Possible Protective Effect of Silver Nanoparticles against Cisplatin Induced Pulmonary Inflammation in Rat Model

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Silver nanoparticles (AgNPs) are gaining interest in medical applications for their prominent antibacterial and antimicrobial potentials. AgNPs possess remarkable anti-inflammatory and antioxidant activities and enhances wound healing. The main objective of the current study was to investigate the therapeutic effect of administration of AgNPs on cisplatin (CP) induced pulmonary inflammation in rats. Sixty male albino rats were used in this study. Rats were divided into 6 groups (n=10). Group I control group. Group II and III control groups received AgNPs at doses (5 and 10 ppm). Group IV CP group received CP (2.5 mg/kg). Group V and VI CP group received AgNPs (5, and 10 ppm). All doses were administered intraperitoneally once a day for 4 weeks. Oxidative stress and antioxidant status, inflammatory mediators, fibrogenic as well as apoptotic markers were determined in lung tissues. The results revealed that rats treated with CP showed remarkable elevation in lung tissues MDA, TNF-α, IFN-γ, IL-6, CRP, Fibrinogen and P53 levels associated with depression in SOD, GSH and CAT activities. However, administration of AgNPs (5 or 10 ppm) to CP group resulted in significant amelioration of the aforementioned parameters in a dose dependent manner. Histopathological investigation of lung tissues of CP group demonstrated disruption of normal lung architecture and lung injury. However, treatment with AgNPs revealed

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significant improvement in lung tissue against CP-induced inflammatory changes and lung tissue damage. It could be concluded that AgNPs exert potent cytoprotective effects via combating oxidative stress, inflammation, fibrogenic and apoptotic markers and repairing histopathological changes in lung tissues.

Keywords: silver nanoparticles; cisplatin; lung inflammation; oxidative stress.

1. INTRODUCTION

The lung is the most susceptible organ to infection and injury. Pulmonary inflammation is usually caused by pathogens, toxins or as a side effect due exposure to chemotherapeutic agents such as cisplatin (CP) [1]. Worldwide, the inflammatory lung diseases are one of the leading causes of death.

Cisplatin (Cis-diamminedichloroplatinum [II]) is a potent antineoplastic agent [2] commonly used in many cancers including testis, ovary, bladder, lung, kidney and head neck cancers [3]. Despite its potent anti-tumoral effect, it has detrimental toxic side effects. The reported side effects include oxidative stress, which affects the lungs and various other tissues and organs [4]. Interstitial inflammation, fibrosis, structural pulmonary damage, and other severe complications have also been reported during cisplatin chemotherapy [5]. These adverse effects of cisplatin-induced pulmonary damage may be due to the ability of cisplatin to produce oxidant-induced inflammatory and fibrotic lesions in lungs [6]. Previous study reported that cisplatin induces ROS formation and generates oxygen free radicals and initiates lipid peroxidation and reduces enzymatic and nonenzymatic antioxidant levels [7]. Moreover, cisplatin induces the expression of a variety of inflammatory chemokines and cytokines, including TNF-α and TNF-α mRNA translation [8] which is considered to contribute to the initiation and extension of the inflammatory process.

The current pharmacological management of inflammation is mainly by two groups of drugs the steroidal anti-inflammatory drugs and the non-steroidal anti-inflammatory agents. However, these conventional drugs are associated with numerous side effects that has compelled the need for identification of alternative substances that can resolve inflammation in a way that is homeostatic, modulatory and efficient.

Silver nanoparticles (AgNPs) are gaining more importance due to their diversified biological properties and potential applications. Silver has been used since ancient times for the treatment of wounds and inflammation and nanoparticles of silver have been developed which have potent antiinflammatory [9] and antioxidant activities [10]. Silver derivatives nanoparticles have drawn great attention due to their broad applications in wound dressings, medical devices and having good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms. Owing to the “altered” physical, chemical or optic properties of silver nanoparticles. They possess a wide spectrum of action [11].

2. MATERIALS AND METHODS

2.1 Chemicals

Cisplatin 1 mg/ml Sterile Concentrate colorless to pale yellow solution, purchased from Hospira Company (UK). Silver nanoparticles (Ag-NPs) dark gray powder (Particles Size Analysis {APS} 30-50nm) 1g dissolved in saline, purchased from Sigma Aldrich (USA). All the other chemicals and reagents were analytical grade.

2.2 Animals

Sixty male wistar rats (200 ± 50g) were used in this study. The rats were obtained from the King Fahd Medical Research Centre’s Animal House Colony in Jeddah. In a temperature-controlled room (25 ± 2°C), the rats have been kept on a 12:12 light/dark cycle. At the King Fahd Medical Research Center's Animal Facility Breeding Colony, the rats had ad libitum access to food and water.

2.3 Experimental Design

Rats were randomised in six groups (n=10). group 1 Control group received only 1ml saline. Group 2 received AgNps (5ppm). Group 3 received AgNps (10ppm) [11]. Group 4 induced group received single interperitoneal dose of CP (2.5mg/kg ) [12]. Group 5 and 6 1h after CP administration animals received AgNPs (5ppm and 10ppm).
Treatments with AgNPs were given interperitoneally for 4 weeks. At the end of the experimental period, animals were fasted overnight and then anaesthetized using urethane (1.4 ml/kg, IP) [12]. All animals were rapidly sacrificed and the lung of each animal was removed by dissection, washed by ice-isotonic saline and blotted between two filter papers. Part of the harvested lungs was homogenized in 9 volumes of 0.1 M potassium phosphate-buffered saline at pH 7.4 to give a final concentration of 10% w/v in Potter Elvehjem homogenizer. Homogenates were centrifuged at 2000 rpm for 20 minutes in cooling centrifuge (Hitachi- Japan). Aliquots of supernatant were kept at −20°C for the biochemical determinations. The other part of each lung was stored in 10% formalin-saline at 4°C, then processed for histopathological investigation.

2.4 Biochemical Analysis

2.4.1 Lung tissues oxidative stress markers

Pulmonary tissues lipid peroxidation (MDA) were estimated by measuring thiobarbituric reactive substances (TBARS) according to [13]. Reduced glutathione (GSH) was evaluated by the method of Ellman [14]. The determination of superoxide dismutase (SOD) were carried out according to [15] and Catalase activity (CAT) in lung tissues were determined as described by [16].

2.4.2 Lung tissues inflammatory mediators

Lung tissues tumor necrosis factor alpha TNF-α, Interferon gamma (IFN-γ) (Cat # KHC4021), Interleukin -6 (IL-6) (Cat # BMS213-2) and C-reactive protein (CRP) (Cat # KHA0031) were determined enzyme-linked immunosorbent assay following the instruction of the manufacturer using ELISA kits (Invitrogen Corporation Camarillo, CA, USA) and microtiter plate reader (Fisher Biotech, Germany).

2.4.3 Lung tissues fibrogenic and apoptotic markers

The concentration of fibrinogen (FGN) and P53 in lung tissues were estimated by Enzyme-Linked Immunosorbent Assay (ELISA) sandwich technique using kit purchased from (Bioassay Technology Laboratory, Shanghai, China). Microplate Reader ELx808 (U.S.A).

2.4.4 Histopathological investigation

Lung samples were fixed 10% formalin-saline, embedded in paraffin, and partitioned using a microtome (5-μm thick) (Leica, Berlin, Germany). Hematoxylin and eosin solution were used to stain the sections, which were mounted on glass slides. A light microscope was then used to examine them.

2.5 Statistical Analysis

Values of the biochemical assays were given as mean ± standard error (SE). The data have been analysed using the Statistical Package for the Social Sciences (SPSS) version 11 Statistically significant differences between groups were determined using Analysis of Variance (ANOVA), followed by LSD multiple comparison tests. P< 0.05 was considered to be significant.

3. RESULTS

3.1 Effect of AgNPs Administration on Lung Oxidative Stress Markers

Administration of CP (2.5 mg/kg) resulted in significant elevation in MDA level (267.26%) associated with significant decrement in the levels of GSH (-48.77%), SOD (-75.68%) and CAT (-65.32) as compared to normal control group. The treatment of CP intoxicated rats with AgNPs (5ppm, 10ppm) led to significant amelioration in MDA level as well as significantly improved GSH, SOD and CAT activities as compared to CP group, likely due to the antioxidant effect of the AgNPs.

3.2 Effect of AgNPs Administration on Lung Inflammatory Mediators

Significant elevations in lung tissues TNF-α, IFN-γ (Fig.2a), IL-6 and CRP (Fig. 2b) (112.34%, 82.18%, 117.46% and 192.89%, respectively) following administration of CP as compared to control group. On the other hand, treatment CP rats with AgNPs either 5ppm or 10 ppm significantly reduced these inflammatory mediators in a dose dependent manner as compared to CP treated group.

3.3 Effect of AgNPs Administration on Lung Fibrogenic and Apoptotic Markers

The current data elucidated that induction of lung injury by CP led to a significant increment in both P53 (295.52%) and FGN levels (223.72%) (Fig.3) as compared to normal control group. Upon treating induced group with AgNPs (5ppm or 10ppm doses) resulted in a significant
suppression in both P53 (-61.89%, -72.45%) and FGN levels by -47.13%, -57.43% respectively.

3.4 Histopathological Investigation

Lung sections of normal control, AgNPs (5ppm) and AgNPs (10 ppm.) groups exhibited normal histological features (Fig. 4a, 4b, 4c). Lung sections of group received CP (Fig.4d) showed remarkable decrease in opened normal alveoli with marked increase in spaces between alveoli due to inflammatory cells infiltrate and hemorrhage associated with damage of bronchial lining epithelium with cells desquamated to the lumen. Lung sections of induced group treated with AgNPs (5ppm) (Fig. 4e) showed amelioration in lung architecture. Most alveoli looked normal with few only appeared dilated. Bronchioles showed normal lining epithelium with lumina free of any inflammatory cells or secretion but there is slight thickening of muscle layer and nearby blood vessels. Also, Lung sections of induced group treated with AgNPs (10ppm) (Fig. 4f) revealed significant improvement against CP-induced inflammatory changes and lung tissue damage the alveoli and alveolar sacs nearly looked normal and free from cell debris or inflammatory cells.

![Graph](image1.png)

**Fig. 1a.** Data were expressed as mean +/- SE (a): significance versus control group; (b): significance versus AgNPs (5ppm) group ;(c): significance versus AgNPs (10 ppm) group ;(d): significance versus CP group. Significance was made using OneWay ANOVA(LSD) test. Statistically significant at P≤ 0.05

![Graph](image2.png)

**Fig. 1b.** Data were expressed as mean +/- SE (a): significance versus control group; (b): significance versus AgNPs (5ppm) group ;(c): significance versus AgNPs (10 ppm) group ;(d): significance versus CP group. Significance was made using OneWay ANOVA(LSD) test. Statistically significant at P≤ 0.05
Fig. 2a. Data were expressed as mean +/- SE (a): significance versus control group; (b): significance versus AgNPs (5ppm) group; (c): significance versus AgNPs (10 ppm) group; (d): significance versus CP group. Significance was made using OneWay ANOVA(LSD) test. Statistically significant at P≤ 0.05.

Fig. 2b. Data were expressed as mean +/- SE (a): significance versus control group; (b): significance versus AgNPs (5ppm) group; (c): significance versus AgNPs (10 ppm) group; (d): significance versus CP group. Significance was made using OneWay ANOVA(LSD) test. Statistically significant at P≤ 0.05.

Fig. 3. Data were expressed as mean +/- SE (a): significance versus control group; (b): significance versus AgNPs (5ppm) group; (c): significance versus AgNPs (10 ppm) group; (d): significance versus CP group. Significance was made using OneWay ANOVA(LSD) test. Statistically significant at P≤ 0.05.
Fig. 4. Histopathological examination of lung tissues of normal control rats (a) rats received AgNps (5ppm) (b) and rats received AgNPs (10ppm) (c) showing normal structures. Rats received CP showed marked decrease in opened normal alveoli (thin black arrows) with marked increase in spaces between alveoli due to inflammatory cells infiltrate (white stars) and hemorrhage (red regions) (d). Lung tissues of rats received CP+ AgNps (5ppm) revealed most alveoli looking normal (black arrows) with few only appeared dilated (stars). Bronchioles (black arrows) showed normal lining epithelium with lumina free of any inflammatory cells or secretion but there is slight thickening of muscle layer and nearby blood vessels (e). Rays received CP+AgNPs (10ppm) showed normal alveoli and alveolar sacs free from call debris or inflammatory cells (f).H&E x100 and x200

4. DISCUSSION

Cisplatin is one of the most effective antitumor drugs and used widely [17]. The main cellular target of CP is to deform the natural structure of the DNA which cause DNA damage in cells, block cells division and result in apoptotic cell death [18,19]. It has many side effects appeared to affect the inner body organs. The cytotoxic activity of CP is attributed to pleiotropic mechanisms including inflammation, apoptosis, and necrosis, which affects the lungs and various other tissues and organs [18,20]. The present study was conducted to evaluate the potential protective effects of silver nanoparticles against lung inflammation induced by cisplatin in rats.
In the present study, administration of CP led to a significant elevation in lipid peroxidation level in lung tissue that was revealed by increasing of MDA level. Additionally, resulted in a significant decline in antioxidants detected by decreased levels of GSH, CAT and SOD as compared to control group. Cisplatin induces ROS formation and generates oxygen free radicals, such as hydrogen peroxide, superoxide anions and hydroxyl radicals. The hydroxyl radical is capable of abstracting a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate lipid peroxidation. These radicals can evoke extensive tissue damage, reactivity with macromolecules, such as membrane lipids, proteins and nucleic acids [21]. Thus, an alteration in enzymatic and nonenzymatic antioxidant status indicates that they play an important role in combating free radical induced oxidative stress on the tissue. On the other hand, Nanosilver therapy suppressed MDA level and augmented GSH, SOD and CAT activities. This antioxidant effect of AgNPs were previously reported Negahdary et al., [22]. The possible mechanism of antioxidant activity of Ag NPs includes reductive, electron donating and radical scavenging abilities [23]. Afifi and Abdelazim [24] reported that AgNPs (10 mg/kg) administration to diabetic rats resulted in prevention of the decrease in the activity and mRNA expression levels of SOD, CAT, GRD and GPx and amelioration of the oxidative stress induced by impairment of glucose homeostasis. The reduction of MDA level in the rats administered with AgNPs was due to the ability of AgNPs to improve the antioxidant power of the cells [25].

The current study also revealed that CP administration resulted in vigorous inflammatory responses as evidenced by marked increment in lung tissue TNF-α, IFN-γ, IL-6 and CRP levels as compared to control group. These results are consistent with those of Chauhan et al., [26] and Ali et al., [27]. Cisplatin causes extensive cellular damage and forms a platinum-based DNA adduct (Pt-DNA), which activates the p38 mitogen-activated protein kinase (MAPK) pathway and inflammatory pathway [28]. Cisplatin induces the expression of a variety of inflammatory chemokines and cytokines, including TNF-α and TNF-α mRNA translation. The increase in TNF-α mRNA translation was dependent on cisplatin-induced activation of p38 MAPK and phosphorylation of Mnk1 and eIF4E [8]. Zhang et al., [29] reported that inflammatory cytokines and chemokines are extensively produced in early phases of cisplatin toxicity including interferon γ (IFN-γ), keratinocyte chemotactant (KC) and granulocyte-colony stimulating factor (G-CSF). Macrophage is one of the most important cells in the production of IFN-γ and TNF-α which mediated cisplatin cytotoxicity. Chauhan et al., [26]. C-reactive protein (CRP), a member of the pentraxin family of plasma proteins, is one of the most distinctive acute phase reactants. In response to inflammation, cell damage or tissue injury, plasma level of CRP rapidly and dramatically increases. CRP might be involved in the regulation of lung function and may participate in the pathogenesis of various pulmonary disorders [30]. Chraibi et al., [31] reported that CP was characterized by increased TNF-α, IL-6 levels, these pro-inflammatory cytokines lead to increased levels of C-reactive protein (CRP) with a demonstrable decrease in the anti-inflammatory cytokines. On the contrary, A revealed a significant suppression in the levels of the inflammatory mediators TNF-α, IFN-γ, IL-6 and CRP was observed following the administration of CP rats with AgNPs (5ppm and 10ppm) in a dose dependent manner as compared to CP group. These results were consistent with those of Nadworny et al., [32] and Wong [9]. Mechanisms of anti-inflammatory effects of Ag-NPs have been proposed by reducing IFN-γ and TNF-α by macrophages [9]. It can be presumed that nanosilver anti-inflammatory effect is due to its strong inhibitory effect on the production of Th1cells that secrete the inflammatory cytokines TNF-α, IL-1β and INF-γ which are involved in cellular immunity and in chronic inflammatory disorders [33]. Also, The NF-KB transcriptional pathway is a known signal transducer of TNFα, and is stimulated by the binding of TNFα to its receptor TNFR1, AgNPs can reduce the expression of TNFR1, resulting in a reduction in TNFα-induced signal transduction in a lung epithelial cell line [34]. AgNPs induced a reduction in the activation of the TNFR1/NF-KB transcriptional pathway, resulting in a reduction in the TNFα-induced inflammatory response [35]. In the same trend, Tian et al., [36] reported that AgNPs suppress the production of the early pro-inflammatory cytokines, such as IL-6, but promote the production of an anti-inflammatory cytokine IL-10 after skin injury, which would indicate that AgNPs suppress early inflammation. In fact, IL-6 is the only known cytokine capable of inducing all acute-phase proteins involved in the inflammatory response. As such, the induction of CRP by IL-6 may be one step in the pathobiology of inflammation [37]. It could be assumed that AgNPs administration reduces IL-6, which return.
causes depression in CRP level as indicated by the results of the current study.

The current data indicated an activation in fibrogenic and apoptotic markers in CP treated rats as observed by significant upregulation in FGN and P53 levels as compared to control group. These findings in accordance with those of Stewart et al., [38] and Sun et al., [39]. Marchetti et al, [40] reported that CP induces upregulation of both IL-6 and IL-8 via activation of the NFkB signaling pathway. IL-6 interacts with several target cells to initiate a variety of biological activities, including the stimulation of hepatocytes to produce plasma proteins, e.g. C-reactive protein (CRP), alpha-1-antitrypsin (AAT), alpha-1-acid glycoprotein (AGP) and fibrinogen [41]. In addition, Oo et al., [42] reported that increased oxidative stress and the production of excess free radical may lead to increased endothelial injury and eventually to fibrinogen production. Apoptosis is a form of cell death, and as such it has been frequently induced by chemotherapy agents. Cisplatin-induced damages are considered to be an important trigger of p53 activation that leads to cell apoptosis [19]. Increased cisplatin induced p53 activation, resulting in apoptotic cell death [39]. A primary mechanism by which p53 induces apoptosis is through transcriptional activation and repression of target genes whose promoters contain p53-binding sites. These genes may activate apoptotic process via multiple pathways. The protein p53 activates a host of other genes that lead to cell cycle arrest and activation of DNA repair [19]. As shown by the results of the study by Chtourou et al., [43] increased level of p53 induced by cisplatin leads to the induction of pro-apoptotic genes and, finally, apoptosis. Conversely, Lung injured group received AgNPs (5ppm and 10 ppm) exhibited a significant decrement in both lung FGN and P53 levels as compared to the CP group. These findings were in accordance with those of Asghar et al., [44] and Fawzy et al., [45]. AgNPs have potent anti-inflammatory effects that are capable of reducing inflammatory mediators, i.e., TNF-α, interleukin-1 (IL-1), IL-6, IL-10, and interferon-γ (IFN-γ) [36]. TNF-α and IL-6 appear to play a central role in the development of the acute phase processes during inflammation and share the ability to induce acute-phase proteins such as fibrinogen [46]. Together with other cytokines, TNF-α regulates both fibrinogen and CRP synthesis and, via induction of IL-8, activates neutrophils [47]. So the reduction in the inflammatory mediators caused by treatment with AgNPs could in turn cause the inhibition of fibrinogen. Also, Fehaid and Taniguchi [34] studied the effect of silver nanoparticles on the reduction of apoptosis in lung epithelial cells and reported that a low concentration of AgNPs enhanced the protective effect against apoptosis induced by TNFα. TNFα binds to TNFR1 specifically and enters the cells, and then TNFα is released from its receptors, freeing the receptors to return to the cell membrane to bind more TNFα molecules. This cycle would induce TNFα-signal transduction, leading to apoptosis. AgNPs, binds specifically with the same receptor, forming a TNFR1-TNFα-AgNPs complex, thus disturbing the receptor’s shape, molecular weight, and characteristics, leading to disturbance of its normal pathway. AgNPs-TNF1 complex hinders the reexpression pathway of the receptors on the cell membrane leading to decrease in the TNFα signal transduction and its apoptotic effect. Further in vitro experiments demonstrated that down-regulation of TNF-α in OGD/R cells could increase cell viability and decrease apoptosis and p53 expression [48].

5. CONCLUSIONS

The present study indicated that silver nanoparticles at a dose 5-10 ppm could provide powerful protection against cisplatin induced pulmonary inflammation in a dose dependent manner. AgNPs exerted potent cytoprotective effects via regulating several pathways involved in pulmonary inflammation process. This study demonstrated that AgNPs could possess anti-oxidant, anti-inflammatory, anti-fibrotic and anti-apoptotic effects as well as overcome most of the histopathological adverse effects of cisplatin on lung tissues.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.
**ETHICAL APPROVAL**

Animal Ethic committee approval has been taken to carry out this study.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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