Effect of silver nanoparticles that extracted by *Staphylococcus aureus* on cellular immunity in rabbits

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Abstract

All people in developed countries are exposed to metal nanoparticles that are used in a large number of applications including medical materials, its effect on immunity is less known .The aim of this study was effect of silver nanoparticles that extracted by *Staphylococcus aureus* on cellular immunity six adult males rabbits were used, three of them Injected with AgNPs (50µg/ml subcutaneously). The results were appeared E-rosette forming T-lymphocytes that increased 50.50% in test group, while control group was 27.5%. Indurations and erythematic in skin test when intradermal injected with immunized rabbits with AgNPs. CD4 and CD8 concentrations were significantly increased (42.80, 3.20ng/ml) compared with control (26.58, 1.7ng/ml respectively). The conclusion of this study AgNPs increased T cell induction.

Keyword: silver, nanoparticles, CD4, CD8, interlukin, E rosette

INTRODUCTION

Silver nanoparticles, which could induce numerous deleterious effects on human health and the environment. In nanomedicine, they are used in diagnostic imaging, vaccines, cancer therapy and drug delivery (1).

The adverse effects of silver nanoparticles on organs can extend into the cardiovascular or central nervous system, thereby causing neurotoxicity or immunotoxicity The cytotoxicity and genotoxicity of silver nanoparticles depends on many factors such as it concentration, dispersion, size and surface functionalization, reports have indicated that the size of silver nanoparticles is an important factor for cytotoxicity and genotoxicity probably acting through apoptosis and necrosis mechanisms (2).

Although the Ag-NPs have been considered as safe compounds at low doses, Amiri *et al*(3)their results indicate that prenatal exposure to low doses of Ag-NPs for rat is able to induce behavioral and cognitive abnormalities in adulthood. Also found that these effects are at least partly associated with hippocampal mitochondrial dysfunction and the activation of sterile inflammation during early stages of life. Increasing experimental evidences suggest that cells of immediate immunity react to metal nanoparticles by receptors Toll like receptor as pathogenic agent and act as signal transducer ending up with activation of the innate immune response and proinflammatory cytokine production(4, 5) the aim of this study to indicate the effect of AgNps on some cellular immunity parameters.

MATERIALS AND METHODS

Silver nanoparticles prepared previously by using local isolate *Staphylococcus aureus* (6). In this study, six adult males rabbits were used to detect immune response for AgNps .It was kept in cages specialized for animals in laboratory animals house. Three rabbit injected with AgNPs produced by *S. aureus*. Other rabbits group was considered as control and it was injected with normal saline. The injection period was contained for three weeks, one injection (subcutaneous of AgNPs 50µg /ml) for each week, In the fourth week of experiment the groups of rabbit were left without injection, and in the fifth week the rabbit groups were anesthetized with chloroform, and it was sacrificed to take blood samples from the heart directly (7)**.** E-Rosette Forming test was done according to the method of (8).

Skin test (Delayed type hypersensitivity). This test was done for AgNPs primed rabbits by injection intradermal 0.1 ml of antigens in all animals and recording the observed skin change after (4,24,48,72) hrs by measuring the diameters of the induration, erythema and necrosis by ruler in comparison with control animals (9). Cluster of Differentiation CD4 and CD8 Assay.

The following procedures are performed at room temperature according to manufacturer's instructions (Elabscience–china).Interleukin-2 according to manufacturer's instructions (Koma biotech–korea).

RESULTS AND DISCUSSION

Silver nanoparticles immunized in rabbits induce the T ell this occurred by using E- rosette forming T- lymphocytes that increased in test group compared with control (table 1) This indicated CD2 was increased CD2 is a lymphocyte surface glycoprotein that expressed on all thymocytes, T-cells and NK cells, the heterotypic interaction Rosetting between CD2 and its major ligand leukocyte function antigen-3 (CD58) which expressed by most nucleated cells as well as by erythrocytes enhances T cells antigen recognition. CD2 engagement by LFA-3 expressed on an Antigen Presenting Cell stimulates T-cell proliferation and differentiation, its role in stabilizing cell-cell contact the interaction between CD2 and CD58 delivers an activating signal to the T-lymphocyte (10). This result agree with Ikramullah *et al.*(11) who found that concentration of lymphocytes were increased with the time after exposing to different dilution of AgNPs with different time periods.

Skin test the results appeared signs of delayed type hypersensitivity DTH table 2. DTH response require prior immunological sensitization to a specific antigen and thus are categorized by memory T-cell response, they can identify specific antigens to which the host has already made an immune response and it provide an index of the current T-cell reactivity to specific recall antigens ,the real of redness and sometime the degree of swelling that can be measured to provide an index of DTH reactivity (12). The results agrees with Lison and Muller (13) study of cerium oxide nanoparticles they were found that may induce DTH through the induction of pro-inflammatory cytokines, predominantly by Th1 responses.

Yang *et al*. explained that AgNPs induced the formation of the inflammation some, caspase-1 activation and then release of mature IL-1β from human blood monocytes (14).

Table 1: E- rosette forming T- lymphocytes in rabbits immunized with AgNPs

Groups of rabbits	E- rosette forming T- lymphocytes (%)Mean± sd
AgNps of *Staphylococcus aureus*	50.50 ± 8.49*
Control (normal saline)	27.5 ± 12.3

*Significant at P<(0.05).

Table 2: Skin reactions in rabbits immunized with silver nanoparticles

Time after intra-dermal injection/hr	normal saline	AgNPs	
	no sign of skin reaction	Indurations mm mean ± S.D	Skin appeared
4		1.2±0.4	Erythematic, *pus*
24		10.5±2.0	Erythematic,*pus*
48		10 ± 0.6	Erythematic,necrosis
72		7±2	Erythematic,necrosis

Table 3: Concentrations of CD4 and CD8 (ng/ml) in rabbits immunized with silver nanoparticles.

Groups of rabbits	sd ng/ml± Mean	
	CD4	CD8
AgNps of *Staphylococcus aureus*	42.80 ± 4.25*	3.20 ± 0.7*
Control (normal saline)	26.58 ± 8.30	1.7 ± 0.3

*Significant difference with control at P<(0.05).

Table 4: Effect of AgNps on interleukin 2 concentrations in rabbits

Groups of rabbits	IL-2 concentrations Pg/ml mean ± SD .
AgNps of *Staphylococcus aureus*	130. 2667± 5.650*
Control (normal saline)	142.1667 ± 5.460

*Significant at P<(0.05).

Table- 3-appeared the concentrations of CD 4 and CD8 (42.80, 3.20 ng/ml) significantly increased compared with control(26.58, 1.7ng/ml) this result agreed with (15)who used oral administration of different doses (0.25, 2.5, 25 ppm) of commercial silver nanocolloid on hematological parameters in mice ,he found an increase in CD4+/CD8+ T cell distribution . In preset study AgNPs that activate T cell this approved by the results of table 3,the interleukin 2 concentrations were increased in rabbit immunized with AgNPs. The conclusion of this study silver nanoparticles synthesis by *S. aureus* induce specific cell immunity.

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