Toxicity Assessment of Nanosilver Wound Dressing in Wistar Rat

Sepideh Arbabi Bidgoli[1], Moujan Mahdavi[2], Seyed Mahdi Rezayat[1,3,4], Mitra Korani[3], Amir Amani[4], and Parisa Ziarati[5]

[1] *Department of Toxicology & Pharmacology, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran*

[2] *Young Researchers Club (YRC), Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran*

[3] *Department of Pharmacology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[4] *Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[5] *Department of Medicinal Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran*

Received: 10 Apr. 2012; Received in revised form: 10 Dec. 2012; Accepted: 4 Jan. 2013

Abstract- Antibiotic resistance to microorganisms is one of the major problems faced in the field of wound care in burns patients. Silver nanoparticles have come up as potent antimicrobial agent and are being evaluated in diverse medical applications ranging from silver based dressings to silver coated medical devices. We aimed in present study to test the release of nanosilver from nanosilver wound dressing and compare the dermal and systemic toxicity of nanosilver dressings in a repeated dose (21 days) model. Under general anesthesia, a limited standard 2nd degree burns were provided on the back of each rat in all treatment, negative control (simple dressing) and 5% silver nitrate groups, each contained 5 male wistar rats. According to the analysis made by atomic absorption spectrometry, the wound dressings released 0.599 ± 0.083 ppm of nanosilver during first 24 hrs of study. Daily observations were recoded and wounds were covered with new dressings each 24 hrs. Burn healing was observed in nanosilver wound dressing group in shorter time periods than the control groups. In toxicity assessment, this dressing didn't cause any hematological and histopathological abnormalities in treatment group but biochemical studies showed significant rise of plasma transaminase (ALT) at the endpoint (21 days) of the study (P=0.027). Portal mononuclear lymphoid and polymorphonuclear leukocyte infiltrations in three to four adjacent foci were recognized around the central hepatic vein in treatment group. Mild hepatotoxic effects of nanosilver wound dressing in wistar rat emphasize the necessity of more studies on toxicity potentials of low dose nanosilver by dermal applications.

Acta Medica Iranica, 2013; 51(4): 203-208.

Keywords: Nanosilver; Safety; Silver nitrate; Toxicity; Wound dressing

Introduction

Nanosilver has been used as antiseptic agent for more than a century but in recent years (1) it has found increasing global attentions because of its stronger biocidal characteristics in comparison to the silver (2). Silver nanoparticles have come up to the market by many industries with diverse medical applications ranging from silver based dressings to silver coated medical devices in band, pad, gloves, catheter cover, wound dressing etc (3,4). Because of very few data on the safety of silver nanoparticles which is increasingly used in industry and its direct contact with the skin, the dermal and systemic toxicity of this nanoparticle should be carefully determined by *in vitro* and *in vivo* models to define its safety thresholds for regulatory purposes (5,6).

According to our recent study, histopathological abnormalities of high doses (>100 μg/kg) of silver nanoparticles in the skin, liver and spleen of male guina pigs could be appeared by dermal administration in a dose-dependent manner in comparison to silver nitrate (7). In fact the most important toxicological concern of nanosilver is its redox activities and its potential to transport across cell membranes which may causes some toxic interactions with sub cellular organelles (8). Nanosilver crystallines could be toxic to keratinocytes and fibroblasts which are even more sensitive to the nanoparticle than keratinocytes (9). Therefore it is

Corresponding Author: Sepideh Arbabi Bidgoli
Associate Prof. of Toxicology & Pharmacology, Islamic Azad University, Pharmaceutical Sciences Branch (IAUPS), No 99, Yakhchal, Gholhak, Dr. Shariati, Tehran, Iran.
Tel/Fax: +98 21 22600037, E-mail: arbabi.s@iaups.ac.ir, sepideharbabi@yahoo.com

necessary to balance the healing potencies and toxic effects of each nanosilver contained wound dressings on skin and other organs before any clinical use (10,11). We aimed in the present study to compare the long-term dermal and systemic toxic effects of nanosilver burn dressing with dermal silver nitrate in male wistar rat.

Materials and Methods

Nanosilver burn dressing

The nanosilver burn dressings were kindly provided by Emad Pharmaceutical Company in sterile packages. All dressings were humidified with sterile distilled water before any application according to the manufacturer's instruction.

Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM)

AFM analysis was performed by Maharfanabzar Co. in Iran. Scanning electron microscopy was performed by KTH University in Sweden to test the presence and the morphology of nanosilver and to confirm the AFM results. Both techniques showed cluster of nanocrystaline silver with distribution in nanometric sizes.

Migration analysis by atomic absorption spectrometry

The extent of nanosilver release from wound dressing was performed by atomic absorption under the optimum conditions. The physiologic solution was provided as the receptor fluid by dissolving 2.38 g of Na_2HPO_4, 0.19 g of KH_2PO_4 and 9 g of NaCl in 1 l of milli Q water and the final pH was adjusted to 7.35. After preparing fresh solutions, nanosilver dressings were cut in the sizes of 3.29 cm^2 and remained in this physiological solution for 24 hrs at 25°C (11). The exact levels of nanosilver migration from wound dressings were determined by an electro-thermal atomic absorption spectrometry after 24 hrs. All salts were purchased from Merck Company for this experiment.

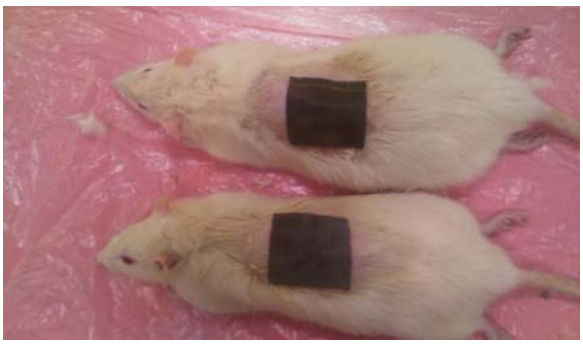
Experimental animals and housing conditions

Experimental male wistar rats were obtained from Pasteur Institute of Iran at 4-8 weeks of age and 100 ± 20 g body weight. Each three rats were housed in separate stainless steel cages and allowed to adapt to the conditions of the animal house for 10 days before starting the main experiment. The animals were maintained on a 12 h dark/light cycle at about 22±3ºC and allowed free access to standard laboratory diet (Pars Co.) and tap water *ad libitum* during the experiments.

Sub-chronic dermal toxicity studies

All of 45 rats were randomly divided into three groups (15 males /group). The back zone of each animal was shaved in 6 x 4 cm. At the beginning of study and under the routine general anesthesia conditions for animals, a limited standard 2nd degree burn was produced on the back of each rat in 2 of 3 groups by hot stamps (treatment and silver nitrate groups). In the main intervention group the wounds were covered with nanosilver dressing; for negative control group the dressing was a simple dressing (without any burning), and the positive control group was treated with 5% silver nitrate (Figure 1). All dermal changes were recorded in the site of application in treatment groups. In dermal toxicity tests, 0.5% concentration of $AgNO_3$ was kept as positive control and a comparative control of simple wound dressing was kept as negative control. The duration of experiments lasted for 21 days.

All of wound dressings were humidified by sterile distilled water in all groups during this 21 days study and all clinical conditions were observed and documented daily. This animal study was conducted according to the NIH guideline (10).



(A)

(B)

Figure 1. A: Second degree burns were induced in rats during general anesthesia and coated by nanosilver containing wound dressing B: Male wistar rats after complete dressing.

Clinical examinations

Clinical signs were observed and weights were recorded once daily. The recorded items were divided to three categories:

Cageside observations contained home-cage activity, feces amount, feces color, feces consistency, urine amount, urine color and behavior while removing from cage.

Neurological Examination contained tail elevation, abnormal gait, ataxic gait and head Position.

Physical Examination included death, hair coat, mucus membrane/eye/skin color, body temperature, respiratory rate, respiratory character, lacrimation, salivation amount and eye prominence.

Hematological studies

Various hematological parameters including hematocrit (Hct), hemoglobin concentration (Hb), mean corpuscular Hb (MCH), mean corpuscular Hb concentration. (MCHC), mean corpuscular volume (MCV), total leukocytes (WBC) and platelet count were determined in three groups by automated blood analyzer in the main laboratory of Imam Khomeini university hospital at days 21 and 45 of present study.

Biochemical assays

Biochemical parameters measured in three animals/group with an automated biochemical analyzer in the same laboratory and consisted of albumin, total cholesterol, LDL, HDL, total protein, fasting triglycerides, total protein (TP), creatinine, urea nitrogen, aspartate aminotransferase (AST or SGOT), alanine aminotransferase (ALT or SGPT), alkaline phosphatase (ALP), total bilirubin, glucose, calcium, phosphorus potassium, sodium in days 21 and 45 of study.

Pathologiscal studies

Skin, liver, spleen, stomach, kidneys, lungs, testes and hearts were removed from 3 groups whose blood and serums were assayed for hematological and biochemical studies. Organ weights were recorded and absolute and relative organ weights were compared in each group with related control. The tissues were fixed in 10% buffered formalin and dehydrated in graded series of alcohol, cleared in xylene and embedded in paraffin wax. Multiple sections from each block were prepared at 5 µm and stained with haematoxylin and eosin (H&E).

Statistical analysis

Values were expressed as means ± SD. To compare groups, homogeneity of variances was evaluated first. When variances were not significantly different data were analyzed by one-way analysis of variance (ANOVA) and the Student's t-test. When variances were considered significantly different, Man Whitney U test for comparison of two variables and Kruskall Wallis H test for comparison of more than two variables were used. A significant difference was accepted with $P<0.05$.All statistical methods were performed by SPSS 16.

Results

SEM and AFM analysis

According to the AFM and SEM results, this product didn't contain individual nanoparticles; hence there was no particle release and it seemed the antibacterial efficacy maintain by dissolution of silver clusters. Both techniques showed clusters of nanocrystaline silver with distribution in nanometeric levels (Figure 2).

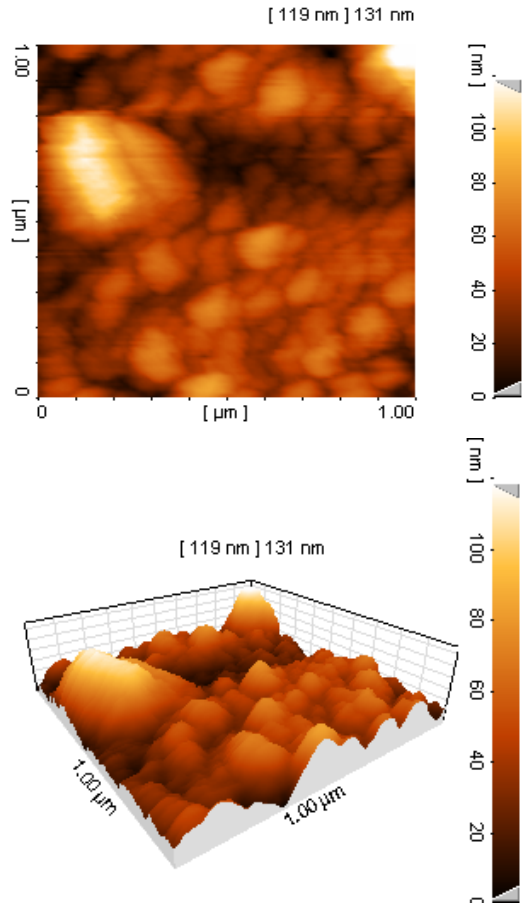


Figure 2. Cluster of nanocrystaline silver with different range of distribution in nanometric scale.

Nanosilver release

Migration amount of nanosilver into buffer solution from wound dressing was determined for one end point at 24 hrs. According to the analysis made by atomic absorption spectrometry, the wound dressings released 0.599 ± 0.083 ppm of nanosilver during 24 hrs study. The amount of silver migration was observed in an increasing manner with storage time and temperature (data was not showed).

Dermal toxicity study

During 21 days of study no deaths and no signs of toxicity were recorded in the three groups moreover the burn healing process was more acceptable in the cases when compared with control positives. Based on the lack of mortality in the male Wistar rats at the limit test, interventions were continued for the next 21 days of this study although all animals looked healthy with normal physical activities during the next 21 days of study.

Growth rate

Weights of animals from all three groups were recorded continuously until the final step of this study. Although they didn't showed any significant weight gain for the duration of the study but no statistically significant changes were also observed between groups with respect to body weight at any time point evaluated. No significant differences were also recorded in food and water consumption of animals during this study. Table 1 compares the mean weights (SD) of animals during this 21 days study.

Hematological Studies

Blood samples of animals were analyzed at the baseline, days 10 and 21 of study and compared at each endpoint with controls. No significant change was detectable in three points between cases and two control groups. Table 2 compares the levels at the endpoint of study.

Biochemical Studies

Daily intervention with nanosilver wound dressing caused significant rise in ALT levels (P=0.027) at the endpoint of study. Table 3 shows the clinical chemistry values in three different groups.

Necropsy studies

Absolute weights of kidney (P=0.038) and liver (P=0.036) were increased in treatment group. Other organs including heart, lung, testis and spleen remained unchanged from corresponding control groups.

Pathological studies

Pathological studies were performed on H&E stained slides at baseline, mid point and day 21 of study. No pathological lesion was detected in heart, lungs, spleen, kidney, and testis of animals during the treatment periods. Portal mononuclear lymphoid and polymorphonuclear leucocytes infiltrations in three to four adjacent foci were detected around the central hepatic vein in treatment group.

Table 1. Weight change patterns (g) of three groups of male wistar rats in 21 days of study.

Time intervals (day)	Mean weights (SD)			*P*-value		
	G1*	G2**	G3***	G1 & G2	G2 & G3	G1&G3
Day 0	195.33(8.08)	197.00(10.81)	203.33(16.25)	0.593	0.498	0.294
Day 14	211.66(10.40)	220.33(1.52)	211.00(8.18)	0.053	0.182	0.607
Day 21	203.66(10.40)	209.66(3.78)	206.33(14.01)	0.127	0.214	0.743

*Nanosilver Dressing ** Simple Dressing *** Silver Nitrate

Table 2. Comparison of hematological values between three groups of male wistar rats at 21 days of study.

Hematological Variables	Treatment groups			*P*-value		
	G1*	G2**	G3***	G1 & G	G2& G3	G1 & G3
Total Erythrocyte Count /RBC (10^{12}/L)	7.90 (1.14)	5.66(1.45)	7.90(0.42)	1.00	0.856	0.926
Total Leukocytes /WBC (10^{9}/L)	3.65(2.19)	7.90(0.42)	5.66(1.45)	0.293	0.084	0.990
Hgb(g/dl)	15.15(1.90)	15.00(0.40)	15.00(0.40)	0.895	0.441	0.897
HCT (%)	42.25(5.02)	44.43(1.47)	44.43(1.47)	0.501	0.409	0.700
PLT (10^3/UL)	761.2(185.96)	657.2(140.74)	657.2(140.74)	0.522	0.953	0.760
Poly(%)	23.00 (1.41)	23.00(2.64)	23.00(2.64)	1.00	0.096	0.185
Lymph (%)	77.00(1.41)	74.66(3.05)	74.66(3.05)	0.402	0.141	0.185

*Nanosilver Dressing ** Simple Dressing *** Silver Nitrate

Table 3. Comparison of biochemical values between three groups of male wistar rats at 21 days of study.

Biochemical Variables	Treatment Groups			*P*-value		
	G 1	G2	G3	G1 &G2	G3 & G2	G1&G3
FBS(mg/dl)	118.2(22.94)	112.2(23.43)	132.2(47.14)	0.767	0.553	0.675
Urea(mg/dl)	40.66(3.51)	43.00(12.49)	51.00(4.35)	0.771	0.354	0.033*
Creatinine (mg/dl)	0.46(0.57)	0.43(0.057)	0.50(0.023)	0.519	0.116	0.374
Cholesterol(mg/dl)	73.00(12.16)	51.66(11.23)	65.33(9.29)	0.090	0.180	0.435
Triglyceride(mg/dl)	54.66(2.51)	53.33(23.86)	43.66(16.16)	0.928	0.592	0.309
HDL(mg/dl)	45.00(3.46)	37.33(7.57)	44.00(6.00)	0.186	0.298	0.815
LDL(mg/dl)	21.00(4.58)	13.33(3.78)	19.66(3.21)	0.089	0.092	0.701
AST(Iu/L)	197.2(38.69)	180.2(22.18)	2.43E2(102.99)	0.560	0.361	0.506
ALT(Iu/L)	59.66(3.05)	47.33(5.50)	51.66(4.50)	0.027*	0.351	0.064
ALP(Iu/L)	293.2(52.57)	265.2(59.35)	2.962(121.12)	0.574	0.708	0.967
Ca(mg/dl)	9.43(0.35)	9.46(0.05)	9.66(0.50)	0.879	0.523	0.546

*Nanosilver Dressing ** Simple Dressing *** Silver Nitrate

Discussion

The dermal use of safe and effective topical anti-microbial agents (TAAs) is a very important issue in burn healing because the patients who are suffering from major burns are at higher risk to both cutaneous and systemic infections. Prior to the routine uses of TAAs, burn wound sepsis was listed as the major cause of death in 60 % of burns mortality. After the routine application of topical silver nitrate in 1965 (12), a range of Ag-containing bandages have come into the commercial use but after introducing the nanosilver some public concerns were released over the potential adverse effects of nanosilvers (13). Nowadays the widespread use of silver nanoparticles in commercial products, from silver based dressings to silver coated medical devices, will likely result in an unknown spread of silver into the body and environment therefore the quantification and characterization of the nanosilver released from medical products is an important regulatory issue needed to predict the possible harmful effects of nanosilver in the human and environment (14). This study has tried to characterize and quantify the possible toxicity potentials of a commercial nanosilver coated wound dressing product (Agicoat ®) by dermal application in rat and identified mild hepatotoxic effects of this product by continuous long term application on burned skin .

Most of toxicological studies on nanosilver are limited to inhalational (15,16) or oral routs of administration (16,17) but the most popular rout of nanosilver administration is dermal use via medical products with undetermined systemic and topical toxicity potentials (18). This study has identified the existence of nanocrystaline silver clusters with nanometeric levels in Agicoat ® burn dressing by AFM and SEM and the release of nanocrystaline silver in the first 24 hours of exposure to the textile were proved by atomic absorption analysis. We used rat models to assess the possible toxic effects of this nanomaterial in a subchronic study. Despite of its safety profile in clinical, hematological and pathological studies, significant rise of ALT (P=0.027) was identified at the end point of study. To confirm the hepatotoxic potentials of this nanomaterial, pathological studies were conducted and the portal mononuclear lymphoid and polymorphonuclear leucocytes infiltration in three to four adjacent foci around the central hepatic vein in the treatment group was identified which were not appeared in two control groups.

Our recent study in guina pig clearly showed that high dose dermal application of nanosilver may cause histopatholgical abnormalities to the skin, liver and spleen of animals which could be magnified by increased concentration in longer terms of exposures (7). The same extent of toxic response was not detectable in this study on rat because of lower dermal doses and shorter periods of application but low toxic responses of liver by significant rise of ALT, and minor pathological changes have emphasized the hepatotoxic potentials of this nanoparticle even in lowest doses (0.599±0.083 ppm during 24 hrs) comparing to control groups. In conclusion, present results have indicated obviously the mild hepatotoxic effects of nanosilver even via its release from the textile during dermal application on the burned skin of wistar rat. Further studies are necessary to confirm this preliminary data which was showed by serological and pathological abnormalities of the liver. Present results also emphasized the necessity of more

studies on the possible toxic responses on the liver and other organs by electronic microscope and analysis of specific molecular markers. Efficacy and safety of nanosilver wound dressing should be assessed also in the future human clinical studies.

Acknowledgements

The authors acknowledge Emad Pharmaceutical Company that has provided the Agicoat® and supported this study but was not involved in design and conduct of this study.

References

1. Costanza J, El Badawy AM, Tolaymat TM. Comment on "120 Years of Nanosilver History: Implications for Policy Makers" .Environ Sci Technol 2011;45(17):7591-2.
2. Park SH, J-H Im JH, Im JW, Chun BH, Kim JH. Adsorption Kinetics of Au and Ag Nanoparticles on Functionalized Glass Surfaces. Microchemical Journal 1999;63(1):71-91. http://www.sciencedirect.com/science/article/pii/S0026265X99917691
3. Soni I, Salopek-Bondi B. Silver nanoparticles as antimicrobial agent: a case study on E.coli as a model for Gram-negative bacteria. J.Colloid Interface Science 2004;275(1):1770-82.
4. Crosera M, Bovenzi M, Maina G, Adami G, Zanette C, Florio C, Filon Larese F. Nanoparticle dermal absorption and toxicity: a review of the literature. : Int Arch Occup Environ Health 2009;82(9):1043-55.
5. Teodoro JS, Simões AM, Duarte FV, Rolo AP, Murdoch RC, Hussain SM, Palmeira CM. Assessment of the toxicity of silver nanoparticles in vitro: A mitochondrial perspective. Toxicol In Vitro 2011;25(3):664-705.
6. Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, de Camargo ER, Barbosa DB. The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. Int J Antimicrob Agents 2009;34(2):103-10.
7. M Korani, SM Rezayat, B Minaee, Arbabi Bidgoli S, Gilani K, Adeli S .Acute and subchronic dermal toxicity of nanosilver in guinea pig. Int J Nanomedicine 2011;6:855-62.
8. Poon VK, Burd A. In vitro cytotoxity of silver: implication for Clinical wound care. Burns 2004;30(2):140–7.
9. Paddle-Ledinek JE, Nasa Z, Cleland HJ. Effect of different Wound dressings on cell viability and proliferation. Plast Reconstr Surg 2006;117(7 Supple):110S–8S.
10. Cuttle L, Naidu S, Mill J, Hoskins W, Das K, Kimble RM. A retrospective cohort study of Acticoat versus Silvazine in a pediatric population.Burns 2007;33(6):701-7.
11. 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):1875-82.
12. National Research Council (US) Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research. Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research, Washington (DC): National Academies Press (US); 2003.
13. Fraser JF, Bodman J, Sturgess R,Faoagali J, Kimble RM. An inviro study of the anti-micribial efficacy of a 1% silver sulphadiazine and 0.2% chlorhexidine digluconate cream,1% silver sulphadiazine cream and a silver coated dressing.Burns 2004;30(1):35-41.
14. Siver S, Phung LeT, Silver G. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. Microbial biotechnol 2006;33(7):627-34.
15. Geranio L, Heuberger M, Nowack B. The behavior of silver nanotextiles during washing. Environmental Science & Technology 2009;43(21):8113–8.
16. Stebounova LV, Adamcakova-Dodd A, Kim JS, Park H, O'Shaughnessy PT, Grassian VH, Thorne PS. Nanosilver induces minimal lung toxicity or inflammation in a subacute murine inhalation model. Part Fibre Toxicol 2011;8(1):5.
17. Hollinger MA. Toxicological aspects of topical silver pharmaceuticals. Crit Rev Toxicol 1996;26(3):255–60.
18. Tang J, Xi T. Status of biological evaluation on silver nanoparticles. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi 2008;25(4):958-61.