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Original Article

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A study of nanosilver dermal toxicity in mice balb/c

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Abstract

As a result of growing applications of manufactured nanoparticles, there is an increasing concern about possible side effects of exposure to these materials on human and environment. Nanosilver (n-Ag) has found wide use in medical applications including development of silver-based dressings and silver-coated medical devices. However, toxicity of these particles clue to their dermal use has not been fully identified. In this study, we analyzed the potential dermal toxicity of n-Ag bandage (100 μg/mL) over a period of 3 and 7 days in mice balb/c. Silver nitrate bandage (100 μg/mL) was applied in the positive control group. We found no cutaneous inflammatory responses such as erythemia and/or eschar for n-Ag. However, exposure to these nanoparticles resulted in considerable increase of hepatic necrosis biomarkers viz. ALT and AST. On the other hand, TGF-beta1 levels, an anti-inflammatory biomarker was found to be decreased significantly in mice blood sera following use of n-Ag bandage. No mortality was observed in n-Ag treated groups. Also, differences between n-Ag treated groups and positive control in hepatic and anti-inflammatory biomarkers was found to be significant.

Briefly, our results reveal that dermal application of n-Ag does not lead to any external changes and observable inflammatory responses in animal skin. However, it can alter the level of some biomarkers related to liver function in blood serum of samples.

Keywords: Nanosilver, Dermal toxicity, Cutaneous inflammation, Hepatic biomarkers, TGF-beta1.

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Introduction

Nanoparticles can exhibit size-related properties that differ significantly from those observed in bulk form. Nanoparticle can influence the physicochemical properties of the other materials. In particular, they can interact with biological tissues in significant ways (Paull et al., 2003). The growing applications of manufactured nanoparticles, which are mostly composed of metal and metal oxides is increasing their environmental exposure. One of the major toxicological concerns associated with manufactured nanomaterials is that some of them are redox active and can be transported across cell membranes and interact with subcellular organelles (Stebounova et al., 2011). Hence, evaluation of their toxicity in acute and subchronic exposure is essential.

The medical use of silver dates centuries back. Many of the industrial silver compounds, including nitrate, chloride, bromide, acetate, oxide, sulfate, and cyanide can be released in to the environment from various sources (Rosenman et al., 1979; Weast et al., 1988). Silver can be found in low levels in many tissues but without any clear physiologic function (Rosenman et al., 1979; Wan et al., 1991). However, dermal exposure to high doses of silver causes agrarian and mild allergic responses (Stokinger, 1981). Nanosilver (n-Ag) has recently been recognized as antimicrobial agents and is finding diverse medical applications such as silver-based dressings and, silver-coated medical devices. This nanoparticle can damage bacterial cell wall and cause cell death. n-Ag have already been tested in various fields of biological science including drug delivery, water treatment, and as an antibacterial compound against both Gram (+) and Gram (-) bacteria (Soni and Salopek-Bondi , 2004; Nowack et al., 2011). The number of n-Ag -containing products has grown from less than 30 in 2006 to over 300 at the beginning of 2011. One of the most frequent applications of these nanoparticles is in development of bacteriostatic coatings for prevention of infection or as deodorants. It is estimated that approximately 280 tons of n-Ag is produced for use in commercial or industrial applications and their level of applications is expected to quadruple by 2015 (The Silver Institute, 2011). However, an adequate assessment of the long-term effects of n-Ag exposure on human physiology and their release into the environment is lagging behind the rapid increase in the commercialization of n-Ag products. Although use of Ag at nano scale can improves the therapeutic effects of silver, its safety has remained controversial. In the present work, considering the importance of dermal exposure to n-Ag by using the different health and cosmetic products, we assessed the dermal toxicity of n-Ag low concentration in mice balb/c. The concentration of n-Ag used in this study is not environmentally relevant but provides a model to examine dermal toxicity.

Materials and Methods

Nanosilver solution was purchased from Nano-shop Co., Tehran, Iran. The particle size and purity were 40 nm and 98%, respectively. Thirty healthy adult male mice with a body weight of 30-35 gr were obtained from animal house of Shahrekord Azad University and randomly divided into four groups (negative and positive controls, sham-operated plus experimental ones). Sham-operated animals received only normal saline-containing bandage. All mice were kept in stainless steel cages and allowed to adapt to the conditions of the animal house for 14 days before the experiments. The animals were maintained on a 12 hour dark/light cycle at 22 ± 3 °C and allowed free access to a standard laboratory diet and tap water ad libitum. An area of 0.90 cm×0.90 cm of the back zone of each animal was shaved for treatment. The shaved and

rubbed areas were covered with sterile gas and fixed with cloth glue (Photo 1). The shaved skin of experimental animals was rubbed once with the test material at 100 μg/ml and skin of positive controls was rubbed once with 100 μg/ml of Silver nitrate (AgNO3) solution and kept separately for 3 and 7 days. The other parts of body in all treatment groups were kept untreated as negative controls. At the end of exposure periods residual test gas was removed using water.

Cutaneous inflammatory responses (erythemia and eschar), were detected in the test group on the last days. The changes in the hepatic necrosis biomarkers namely alanine transaminase (ALT) and aspartate transaminase (AST) were analyzed using commercial kits. TGF-beta1 level was determined using ELISA immunoassay kit (Quantikine ELISA, USA & Canada, MN, R&D Systems, Inc.) in mice blood sera. All animal studies were conducted according to the US National Institute of Health guidelines (NIH publication no. 85-23, revised 1985).

Mean values and standard deviation of mean were calculated and expressed as Mean±SD. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post-test. $P < 0.05$ was considered as statistical significance. Statistical analyses were carried out using the SPSS Version 16 Software.

Results

No mortality, abnormal behavior and obvious weight differences were found after treatment periods of 3 and 7 days. The test site examined immediately after skin treatment periods for sings of erythemia and eschar. No indication of appreciable skin reactions or inflammatory responses was detected in both n-Ag and AgNO3-treated animals compared to the negative controls.

Sera that were collected from blood samples on day 3 and 7 of the study period were used to estimate liver necrosis biomarkers, AST and ALT, for all the groups. Results showed that both AST and ALT levels were increased significantly in all treated groups in comparison with sham and negative control groups (Table 1). The level of inflammatory biomarker TGF-β1 was found to be decreased significantly as compared with sham and control groups during treatment periods (Table 2).

Table 1: Changes in liver necrosis biomarkers after 3 and 7 days following treatment with nanosilver (n-Ag).

	Liver necrosis biomarkers (IU/L)			
	AST		ALT	
	3 days	7 days	3 days	7 days
Negative control	14.65± 0.61	14.05± 1.01	11.45± 0.91	11.88± 1.31
Sham (Only bandage)	15.45± 1.81	14.65± 2.01	12.43± 1.41	12.65± 2.14
Positive control (100 μg/ml)	20.17± 4.01 *	22.55± 1.11 *	18.41± 2.21 *	19.25± 3.01 *
Nanosilver (100 μg/ml)	24.47± 3.11 * #	26.75± 2.21 * #	19.55± 1.51 *	22.14± 2.82 * #

Datas are given as mean±SD for n = 10. Values are statistically significant at *p< 0.05.
* compared to sham group. , # compared to positive control group.

Table 2- Changes in blood inflammatory biomarker TGF-β1 after 3 and 7 days following treatment with nanosilver (n-Ag).

	TGF-β_1 (ng/ml)	
	3 days	7 days
Negative control	57± 20	57± 22
Sham (Only bandage)	58± 22	58± 19
Positive control (100 μg/ml)	52± 13 *	51± 21 *
Nanosilver (100 μg/ml)	48± 21 * #	47± 21 * #

Datas are given as mean±SD for n = 10. Values are statistically significant at *p< 0.05.
* compared to sham group. , # compared to positive control group.

Discussion

Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. The properties of these materials depend on their size and composition. Silver has been used in topical gels, cloths and bandages due to its wide-spectrum antimicrobial activities (Slawson et al., 1992).

However, increased application of n-Ag products is accompanied by possibility of their adverse effects on humans. Discoloration of the conjunctiva and cornea in some workers is reported after inhalational exposure to silver dust (Moss et al., 1979). Because of public concern on the potential adverse effects of n-Ag, we assessed the toxicity potentials of a certain concentration of n-Ag in dermal application, compared to AgNO3 as positive control.

Our data showed that the activities of both AST and ALT were significantly higher in n-Ag and AgNO3-treated mice which indicate a possible dysfunction in liver. AST and ALT are reliable determinants of liver parenchymal injury (Moss et al., 1987). The increased activities of AST and ALT in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro et al., 1993), which implies the hepatotoxic effect of n-Ag. Reports show that many medical devices loaded with silver can release silver ions (Ag+), which could be transported by the blood circulation and be accumulate in some organs such as the liver and kidney, thereby inducing hepatotoxicity and renal toxicity, respectively (Stepien et al., 2009). We showed that dermal exposure to n-Ag, with properties similar to those of Ag +derived from AgNO3 can be translocated in the body and lead to histopathologic changes in the liver.

Transforming growth factor-β (TGF-β) proteins and their antagonists have entered clinical trials. These multi-functional regulators of cell growth and differentiation, induce extracellular matrix proteins and suppress the immune system. These effects render TGF-βs useful in treatment of wounds with impaired healing, mucositis, fractures, ischemia-reperfusion injuries, and autoimmune disease (Flanders and Burmester, 2002). TGF-β knockout mice are indistinguish-

able from wild type litter mates, but show a severe multifocal organ-dependent inflammatory cell infiltration in heart, stomach, liver, diaphragm, lung, salivary gland and pancreas, leading to abnormal functions (Dang et al., 1995). Our results also showed a marked decrease in blood serum levels of TGF-β1. This change may lead to n-Ag negative effects on liver and other sensitive organs such as kidneys where inflammatory reactions occur.

Meanwhile, application of TGF-β blockers may be valuable in stimulating an immune response toward metastases (Fakhrai et al., 1996). However, to find no-observable-adverse-effect level (NOAEL) of n-Ag for dermal application, further studies are required. Taken together, our results reveal that dermal application of n-Ag does not lead to any external changes and observable inflammatory response in animal skin. However, it can alter the level of some biomarkers in serum blood samples related to liver function.

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