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Original Article

Comparison the Effect of Silver Nanoparticles and Silver Oxide (Ag_2O) on Oxidative and Nitrosative Stress Biomarker in Human Lymphocytes

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ABSTRACT

Silver nanoparticles (Ag NP) have an interesting potential in drug delivery, gene therapy, molecular imaging and medicine in the cure of diseases that require maintenance of circulating drug concentration or targeting of specific cells or organs. The present study focused on the effect of exposure of AgNP and silver oxide on oxidative stress and nitrosative stress biomarkers in human lymphocytes. In lymphocytes samples evaluated oxidative stress biomarkers such as total antioxidant capacity (TAC), catalase activity (CAT) and nitrosative astress biomarker such as assay nitric oxide radical (NO) after 24,48 and 72 hours. The results showed that AgNP 5 Mmol/ml after 48 hours caused a increasing in the TAC compared to control group. AgNP5 Mmol/ml decreased NO level after 24 hours. Also CAT activity decreased in AgNP5 Mmol/ml compared with silver control group after 72 hours. At all alteration oxidative stress biomarkers in AgNPs was similar to AgO_2 groups. Therefore, they may induce oxidative and nitrosative stress expect AgNP5 Mmol/ml in human lymphocyte.

Keywords: Silver nanoparticles , Lymphocyte, Oxidative stress, Nitrosative stress

1. INTRODUCTION

Nanotechnology has been an ever growing area of research and opportunities during the new years. Because of the novel chemical and physical properties of supplies of nanomaterials have been consumed to make new consumer products as well as applications

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for life sciences in the all word [1].). Nanoparticles (NPs) are a promising class of functional materials. Silver nanoparticles (AgNPs) are used in many customer products. Also to their medical uses, AgNPs are consumed in food and clothing industry, household, paints and yields [2]. Also, AgNPs are held up in the several medical products making, include implants, catheters, and other equipment to prevent infection [3]. While the use of AgNPs is increasingly common in medicine and in each day life, comprehensive biologic and toxicological information is deficient [4].). Furthermore, AgNPs have been used extensively for antimicrobial cure. These antimicrobial properties are retained in AgNPs and exploited in e.g. nanofiber mats, bandages, wound dressings and ointments [5, 6]. In addition, AgNPs have been used to stop bacterial colonization on different surfaces such as catheters, prostheses and clothing [7, 8]. Several different mechanisms contributed to AgNPs toxicity, notably the production of excess reactive oxygen species (ROS). ROS are both physiologically essential and potentially destructive. ROS. Nevertheless, some of the important and basic characteristics of AgNPs, including the mechanism of cytotoxicity ,the procedure of cellular uptake, the intracellular location and the translocation of AgNPs, stay unclear [9]. Additional studies are needed to focus on the processes of nanoparticle in cell interactions, their intracellular fate and their relationships may contribute to tissue damage in many pathophysiological. Therefore, understanding the detailed mechanism of cell-specific effects of AgNPs will be helpful for assessing their risk [10].

There is no sufficient data on the oxidative and nitrosative stress property of AgNPs in the human lymphocytes in vitro. Thus, this study aimed to determine the effect of AgNPs at several different doses compared with silver oxide (AgO_2), in nitric oxide, total antioxidant capacity and catalase activity levels in human lymphocytes after various times,24,48 and 72 hours.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

2, 4, 6 tri pyridyl-s-tiazine (TPTZ), H_2O_2 ,nitric oxide kit(Biovision companey)and silver oxide (AgO_2) were used in this study.Therfore, all chemicals were bought from the Sigma. In this sturvay, AgNPs (100 nm) were bought by Notrino compony. Also, AgNPs were diluted in deionized water.

2.2 Isolation of Lymphocytes

In this in vitro study, blood sample (5 ml) was gathered from a healthy human donor in conical centrifuge tube and centrifuged at 3,000 rpm for 15 min. The whitish portion of blood, produced just lower the upper plasma layer, was separated and collected into fresh microcentrifuge tubes. Then, to remove contaminant RBC, collected cells were washed twice with 0.5 ml NH4Cl (0.85 %). Lymphocytes that appeared in the form of white pellet were consequently suspended again into PBS (phosphate buffer saline) and stored at 4 °C.

2.3 Incubation of human lymphocytes with AgNPs and AgO_2

Human lymphocytes were incubated with AgNPs and AgO_2 at the following nanoparticle concentrations: 5, 50, 250 and 500 mmol/mL, for 24,48 and 72 h. Following treatment, the lymphocytes were processed for oxidative & nitrosative stress biomarkers.

2.4 Oxidative& nitrosative stress biomarkers

2.4.1 Measurement of total antioxidant capacity (TAC)

TAC was calculated by the ferric reducing ability of plasma (FRAP) method. This procedure is based on the ability of plasma to reduce Fe^{3+} to Fe^{+2} in the presence of TPTZ. A complex with blue color and maximum absorbance appeared in 593 nm with reaction of Fe^{2+} and TPTZ [11].

2.4.2 Measurement of catalase (CAT) activity

CAT activity was measured in the samples by measuring the absorbance reduction at 240 nm in a reaction H_2O_2 medium containing (10 mM), sodium phosphate buffer (50 mM, pH = 7.0). At the end, one unit of the enzyme is distinct as 1 mol H2O2 as substrate consumed/min, and the exact activity is noticed as units/ml sample [12] .

2.5 Statistical analysis

In this study, all the results were showed as Mean and standard error values as Mean±SE. All data were analyzed with SPSS Version: 18 employing one-way ANOVA followed by Tukey post hoc test. Then, differences between groups was measured significant when $P < 0.05$.

3. RESULTS

NO

AgNPs caused a significant decrease in LPO level in 5 and 50mg/kg when compared to control group and AgO_2 5, 50,250 and 500 mg/kg after 24 hours($p < 0.05$). Also, Ag NPs caused a significant decrease in LPO level when compared to control and AgO_2 groups after 48 hours ($p < 0.05$). No significant difference was observed LPO between groups after 72 hours, Figure, 3.

TAC

TAC increased in 5and 50mg/kg when compared to control group significantly, after 24,48 hours ($p < 0.05$). AgO_2 caused a significant decrease in TAC level when compared to control group after 24, 48 and 72 hours ($p < 0.05$), Figure, 2.

CAT

AgNPs caused a significant decrease in CAT activity in 5, mg/kg when compared to control group after 24 hours ($p < 0.05$). No significant difference was observed CAT activity between groups in AgO_2 after 24, 48 and 72 hours, Figure, 1.

4. DISCUSSION

The purpose of this investigation was to evaluate potential mechanism involved in AgNPs compared to AgO_2 in oxidative and nitrosative stress status in human lymphocyte.

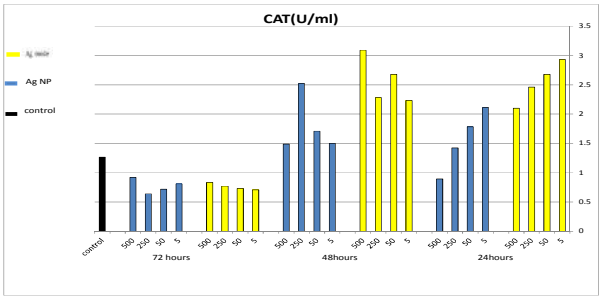

Fig 1: Catalase activity in human lymphocytes incubated after 24, 48 and 72 hours

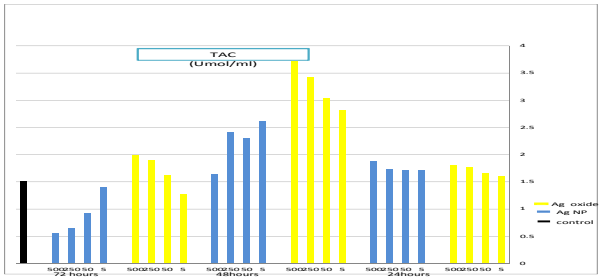

Fig 2: Total antioxidant capacity in human lymphocytes incubated after 24, 48 and 72 hours.

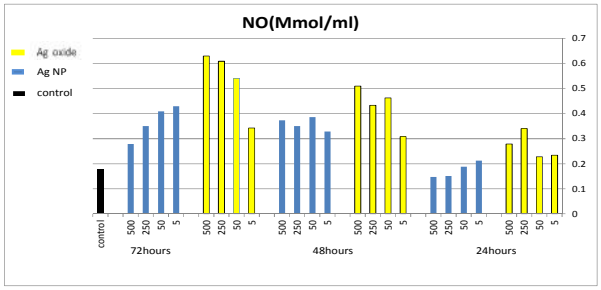

Fig 3: Nitric oxide concentration in human lymphocytes incubated after 24, 48 and 72 hours

The current study aimed to investigate the biochemical effects of AgNPs an in vitro model lymphocyte consequent from human blood. AgNPs are already in use in several industries, exposing humans on a daily basis [4]. Additionally, investigators demonstrated increased ROS production and increased cell lethality in rat liver cells after exposure to nanoparticles, consistent [13, 14] Previous results have demonstrated a clear deleterious effect of nanoparticles such as AgNPs on mitochondrial role in the kidney, lung, nervous

system and liver [15]. The present study provides the first evidence that AgNPs act as AgO2 induction oxidative toxic stress and induces inflammatory response especially in higher doses in various time. Also, this study has demonstrated that AgO_2 and AgNP in higher doses causes ROS increasing,that can have the capability to induction oxidative damage in isolated human lymphocytes. Our findings provide the evidence that AgNPs and AgO_2 can increase ROS production, and decrease antioxidant defense in a biological system. Furthermore, inflammatory cells also produce soluble intermediaries, such as metabolites of arachidonic acid, cytokines, and chemokines, that act by supplementary recruiting inflammatory cells to the site of damage and producing more ROS. More studies are essential to explain the mechanism of action of these microscopic structures, to ascertain the implication of their broad use. Our results showed that AgNP can to decrease the oxidative toxic stress in low doses, as shown by a decreased CAT activity, NO level and increase TAC level in 5 and Mmol, but in 500 Mmol decrease TAC level and increase CAT activities in this group compared the other groups.

In conclusion, the present studies show that after treatment for 24, 48 and 72 hours, AgNPs and AgO_2 are able to induce oxidative toxicity in human lymphocytes. The results suggest that ROS intermediates are responsible for Ag-induced oxidative damage in experimental lymphocytes.

5. ACKNOWLEDGEMENT

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