Nanomedicine Journal

Received: Feb. 15, 2014; Accepted: Mar. 12, 2014
Vol. 1, No. 4, Summer 2014, page 285-291

Online ISSN 2322-5904
http://nmj.mums.ac.ir

Original Research

Subacute dermal toxicity investigation of nanosilver on serum chemical biomarkers in male mice

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Abstract

Objective(s): Nanosilver is one of the most widely used nanomaterials due to its strong antimicrobial activity. Thus, because of increasing potential for exposure of human to nanosilver, there is an increasing concern about possible side effects of these nanoparticles. In this study, we tested the potential dermal toxicity of nanosilver bandage on serum chemical biomarkers in mice.

Materials and Methods: In this study, 20 male BALB/c mice were randomly allocated into the treatment and control groups (n=10). After general anesthesia and shaving the back of all animals in near the vertebral column, in the nanosilver group, a volume of 50µl of 10 µg/ml of nanosilver solution (40 nm), and in the control group the same amount of distilled water was added to the sterile bandage of mice, then the bandages were fixed on the skin surface with cloth glue. After 3 and 7 days, the bandages were opened and serum levels of blood urea nitrogen (BUN), creatinine (Cr), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by using standard kits for two groups of mice.

Results: In treatment group, a significant increase in ALT, AST and BUN levels were observed compared with control group during experiment periods ($p<0.05$), but there wasn't a significant increase in Cr level in treatment group during experiment periods ($p>0.05$).

Conclusion: The present results indicated that the dermal absorption of 10 µg/ml nanosilver (40 nm) can lead to hepatotoxicity and renal toxicity in mice.

Keywords: Dermal toxicity, Hepatic biomarkers, Nanosilver, Renal function parameters

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Introduction

Silver has been used in human health care for centuries due to its antibacterial and anti-inflammatory properties (1). Silver and its compounds were used to counter bacterial infections in wounds and burns (2). Nanotechnology is a rapidly growing field for use of nanomaterials in new products (3). Nanosilver is one of the most widely used nanomaterials due to its strong antimicrobial activity (4, 5), for example, it has been used in medical products such as wound dressing, surgical insruments (6), coating of catheters and implant material (7). Thus, there is a concern about possible dermal toxicity (8). Researchers reported that nanosilver enhanced synthesis of heat shock protein and outer membrane protein. Thus, nanosilver represents a strong stressogenic agent towards bacteria (9). In addition, It was observed that nanosilver has a potential for catalyze protein structure by binding silver ions with functional groups of amino acids (10). Both *in vitro* and *in vivo* studies of nanosilver toxicity have shown that nanosilver may have negative effects in human health with the use of nanosilver products (11). It has been reported that there is a dose-dependent accumulation of silver content in tissues including blood, liver, lungs and kidneys following oral exposure to nanosilver (60 nm) in rats (12). A study has reported primary DNA damage and cytotoxicity in cultured mammalian cells by nanosilver (13). It has been reported induction of caspase-3 activity and DNA ladder formation, evidence of induction of apoptosis in bovine retinal endothelial cells (BRECs) following 24 h of exposure to nanosilver (50 nm) (14). Researchers reported that nansilver (< 100 nm) can cause histopatholgical abnormalities to the skin, liver and sleep of guina pigs in a dose and time-dependent manner following dermal absorption of nanosilver (15). Researchers reported the accumulation and histopathological changes in rat liver after systemically exposed to nanosilver (10-15 nm) (16). There are more studies on toxicity of nanosilver, but only very few studies has been conducted to assess the dermal toxicity of nanosilver, although nanosilver has many applications in medical products and it can be absorbed almost through the skin. Therefore, in this study, we present our findings on dermal toxicity of nanosilver in adult male BALB/c mice.

Materials and Methods

Nanosilver

Nanosilver solution was purchased from Nano-shop Co., Tehran, Iran. The particle size and purity were 40 nm and 98%, respectively.

Mice and housing condition

Twenty healthy adult male BALB/c mice with a body weight of 30-35 gr were obtained from animal house of Shahrekord Azad University and randomly divided into two groups (control and treatment). 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 nanosilver group, a volume of 50µl of 10 µg/ml of nanosilver (diluted with distilled water), and in the control group the same amount of distilled water was added to the sterile bandage of mice. The shaved areas were covered with sterile bandage and fixed with cloth glue (Figure 1), and kept separately in cages for 3 and 7 days. At the end of exposure periods residual test gas was removed using water. The changes in the hepatic necrosis biomarkers namely alanine transaminase (ALT) and aspartate transaminase (AST) were analyzed by pars azmon kit and the renal function parameters namely creatinine (Cr) and blood urea nitrogen (BUN) were measured

by using standard kits (Man Company and Pars Azmoon Company, respectively) in mice blood sera and were compared between two groups. All animal studies were conducted according to the US National Institute of Health guidelines (NIH publication no. 85-23, revised 1985).

Figure 1. Male BALB/c mice after complete nanosilver coated dressings.

Statistical analysis

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.

Results

The results of study on 10 µg/ml nanosilver (40 nm) showed that the level of BUN as the renal function parameter was increased significantly in treatment group in comparison to control group during experiment periods ($p<0.05$). At 3 days, the level of BUN in treatment group was 27.43±6.07 mg/dL while it was 22.12±5.07 mg/dL in control group, and also, at 7 days, the level of BUN was 29.83±5.18 mg/dL in treatment group, while it was 23.06±4.27 mg/dL in control group. Results also showed that there wasn't a significant increase in Cr level in treatment group during experiment periods ($p>0.05$) (Figure 2).

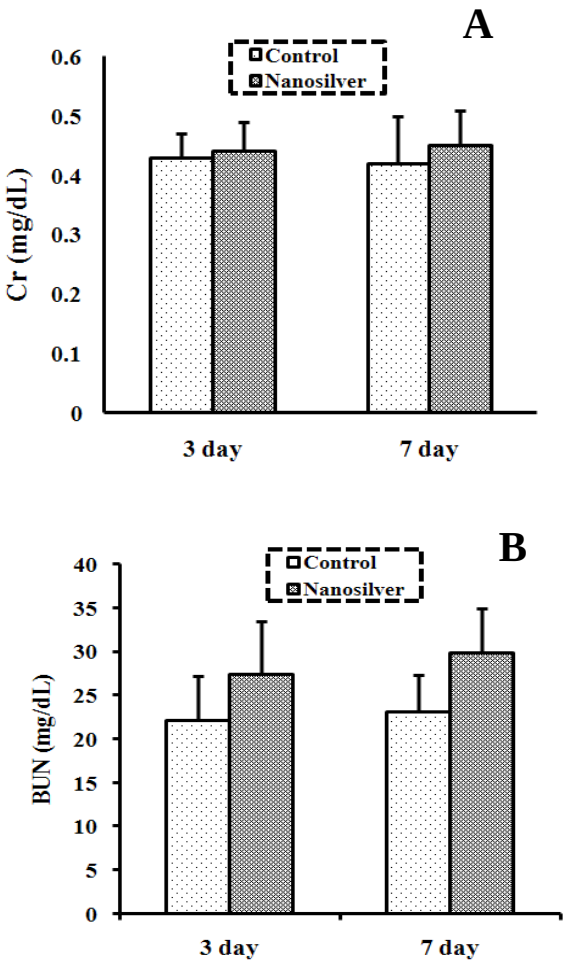

Figure 2. Changes in the renal function parameters after 3 and 7 days following treatment with nanosilver. (A). No significant increase in Cr level in treatment (nanosilver) group during experiment periods ($p>0.05$) (B). A significant increase in BUN level in treatment (nanosilver) group during experiment periods ($p<0.05$). The values shown are Mean±SD; n = 10 per group.

The level of AST and ALT were increased significantly in treatment group in comparison to control group during experiment periods ($p<0.05$) (Figure 3). At 3 days, the level of AST and ALT in treatment group were, respectively, 22.21±3.33 and 20.72±3.09 mg/dL while they were, respectively, 15.45±1.81 and 12.43±1.41 mg/dL in control group, and also, at 7 days, the level of AST and ALT were, respectively, 24.11±2.96 and 22.51±2.75 mg/dL in treatment group, while they were, respectively, 14.65±2.01

and 12.65±2.14 mg/dL in control group. It should be mentioned that any increase in the renal function parameters and hepatic necrosis biomarkers is indicated hepatic and renal dysfunction.

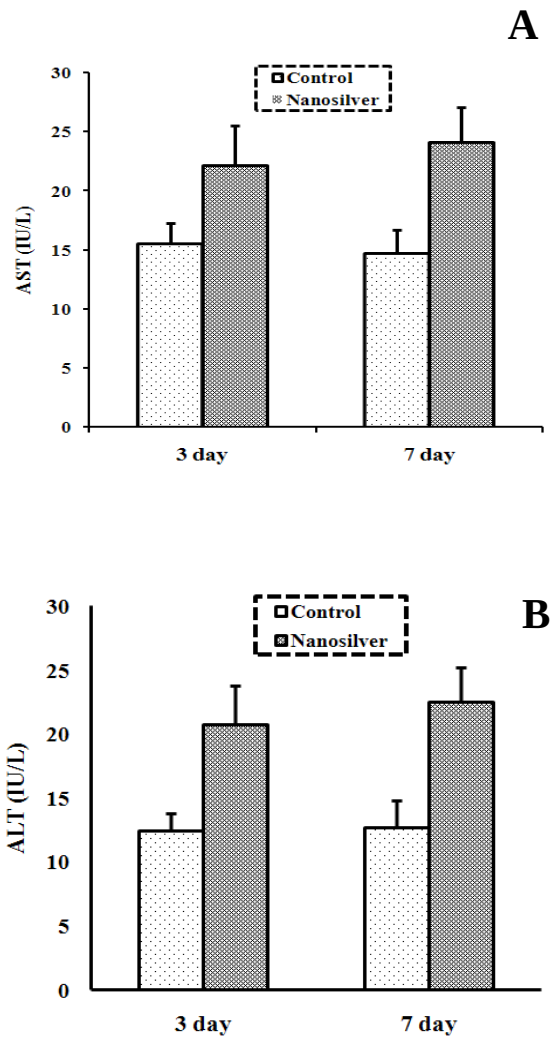

Figure 3. Changes in liver necrosis biomarkers after 3 and 7 days following treatment with nanosilver. (A). A significant increase in AST level in treatment (nanosilver) group during experiment periods ($p<0.05$) (B). A significant increase in ALT level in treatment (nanosilver) group during experiment periods ($p<0.05$). The values shown are Mean±SD; n = 10 per group.

Discussion

Nanosilver plays a strong antibacterial role in the concentration range of 10–50 µg/ml (17). Therefore, in this study we examined the dermal toxicity of nanosilver (10 µg/ml) in mice by measuring renal function parameters and hepatic necrosis biomarkers in serum. The present study showed the renal toxicity and hepato-toxicity of nanosilver.

Researchers have shown that nanoparticles can enter the epidermis and dermis layers through the horny layer of the skin (18). In another study also was found that various nanoparticles can enter the bloodstream after absorbing the dermis (۳).

Tang *et al* (2009) found that nanosilver can enter the body organs such as the kidneys and the liver through the blood stream by subcutaneous injection (۱۹), also nanosilver toxicity on the liver and kidneys was confirmed by Sheng et al (۲۰). Korani *et al* (2013) reported that nansilver (< 100 nm) can cause histopatholgical abnormalities to kidney, heart and bone of guina pigs in a dose-dependent manner following dermal absorption of nanosilver (۲۱). In the present study, the toxic response of kidney was observed by significant rise of renal function parameter (BUN).

Recently, the systemic toxicity of nanosilver-containing dressings on burn wounds after 21 days in rats has been studied. Researchers reported no signi-ficant change of renal function parameters (Cr and BUN) and AST levels, but they found a significant increase in ALT level in nanosilver group which confirmed the hepatotoxic potentials of nanosilver (۲۲). On the other hand, in the present study, nanosilver showed hepatotoxicity and renal toxicity which may be due to different doses and sizes of nanosilver in dressings.

It has been reported that the skin penetration of silver depends on factors such as the concentration and size of silver used in the formulation of nano-products (۲۳).

The majority of toxicological investing-ations on nanosilver are limited to use through mouth (24-31), inhale (32-33) and intravenous injection (34). Daniel *et al* (2009) studied the toxicity of different concentrations of nanosilver trapped in

montmorillonite in Swiss mice. They reported some changes in different biochemical factors in blood and urine such as a decrease in urine creatinine and urea in high dose of Ag (0) montmorillonite (35). Yousef *et al* (2012) evaluated the toxicity of different doses (5 and 10 kg/day) of nanosilver (20 nm) in rates following daily intraperitoneal (IP) injection for 30-days. The results showed a significant increase in BUN and Alkaline phosphatase (ALP) and a significant decrease Cr in low- dose and high -dose groups (36). Kim et al (2008) studied the toxicity of repeated oral doses of nanosilver (60 nm) in rats for 28 days. They reported the hepatotoxicity by a significant increase in ALP and abnormal tissue of liver (13). 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 (37). Several reports showed that nanosilver has the toxicity effects with generating silver ions (Ag^{+}) (17, 38). Silver ions have been reported to interact with thiol groups of the mitochondrial inner membrane, and also generated oxidative stress in mitochondria (39). In addition, it has been shown that the biological effects of nanosilver are dependent on the physical and chemical properties of its (40).

Moudgi et al (2006) found that nanoparticles effects on living cells depend on shape, size and diameter of nanoparticles (5), for example, it has been reported that smaller nanosilver has more accumulation in organs than larger ones after administration of different sizes of nanosilver in Wistar rats (41).

Therefore, nanosilver with having different size and concentration and also generating silver ions has different effects on cells, tissues and organs.

Conclusion

The present results indicate that the dermal absorption of 10 µg/ml nanosilver (40 nm) can lead to hepatotoxicity and renal toxicity. It is proposed that they can do histological investigations, and this study will also be conducted with different concentrations and sizes of nanosilver.

Acknowledgments

This work was fully supported by Deputy Research of Shahrekord University. We thank all those who helped us in this study.

References

1. Chaloupka K, Malam Y, Seifalian AM. Nanosilver as a new generation of nanoproduct in biomedical applications. Trends Biotechnol. 2010; 28(11): 580-588.
2. White RJ. An historical overview of the use of silver in wound management. Br J Nurs 10 (15 Suppl. 2): 3-8.
3. Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005; 113(7): 823-839.
4. Benn T, Cavanagh B, Hristovski K, Posner JD, Westerhoff P. The release of nanosilver from consumer products used in the home. J Environ Qual. 2010; 39(6): 1875-1882.
5. Kowalski Z, Makara A, Banach M, Kowalski M. Zastosowanie preparatów nanosrebra do oczyszczania powietrza z instalacji klimatyzacyjnej zakładów mięsnych. Przemysł Chemiczny. 2010; 89(4): 434-437.
6. Chen X, Schluesener H. Nanosilver: a nanoproduct in medical application. Toxicol Lett. 2008; 176(1): 1-12.
7. Faunce T, Watal A. Nanosilver and global public health: international regulatory issues. Nanomedicine (Lond). 2010; 5(4): 617-632.
8. Arora S, Jain J, Rajwade J, Paknikar KM. Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells. Toxicol Appl Pharmacol. 2009; 236(3): 310-318.
9. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, et al. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J Proteome Res. 2006; 5(4): 916-924.
10. Wzorek Z, Konopka M. Nanosrebro– nowy środek bakteriobójczy. Czas Tech Chemia 2007; 104(1): 175-181.

11. Seaton A, Donaldson K. Nanoscience, nanotoxicology, and the need to think small. Lancet. 2005; 365(9463): 923-924.
12. Kim YS, Kim JS, Cho HS, Rha DS, Kim JM, et al. Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. Inhal Toxicol. 2008; 20(6): 575-583.
13. Kim YJ, Yang SI, Ryu JC. Cytotoxicity and genotoxicity of nano-silver in mammalian cell lines. Mol Cell Toxicol.. 2010; 6(2): 119-125.
14. Kalishwaralal K, Banumathi E, Ram Kumar Pandian S, Deepak V, Muniyandi J, Eom SH, et al. Silver nanoparticles inhibit VEGF induced cell proliferation and migration in bovine retinal endothelial cells. Colloids Surf B Biointerfaces. 2009; 73(1): 51-57.
15. Korani M, Rezayat S, Gilani K, Bidgoli SA, Adeli S. Acute and subchronic dermal toxicity of nanosilver in guinea pig. Int J Nanomedicine. 2011; 6: 855-862.
16. Ji JH, Jung JH, Kim SS, Yoon JU, Park JD, Choi BS, et al. Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats. Inhal Toxicol. 2007; 19(10): 857-871.
17. Kvitek L, Vanickova M, Panacek A, Soukupova J, Dittrich M, Valentova E, et al. Initial study on the toxicity of silver nanoparticles (NPs) against Paramecium caudatum. J Phys Chem C. 2009; 113(11): 4296-4300.
18. Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA. Penetration of intact skin by quantum dots with diverse physicochemical properties. Toxicol Sci. 2006; 91(1): 159-165.
19. Tang J, Xiong L, Wang S, Wang J, Liu L, Li J, et al. Distribution, translocation and accumulation of silver nanoparticles in rats. J Nanosci Nanotechnol. 2009; 9(8): 4924-4932.
20. Tang J, Xi T. Status of biological evaluation on silver nanoparticles. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi. 2008; 25(4): 958-961.
21. Korani M, Rezayat SM, Arbabi Bidgoli S. Sub-chronic Dermal toxicity of silver nanoparticles in Guinea Pig: special emphasis to heart, bone and kidney toxicities. Iran J Pharm Res. 2013; 12(3): 511-519.
22. Bidgoli SA, Mahdavi M, Rezayat SM, Korani M, Amani A, Ziarati P. Toxicity assessment of nanosilver wound dressing in Wistar rat. Acta Med Iran. 2013; 51(4): 203-208.
23. Wasukan N, Kulthong K, Srisung S, Maniratanachote R. A method to evaluate potential dermal exposure to silver in nanoproducts. Proceedings of NanoThailand. 2012; 2012: 1-4.
24. Kim YS, Song MY, Park JD, Song KS, Ryu HR, Chung YH, et al. Subchronic oral toxicity of silver nanoparticles. Part Fibre Toxicol. 2010; 7(1): 20.
25. Maneewattanapinyo P, Banlunara W, Thammacharoen C, Ekgasit S, Kaewamatawong T. An evaluation of acute toxicity of colloidal silver nanoparticles. J Vet Med Sci. 2011; 73(11): 1417-1423.
26. Park K, Park EJ, Chun IK, Choi K, Lee SH, Yoon J, et al. Bioavailability and toxicokinetics of citrate-coated silver nanoparticles in rats. Arch Pharm Res. 2011; 34(1): 153-158.
27. Loeschner K, Hadrup N, Qvortrup K, Larsen A, Gao X, Vogel U, et al. Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. Part Fibre Toxicol. 2011; 8(1): 18.
28. Hadrup N, Loeschner K, Bergström A, Wilcks A, Gao X, Vogel U, et al. Subacute oral toxicity investigation of nanoparticulate and ionic silver in rats. Arch Toxicol. 2012; 86(4): 543-551.
29. Jeong GN, Jo UB, Ryu HY, Kim YS, Song KS, Yu IJ. Histochemical study of intestinal mucins after administration of silver nanoparticles in Sprague–Dawley rats. Arch Toxicol. 2010; 84(1): 63-69.
30. Cha K, Hong HW, Choi YG, Lee MJ, Park JH, Chae HK, et al. Comparison of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles. Biotechnol Lett. 2008; 30(11): 1893-1899.
31. Ahmadi F, Kordestany AH. Investigation on silver retention in different organs and oxidative stress enzymes in male broiler fed diet supplemented with powder of nano silver. Amer-Eurasian J Toxicol Sci. 2011; 3(1):28-35.
32. Sung JH, Ji JH, Park JD, Yoon JU, Kim DS, Jeon KS, et al. Subchronic inhalation toxicity of silver nanoparticles. Toxicol Sci. 2009; 108(2): 452-461.
33. Kim JS, Sung JH, Ji JH, Song KS, Lee JH, Kang CS, et al. In vivo genotoxicity of silver nanoparticles after 90-day silver nanoparticle inhalation exposure. Saf Health Work. 2011; 2(1): 34-38.
34. Tiwari DK, Jin T, Behari J. Dose-dependent in-vivo toxicity assessment of

silver nanoparticle in Wistar rats. Toxicol Mech Methods. 2011; 21(1): 13-24.
35. Daniel SCGK, Tharmaraj V, Sironmani TA, Pitchumani K. Toxicity and immunological activity of silver nanoparticles. Appl Clay Sci. 2010; 48(4): 547-551.
36. Yousef J, Hendi H, Hakami FS, Awad MA, Alem AF, Hendi AA, et al. Toxicity of Silver Nanoparticles after Injected Intraperitoneally in Rats. Journal of American Science. 2012; 8(3): 589-593.
37. Stepien KM, Morris R, Brown S, Taylor A, Morgan L. Unintentional silver intoxication following self-medication: an unusual case of corticobasal degeneration. Ann Clin Biochem. 2009; 46(6): 520-522.
38. Navarro E, Piccapietra F, Wagner B, Marconi F, Kaegi R, Odzak N, et al. Toxicity of silver nanoparticles to chlamydomonas reinhardtii. Environ Sci Technol. 2008; 42(23): 8959-8964.
39. Almofti MR, Ichikawa T, Yamashita K, Terada H, Shinohara Y. Silver ion induces a cyclosporine a-insensitive permeability transition in rat liver mitochondria and release of apoptogenic cytochrome C. J Biochem. 2003; 134(1): 43-49.
40. Panácek A, Kvitek L, Prucek R, Kolar M, Vecerova R, Pizurova N, et al. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. J Phys Chem B. 2006; 110(33): 16248-16253.
41. Lankveld DP, Oomen AG, Krystek P, Neigh A, Troost–de Jong A, Noorlander C, et al. The kinetics of the tissue distribution of silver nanoparticles of different sizes. Biomaterials. 2010; 31(32): 8350-8361.