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Nanosilver and Silver Nitrate induced toxicity in a subacute murine dermal model

ABSTRACT

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Silver salts and nanosilver (NS) are the common antimicrobial used in the treatment of chronic wounds, so they are important to assess their dermal toxicity. Therefore, in this study, the possible dermal toxicity of NS particles and silver nitrate ($AgNO_3$), was tested by determining the serum level of transforming growth factor-$\beta1$(TGF-$\beta1$) and study on of mouse skin tissue. In this study, 30 BALB/c mice were randomly allocated into NS, $AgNO_3$ and control groups (n=10). After general anaesthesia and shaving the back of all animals in near the vertebral column, in NS group, a volume of 50μl 10 μg/ml of nanosilver solution (40 nm), in $AgNO_3$ group, with the same amount of $AgNO_3$ solution (100 μg/ml), in control group, the same amount of distilled water was added to the sterile bandage of mice. The shaved areas were covered with sterile bandage that and fixed with cloth glue. After 3 and 7 days, the bandages were opened and serum level of TGF-$\beta1$ was measured using standard kits and preparation of skin samples for histological analysis. Results showed toxicity of NS and $AgNO_3$ by a significant reduction of TGF-$\beta1$ without any damage to skin.

Keywords: *Nanosilver; Silver nitrate; Dermal toxicity; Skin; TGF-β1.*

INTRODUCTION

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Silver, a white, lustrous metallic element, has been known for a long time. It has been used in various applications, such as jewelry, cutlery and photography [1]. Also, silver salts have been used in wound care, catheter coatings and heart valves [2], which is due to their antimicrobial effects [3]. Nanotechnology is the fast-growing field for product nano-products [4]. Recently, silver nanosized particles (NS) are utilized in various regions including in washing machines, cloths and medical products (such as wound dressing, bandages and medical mask) [5]. NS promotes healing of chronic ulcers by its antibacterial and anti-inflammatory activities [3]. However, there are concerns about its toxic effects [6].

Human can exposure to nanomaterials via several routes, including skin contact, ingestion, inhalation and intravenous administration [7]. The studies showed that NS may cause toxic effects by the generation of reactive oxygen species (ROS) and through the interaction with the thiol groups of proteins [1], in addition, it can cause mitochondrial dysfunction, DNA damage and chromosomal aberrations. However, even low doses of NS have effects of cytotoxicity and genotoxicity [8].

Some studies showed that NS has toxic effects to several cell lines, such as fibroblast, monocytes, liver cells with causing necrosis, apoptosis and ROS generations in vitro experiments [9]. A study has reported primary DNA damage and cytotoxicity in cultured mammalian cells by NS [10]. Several studies have reported argyrosis and decreased vision at night due to the use of silver [11]. It has been reported that NS can induced toxicity in BRL3A rat liver cells [12], and human hepatoma cells by reduced mitochondrial function and LDH leakage and increase in the basal level of ROS [13]. It was also observed significant dose-dependent changes in alkaline phosphatase activity and cholesterol level and also slight liver damage in rats following oral administration of NS (18 nm) [14]. It has been reported that nanosilver induced neuro- and immune-toxicity by modulation of expression of several genes related to motor neuron disorders, neurodegenerative disease and immune cell function [15]. However, NS may have negative impact on human health through its use of consumer products. Therefore, the studying of safety and toxicity of these nanoproducts is very important [16]. In this study, we have designed to investigate the possible dermal toxicity of NS and $AgNO_3$ on the serum level of TGF-β1 and skin of adult male BALB/c mice.

The preparation of Prussian blue (PB) family nanoparticles has emerged as a promising subject in the past few years due to its large number of interesting properties and applications in the nanomagnetic, biosensing, electrochromic, and biomedical devices. So far, several techniques for the preparation of Prussian blue nanoparticles have been reported, in which various agents, such as sol–gel [4], mesostructured silica [5], porous alumina [6] etc were used to stabilize nanoparticles.

EXPERIMENTAL

Nanosilver (NS)

NS solution (8000 ppm) was purchased from Nano-shop Co., Tehran, Iran. The particle size and purity were 40 nm and 98%, respectively.

Mice and housing condition

30 healthy adult male BALB/c mice with a body weight of 24.09±4.8 gr were obtained from animal house of Shahrekord Azad University and randomly divided into 3 groups of 10 (NS, $AgNO_3$ and control groups). 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 in near the vertebral column. In NS group, a volume of 50μl of NS solution (10 μg/ml), in $AgNO_3$ group with the same amount of $AgNO_3$ solution (100 μg/ml), NS and $AgNO_3$solution were diluted with distilled water, and in control group, the same amount of distilled water was added to the sterile bandage of mice. The shaved areas were covered with sterile bandage that and fixed with cloth glue, and kept separately for 3 and 7 days (Figure 1). At the end of exposure periods residual test gas was removed using water. On days 3 and 7 of the study, the transforming growth factor-$β_1$ (TGF-$β_1$) level (an anti-inflammatory cytokine) was determined using ELISA kit (Quantikine ELISA, USA & Canada, MN, R&D Systems, Inc.) in mice blood sera, and also, four animals from each group were killed by ether inhalation in a closed space. The skins were removed. Then, small pieces of skin tissue were fixed in 10% buffered formalin and dehydrated in a graded series of alcohol. The samples were sectioned at 5 μm and stained with hematoxylin and eosin (H&E) for histopathologic studies. 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. The values of P<0.05 were considered as

statistical significance. All statistical analyses were performed by the SPSS (Version 17) software.

Fig. 1. Male BALB/c mice after complete dressing.

RESULTS AND DISCUSSION

NS plays a strong antibacterial role in the concentration range of 10–50 µg/ml (17), therefore, in this study; we used a dose 10 µg/ml of NS. The treated site examined immediately after dermal treatment periods for signs of erythema and eschar. Macroscopic observations showed no indication of appreciable skin reactions or inflammatory responses in both NS and $AgNO_3$ treated animals compared to control group. On days 3 and 7 of the study, the pathological results of the treated skin of mice of NS and $AgNO_3$ groups showed normal skin tissue with no specific injury (Figure 2), that it was the similar of test results of skin tissue in control group (No abnormal change).
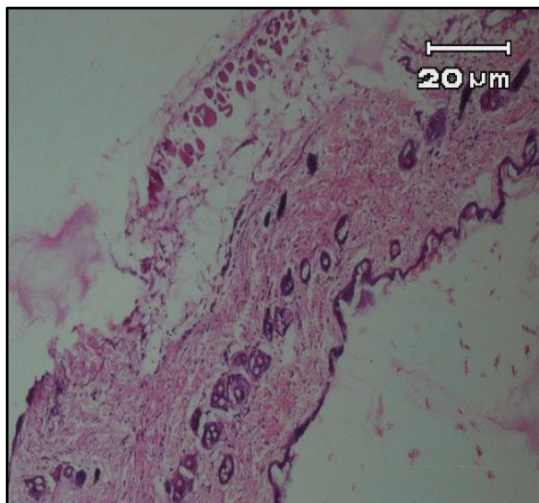

Fig. 2. H&E stained skin section in a toxicity study ($\times$ 10). Skin tissue is normal in all experimental groups.

The results showed that $TGF-\beta_1$ level (An anti-inflammatory cytokine) was decreased significantly in NS and $AgNO_3$ groups in comparison to control group during experiment periods ($p<0.05$). Therefore, on day 3, in NS and $AgNO_3$ groups, the level of $TGF-\beta1$ was respectively, about 8 and 6 ng/mL less than control group, but there was no significant difference between NS group and $AgNO_3$ group ($p>0.05$). On day 7, in NS and $AgNO_3$ groups, the level of $TGF-\beta1$ was, respectively, about 12 and 7 ng/mL less than control group. Also, the serum $TGF-\beta1$ level in NS group was decreased significantly in comparison to $AgNO_3$ group ($p<0.05$) (Figure 3).
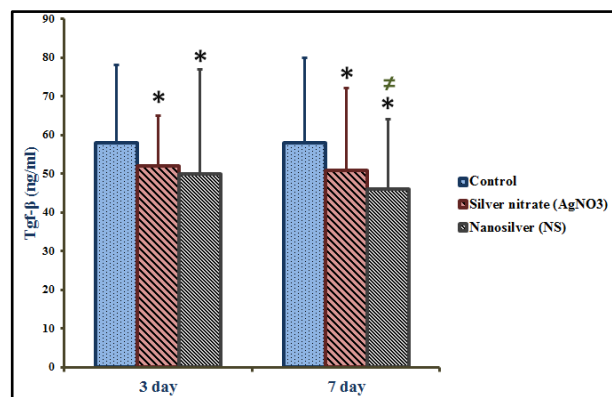

Fig. 3. Changes in anti-inflammatory cytokine ($TGF-\beta_1$) level after 3 and 7 days following treatment with nanosilver (NS) and silver nitrate ($AgNO_3$). A significant decrease in $TGF-\beta1$ level in treatment NS and $AgNO_3$ groups in comparison to control group after 3 and 7 days ($p<0.05$), there was no significant difference between NS group and $AgNO_3$ group after 3-day ($p>0.05$), but the serum $TGF-\beta1$ level in NS group was decreased significantly in comparison to $AgNO_3$ group after 7-day ($p<0.05$). The values shown are Mean$\pm$SD; n = 10 per group, * compared to control group, $^{\neq}$ compared to $AgNO_3$ group.

The present study showed that NS and $AgNO_3$ can inhibit $TGF-\beta1$ production. But, in the study of the treated skin of mice, there were no signs of erythema and eschar, and it was also observed no specific injury in pathological study during experiment periods. NS can be absorbed through the skin, enter the bloodstream [17], bind to plasma proteins, and so it can enter the cells. NS are distributed in organs, including kidney, liver, heart and lymph nodes [18]. It has been reported that the most cultured keratinocytes exposed to extracts of several types of silver containing dressings can be demonstrated cytotoxic [19]. Arora et al. (2009) reported that NS (7-20 nm) can cause the oxidative stress, apoptosis, and decreased

cell viability in fibroblasts and liver cells isolated from Swiss albino mice [20]. The investigations by Korani et al. (2011) showed that NS (<100 nm) and AgNO$_3$ penetrate guinea pig skin caused microscopic changes within the skin, liver and spleen in a dose and time-dependent manner following acute and subchronic dermal exposures of NS with doses ≥100µg/mL [21]. This finding is contrary to our results. In the present study, no abnormal change was detected in the study of skin tissue in all treatment groups that it may be due to differences in the doses used, the size of nanoparticles and animal models. Similarly, Kim et al. (2012) in evaluation of dermal toxicity of NS, reported that rabbits revealed no significant clinical signs and no acute irritation or corrosion reaction for the skin [22].The present results indicate that NS and AgNO$_3$ have inhibition effects on the production of anti-inflammatory cytokine (TGF-β$_1$). TGF-β and their antagonists, multi-functional regulators of cell growth and differentiation, induce extracellular matrix proteins. TGF-β helps promote woundhealing and the treatment of mucositis, fractures, ischemia-reperfusion injuries, and autoimmune disease [23]. TGF-β1 is secreted by a variety cells such as fibroblasts, macrophages, T cells and keratinocytes [23]. Shin et al (2007) evaluated the effects of nanosilver (~1.5 nm) with different doses on the production of cytokines by peripheral blood mononuclear cells (PBMC). The results indicate that nanosilver inhibits cytokines production such as IL-5, INF-γ and TNF-a [24], and in another study, It was also observed that nanosilver can inhibit cytokines production such as IL-6, IL-8 and IL-11 following the effect of nano silver (100 nm) with different doses on human mesenchymal stem cells (hMSCs) [25]. The studies showed that nanoparticles are more effective than large particles [26]. In this study, we also observed that NS (10 µg/ml) and AgNO$_3$ (100 µg/ml) have the same inhibitor effect on TGF-β1 after 3-day, but, on day 7, this inhibitor effect of NS was more than AgNO$_3$. Studied showed that the skin penetration of silver depends on factors such as the concentration and size of silver used in the formulation of nano-products [16]. It has been reported that the biological effects of NS are dependent on the physical and chemical properties [27]. Also it has been confirmed that nanoparticles effects on living cells depend on diameter, size and shape of nanoparticles [28]. There is evidence that showed smaller particles are more toxic than larger ones for animal cells [3]. Some studies showed that silver-coated medical products is able to release silver ions which could be absorbed into the circulation and accumulated in organs such as the liver and kidney and so induced hepatotoxicity or renal toxicity [29]. Therefore, the potential toxic effect of NS is due to release silver ions plus the physical and chemical properties.

CONCLUSIONS

The present results indicate that the dermal absorption of NS (10 µg/ml) and AgNO$_3$ (100 µg/ml) can lead to a decrease in anti-inflammatory cytokine (TGF-β$_1$), that it can indicate that silver can inhibit cytokines production without any damage to skin. However, it was better that the levels of silver in blood were measured in NS and AgNO$_3$ groups during experiment periods, also there is a need for further research with different doses for different periods of time.

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