied with more precautions in relevant industries and also occupational health surveillance should be necessarily considered. According to the application of these NP sin drugs given for ulcer treatment, further investigation is important. With regard to the growing nanotechnological products and the use of nanotechnology in products, modern human life is exposed to biological health risks. Therefore, as an important issue in medical research, we are studying the toxic properties of nanomaterialsin an effort to protect public health.

2. MATERIALS AND METHODS

2.1 Study of Mice and Enzymology Method

Male Wistar strain albino mice (60), weighing 20-38 g were used for this study. They were supplied from the Medical University of Isfahan and were acclimatized before commencing the experiments at suitable conditions of temperature and light for a period of two weeks. The environmental conditions were set at a temperature of 25-27°C, with a relative humidity of 40-60%, a 12h light/dark cycle and the animals had free access to water and food. This study was carried out according to the guidelines approved by the Institutional Animal Ethical Clearance (IAEC) committee. The animals were randomly divided into three classes of which each class consisted of four groups with five animals in each. One group was the control that received 0.3 cc distilled water and the other three groups were fed with 0.3cc ZnONPs, AuNps, and AgNPs at 100,50,and 25 ppm concentrations, respectively, for 15 days (provided by gavage tubes). The NPs were obtained from the Tehran Notrino Company as a colloid solution (concentration of 100 ppm) with an average diameter of 10 nm as determined by transmission electron microscopy (TEM). The mice did not show any symptoms of toxicity such as change in fur color, weight loss, or any other symptoms in terms of morphology and behavior. At the end of the 15-day treatments, all the mice were fasted overnight and were euthanized on the next day to determine the level of toxicity by biochemical analysis. For chemistry analysis, the blood was withdrawn from the hearts of animals to measure LDH enzyme activity at the end of treatment. The serum was isolated by centrifugation(3000 RPM for 15 min), and the LDH activities were measured with an auto-analyzer (Hitachi 902).The addition of a color reagent led to a color reaction, and the absorbance of the colored product was then measured by a colorimeter. The measurement of LDH activity is indicated in equation 1:

$$(1) \qquad \text{Lactate} + NAD^{+} \leftrightharpoons \text{pyruvate} + NADH$$

2.2 Statistical Analysis

Statistical evaluations were conducted by spss19.0. ANOVAs and Dunnett to determine the activity of LDH enzymes, and values $p \leq 0.05$ relative to control were considered statistically significant.

3. RESULTS

3.1 Microscopic Characterization of Nanoparticles

The morphology and size of the synthesized nanoparticles were investigated by TEM. The images showed clearly that the average size of the particles was on the order of 10 nm and that they were relatively uniform in diameter and spherical in shape; the assembly was attached with a computer software programming to analyze the mean size of the particles in the sample (Fig.1). Morphologic studies and examination of the surface of synthesized nanoparticles were performed by a TU-1901 double-beam UV-visible spectrophotometer, a D/Max-RA X-ray diffract meter using CuKa radiation, and a JEM-200CX TEM. Although the TEM operates on the same basic principles as the light microscope, yet in the former the rays descend vertically. The electron microscope consists of a long column on top of which the source of electron rays is mounted. After transmitting through the specimen, electron rays impinge on a photographic film or screen (built of fluorescent materials) and create an image. Since some rays do not pass through the sample, black spots are left on the image and therefore, electron microscope images are black-and-white. Slices in a TEM are much thinner than those in a light microscope, and staining techniques also are different [20].

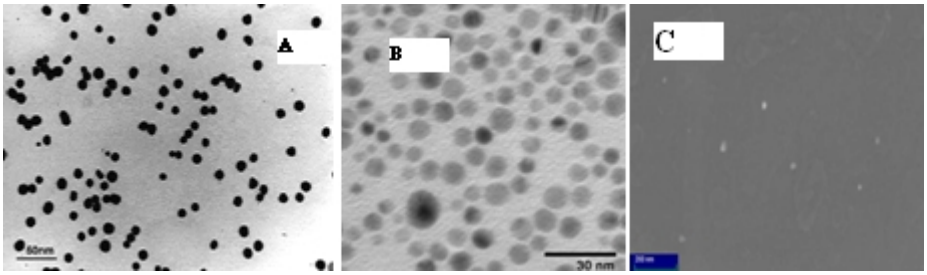

Fig. 1. Image of nanoparticles by TEM. A (AuNPs), B (AgNPs), C (ZnONPs)

The results in Tables 1-3 show that circulating LDH enzyme activity was significantly increased above control with all classes of NPs. For those animals receiving ZnO, each concentration was significantly higher than control.

Table 1. Effect of different concentrations of ZnONPs on LDH enzyme activity.*: significant difference with other groups

Groups	N	Mean	Std. Error	Pvalue
CONTROL	5	608.2000	19.99100	
25PPM	5	796.0000	34.72751	.001***
50PPM	5	1042.4000	38.41562	.000***
100PPM	5	970.0000	10.00000	.000***

When AuNps were administered to the animals, the LDH enzyme activity increased only in the group that received 50ppm NPs. It remains to be established why the activities associated with doses of 25 and 100ppm are less than control.

Table 2. Effect of different concentrations of AuNPs on LDH enzyme activity.*: significant difference with other groups

Groups	N	Mean	Std. Error	Pvalue
CONTROL	5	532.4000	1.96469	
25PPM	5	518.8000	5.59821	
50PPM	5	572.0000	19.59592	0.044*
100PPM	5	514.4000	5.03587	

Administration of AgNPs to the animals resulted in significant increases in LDH activity for the groups receiving 25 and 50ppm NPs. The mean value of activity is higher in the group receiving 100ppm than in controls, but the standard error is also higher; thus, here is no statistically significant difference with control values.

Table 3. Effect of different concentration of AgNPs on LDH enzyme activity. *: significant difference with other groups

Groups	N	Mean	Std. Error	Pvalue
CONTROL	5	544.0000	11.66619	
25PPM	5	641.1000	15.29739	.05*
50PPM	5	647.4000	46.22510	.036*
100PPM	5	553.2000	17.15343	

Interestingly, smaller silver particles are more toxic than larger ones, and nanoparticles in different doses have different effects. The relationship between the consumption dose and its toxic effects is generally not linear [21].

4. DISCUSSION

Nanoparticles have the ability to penetrate into different organs, e.g. liver, kidney, lung, heart and muscle, resulting in tissue damage as assessed by increased serum LDH activity. In one study it was found that administration of ZnONPs was accompanied with signs of toxicity, including an increased level of LDH enzyme activity [22].The present study found a significant increase($p<0.05$) in the LDH level with 50ppm AuNPs, 25 and 50 ppm AgNPs, and 25, 50 and 100ppmZnONPs as compared to the normal group. Using AuNPs, others found a non-toxic effect on the blood chemistry and vital organs [23], but our study showed a significant increase in the level of LDH activity with 50ppm AuNPs as compared to the normal control group (perhaps higher doses of AuNPs are excreted in the urine) [24]. In an earlier study the toxicity effect of ZnO nanoparticles in a cylindrical shape was more obvious than those NPs in a spherical shape [22,23]. In our study we useda spherical shape of ZnONPs, and these showed the toxicity effect on LDH enzyme activity. In another study, the administration of ZnONPs for 4h was accompanied with signs of toxicity, including an increased level of LDH enzyme activity [25], a result confirmed in the present investigation. In another report, the toxicity effect of Zn ONPs in a rat was accompanied with signs of toxicity after 7 days, including an increase in the level of LDH enzyme activity (as found herein), an increase of neutrophils in blood, and an increase of inflammation [26].The effect of AgNPs on cells has been examined, and it was noted that exocytosis of the NPs depended on the cells [27]. This led to a toxicity effect as judged by LDH in 25 and 50ppm doses, and it is possible that the LDH is not being accurately measured in blood at 100ppm [28]. Earlier it was found that the use of silver nanoparticles caused serious damage to skin

[28] because of the essential nature of LDH to skin cells. It has been reported that 60 nm AuNPs, under the exposure conditions tested, are not cytotoxic to the LDH enzyme [25], while our study showed subchronic toxicity of AuNPs with a diameter of 10nm.

5. CONCLUSION

Based on our results using a moderate dosage of each of the three types of nanoparticles, there is an increase in LDH enzyme activity as compared with the control group. Significant difference ($p<0.05$) of the LDH level occurs with the application of a moderate concentration of gold nanoparticles (50ppm),with moderate and low concentrations of silver nanoparticles (50 and25ppm) and in all concentrations of zinc-oxide nanoparticles (100, 50, and 25ppm) as compared with the control group. The results reported herein confirm and extend the observations of others on NPs and LDH enzymic activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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