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Histopathological study on acute toxicity of nanochelating based silver nanoparticles in mouse model

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ABSTRACT

For the therapeutic application and drug delivery of AgNPs in medicine, pharmacy and cosmetic products, it is essential to know the distribution and local or systemic toxicity associated with them. For this purpose, this study was carried out to assess the potential consequences of skin injection of nanochelating based synthesized AgNPs on the mice models. Nanochelating technology used to design and synthesize the AgNPs. The histopathological findings in skin and tissue of micemodels have assessed via histopathological analysis.All samples were visualized by an independent pathologist. The results of each sample have reported as follows, micrograph of the skin cells have shown the normal architecture and cells in all samples. Moreover, histopathological evaluation of samples have shown normal without any significant pathological changes compared to control groups in volume of derm and epiderm, and the number of fibroblast, neutrophil, and macrophage.In summary, this study has observed no obvious decline of immunological performance and morphological signs of skin damage in the mice caused by nanochelating based AgNPs exposure. These findings could provide a fundamental understanding of the intrinsic toxicity associated with nanochelating based AgNPs on biological models. Moreover, this study would arisetypical attentions on the future applications of nanochelating based AgNPs on human, which is valuable for short-term and low dose treatment in nanomedicines.

Keywords: *Histopathological Study, Nanochelating, Silver Nanoparticles, Skin Cytotoxicity*

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INTRODUCTION

Data indicated that patients with healthcare-associated infections(HCAIs) have unique epidemiology and are associated with significant morbidity and mortality[1]. Bacterial infections are the main etiology of HCAIs which consist of a wide range of Gram-positive and -negative bacteria[2]. Multi-drug resistant (MDR) bacteria has spread throughout the world and has become one of the most frequent bacteria among HCAIs[3].

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Infections caused by MDR strains have reached epidemic proportions globally[4]. The overall burden of HACIs, particularly that caused by resistant strains is increasing in many countries in both healthcare and community settings[5]. The emergence of antimicrobial resistance in bacteria as a consequence of antibiotic selective pressure can lead to their spread in conjunction with horizontal and vertical gene transfer mechanisms[6]. Therefore, the development of alternative drug targets or novel approaches for therapeutic or

prophylactic intervention is essential[7].

Nowadays, nanotechnology is used widely in biomedicine, with applications in drug delivery[8]. As knowledge has grown in this field and a multidisciplinary approach employed, targeted therapeutics have been developed which allow the drug of choice to reach the desired site of action in the body, may be used at a much lower dose, hence overcoming the problem of resistance and diminishing other undesirable side effects[9]. Conjugation of small molecule antibiotic drugs onto nanoparticles, silver nanoparticles, for example, is a possible approach to overcome the challenge of bacterial resistance by exploiting the synergistic effect observed in the use of both the drug and the nanoparticle together[9].

Silver nanoparticle (AgNPs) are largely used as bio-markers and biodelivery vehicles in the medicine, pharmacy and in cosmetic products[10, 11]. However, for the therapeutic application and drug delivery of AgNPs, it is essential to know the distribution and local or systemic toxicity associated with them[10, 11]. For this purpose, this study was carried out to assess the potential consequences of skin injection of nanochelating based synthesized AgNPs on the mice models.

MATERIALS AND METHODS

Synthesis of AgNPs

This study was performed using Nanochelating based technology provided by the SodourAhrarShargh Co. To design and synthesize the AgNPs, a method for producing chelate compounds was registered at US20120100372A1 in the United States Patent Office[12]. Self-assembly method has been applied to produce AgNPs as described previously in our published work[13]. Based on electron microscopy assay the mean size of two synthesized AgNPs were about 20-25 nm for NPs (A), and 30-35 nm for NPs (B)[13]. Moreover, these nanoparticles were classified as non-toxic based on our previous reported toxicity. The LD50 of NPs (A) and NPs (B) was estimated 250 mg/Kg and 350 mg/Kg, respectively when administrated intraperitoneal for mice.The IC50 of NPs (A) and (B) were calculated 350 and 700 µg/ml, respectively.

Bacterial strains

The strains used were a Gram-positive and -negative bacteria, including Staphylococcus aureus ATCC 25923, and Acinetobacter baumannii ATCC 19606, respectively. All bacterial strains were obtained from the Institute of Pastor Technology (Tehran, Iran). The strains were recovered from stocks by cultured overnight at 37 °C under aerobic conditions in tryptic soy agar (TSA) plates containing 5% defibrinated sheep blood (Merck, Germany).

Animals

Six to eight-weeks-old male mice (Weight 200±10 g) were obtained from the Pasteur Institute of Iran. All the animal studies were conducted according to the relevant national and international guidelines of the ShahidBeheshti University of Medical Sciences. All the mice were maintained in large group houses under temperature 25 ± 2 °C and 12-hour dark/light cycles with proper access to food and water.

Experimental design

Animals were divided into 22 groups, 6 mice in each group.AgNPs minimum inhibitory concentration(MICs) were determined elsewhere and used here[13], while antibiotics used concentrations was based on the clinical and laboratory standards institute (CLSI) recommendation [14]. Mice were shaved on the back and injected intra-dermally with 10 µl of *S. aureus* (groups A1 to F1) and *A. baumannii* (A2 to F2) with a final concentration of 1×10^7 CFUs using a sterile insulin syringe. At 24 h after the first injection, mice treated with 0.1 mg kg^{-1} and 0.01 mg kg^{-1} NPs alone and simultaneously with vancomycin (1 mg kg^{-1}) and colistin (15 mg kg^{-1}) via intravenous injection (tail vein).

Histopathologicalstudy

The animals were euthanized two days post-treatment and the harvested tissue have fixed in the 10% neutral buffered formalin (NBF, PH. 7.26) for 48 h, then samples processed and embedded in paraffin. Tissue samples decalcified in 5% (w/v) ethylene diaminetetraacetic acid (EDTA) (Sigma–Aldrich, USA) for 20 days. The 5µm thick sections were prepared and stained with heamtoxylin and eosin (H&E). When the pathologist judged that the mid-modiolar region had been reached, two sequential mid-modiolar sections of 6 µm thicknesses were mounted, cover-slipped and stained with haematoxylin and eosin (H&E). Finally, the histological slides were evaluated by the independent reviewer, using light microscopy (Olympus BX51; Olympus, Tokyo, Japan). Also, the

probable inflammation or other lesions have been assessed in different samples comparatively. In addition, any histopathological findings in the skin and tissue have been assessed via histopathological analysis.

Statistical analysis

The results are presented as descriptive statistics in terms of relative frequency. Values were expressed as the mean ± standard deviation (continuous variables) or percentages of the group (categorical variables).

RESULTS

All the samples were visualized by an independent pathologist. The results of each sample have reported as follows, a micrograph of the skin cells shows the normal architecture and cells in all samples (Fig. 1). Moreover, histopathological evaluation of samples shows normal without any significant pathological changes compared to control groups in the volume of derm and epiderm, and the number of fibroblast, neutrophil, and macrophage (Fig. 2).

DISCUSSION

In this study, the pathologic study was carried out in mice models using two different nanochelating based AgNPs. In many previous studies, the pathologic effects of different metal nanoparticles were investigated using mice models[15-17]. Considering that nanoparticles might undergo

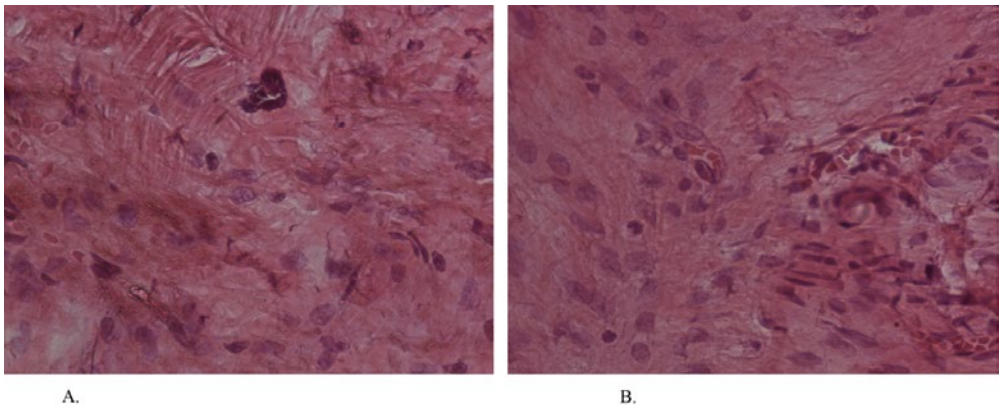

Fig. 1. The pathologicalresults of skin cells micrograph
A. Two days after administration of AgNPs (A); B. Two days after administration of AgNPs (B)

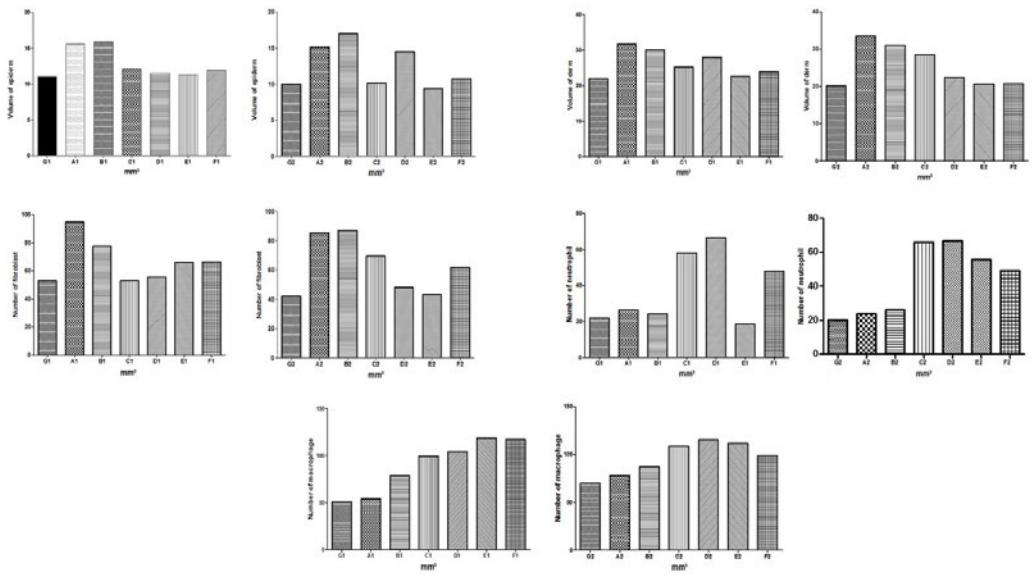

Fig. 2. Histopathological evaluation of samples compared to control groups in volume of derm and epiderm, and the number of fibroblast, neutrophil, and macrophage

degradation and may release silver ions in cellular environments, the intrinsic cytotoxicity of the free nanoparticles should be evaluated[18]. In this study, through TEM and SEM characterization[13], and XRD spectrum measurement(unpublished work), the nanochelating based AgNPs were successfully synthesized. Although the cytotoxicity effects of both synthesized AgNPs were assessed by determination of IC50 and LD50 (unpublished work) *in vitro*, the little study is available to investigate the *in vivo* pathologic effects of AgNPs[19, 20]. However, almost these studies used synthesized nanoparticles rather than nanochelating based technology to study their biological toxicity. The effective concentrations of AgNPs in our study ranged from 1 to 10 mg/ml, In agreement with our results, Hajipour et al. showed antibacterial effects of AgNPs on bacterial strains[21].

Therefore, this information could not reveal that our synthesized AgNPs toxicity in practical applications. Inflammatory potentials related to AgNPs were previously reported [20, 22]; However, little is known about the acute and sub-acutepathobiological effects after nanochelating based technology AgNPs skin exposure. The present study investigated skin effects of AgNPs using a combination of molecular and imaging approaches after 48 h exposure.

The no significant damage and inflammation of the skin demonstrated by the morphological examination of derm and epiderm, and also any changes in the contents of fibroblast, neutrophils and macrophage cells suggested that AgNPs has a no cytotoxicity effect on the mouse skin after nanoparticle injection. After the mice were exposed to the AgNPs nanoparticles, the mice exhibited no significant abnormal pathology changes, which was consistent with the results of Kim et al. [23], and Maneewattanapinyo et al. [24].Finally, as the main limitation of the present study,only one cell line has been investigated because of financial problems.

In conclusion, this study has observed no obvious decline of immunological performance and morphological signs of skin damage in the mice caused by nanochelating based AgNPs exposure. These findings could provide a fundamental understanding of the intrinsic toxicity associated with nanochelating based AgNPs on biological models. Moreover, this study would arise typical attention on the future applications of nanochelating based AgNPs on human, which is valuable for short-term and low-dose treatment in nanomedicines.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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