Journal of Clinical and Medical Images

ISSN: 2640-9615

Research Article

Nanomedicine Approach for the Rapid Healing of Diabetic Foot Ulcers with Silver Nanoparticles

Almonaci-Hernández CA[1&], Luna-Vazquez-Gomez R[2&], Luna-Vazquez-Gomez RA[3], Valenciano-Vega JI[4], Carriquiry-Chequer NI[2], Rembao-Hernández A[2], Gomez-Zendejas ML[5], Almanza-Reyes H[6], Garibo-Ruiz D[7], Pestryakov A[8] and Bogdanchikova N[9*]

[1]Integral and Advance Clinic, Ensenada, Baja California, Mexico

[2]School of Medicine, Campus Ensenada, Autonomous University of Baja California, Mexico

[3]Faculty of Medicine, Campus Mexicali. Autonomous University of Baja California, Mexico

[4]Hospital El Mirador of ISSSTECALI Tijuana, Baja California, Mexico

[5]Faculty of Science, Campus Ensenada. Autonomous University of Baja California, Mexico

[6]Faculty of Medicine and Psychology, Campus Tijuana. Autonomous University of Baja California, Mexico

[7]CONACYT - Center of Nanoscience and Nanotechnology, National Autonomous University of Mexico, Mexico

[8]Research School of Chemistry & Applied Biomedical Science, Tomsk Polytechnic University, Russia

[9]Department of Nanomaterial Physicochemistry Center of Nanoscience and Nanotechnology, National Autonomous

Volume 3 Issue 2- 2020

Received Date: 07 Jan 2020

Accepted Date: 22 Jan 2020

Published Date: 28 Jan 2020

1. Abstract

1.1. Objectives: We present the use of silver nanoparticles (AgNPs) for 3 the treatment of *Diabetic Foot Ulcers* (DFU) of grade 2 and 3 of Wagner classification.

1.2. Methods: Ulcers were daily treated by topical administration of AgNPs (at 1.8mg/mL of metallic silver) in addition to conventional antibiotics.

1.3. Results: In all three cases presented here, a significant improvement in the healing evolution of ulcers was observed. The edges of the lesion reached the point of closure in 2 of the 3 clinical cases.

1.4. Conclusions: This work reports a nanomedicine approach for the successfully treatment of DFU of Wagner classification degrees 2 and 3. Daily topical administration of AgNPs solution with metallic silver concentration of 1.8mg/mL causes a healing improvement of DFU in less than 60 days in average. The results constituted the basis for further studies on the use of AgNPs for the treatment of diabetic and other ulcers from different origins.

2. Keywords

Diabetic foot ulcers; Diabetes mellitus; Silver nanoparticles; Nanomedicine; Chronic ulcers; Wound healing

3. Introduction

Diabetes *Mellitus* (DM) is one of the most common chronic and metabolic diseases. According with the World Health Organization, in 2014 there were 422 million of diabetic people and 1.5 million of people have died due to this illness in 2012 [1,2]. In Mexico DM is the second major cause of death [3]. Impaired wound healing is a common complication and the main cause of hospitalization and lower limbs amputation in patients with DM. This causes what is known as *Diabetic Foot Ulcers* (DFU). The lifetime risk of patients with DM to develop DFU is 25%. As a consequence of DM, it is estimated that a lower limb is lost some where in the world every 30 seconds [4,5].

Conventional treatments for DFU include debridement, administration of antimicrobial agents, use of devices for off-loading, application of silver impregnated dressings, topical administration of cell growth factors, surgical techniques and amputation [6,8]. However, all of them have shown limited clinical success. Due to polymicrobial infections, DFU are known to heal slowly [9,10]. Microbial infections found in DFU consist of 73% aerobic and 27% anaerobic bacteria and also microorganisms with MultiDrug Resistance (MDR) [11,12]. To overcome those infections, silver-impregnated dressings have been used, but the need for constant

***Corresponding Author (s):** Nina Bogdanchikova, Almonaci-Hernández CA, Luna-Vazquez-Gomez R, Department of Centro of Nano Science and Nanotechnology National Autonomous University of Mexico, UNAM, Km. 107 Carretera Tijuana-Ensenada, Ensenada, Baja California, 22860 Mexico, E-mail: nina@cnyn.unam.mx*

Author Contributions: Almonaci-Hernandez CA, Luna-Vazquez-Gomez R, These authors have contributed equally to this article.

Citation: Bogdanchikova N, Department of Center of Nanoscience and Nanotechnology, National Autonomous University of Mexico. Journal of Clinical and Medical Images. 2020; V3(2): 1-7.

reapplications and the deactivation of silver, constitute major limitations [13]. Also, topical administration of cellular growth factors has been demonstrated to be effective to heal DFU. However it is extremely costly and only works for certain wagner stages of DFU [6].

In this sense, nanotechnology has generated new applications for biomedicine through nanomaterials [14].

One of the most used nanomaterials in medical products is silver nanoparticles (AgNPs). These nanoparticles have shown broad microbicidal activity and also anti-inflammatory properties [15, 16]. Therefore, silver and AgNPs are commonly used in dressings for conservative treatment of wounds and burns [16-19]. However, the usage of AgNPs to heal DFU has been scarcely explored [20, 21]. Developed a potent antimicrobial sponge composed of chitosan, hyaluronic acid and AgNPs as a wound dressing to treat DFU infected with antibiotic resistant bacteria [21]. Also, [22] investigated wound healing in diabetic mice, where excised wounds treated with AgNPs completely heal 2.5 days faster than untreated animals [22]. However, although the role of AgNPs in wound healing is well documented, the use of AgNPs to heal DFU in DM patients has never been studied.

Taking this in consideration, the aim of this study was to investigate the potential use of AgNPs to treat DFU in patients with DM. Herein it is presented three cases of patients with DFU classified as wagner ulcers 2 and 3. Patients were treated with a conventional antibiotics schedule and additionally AgNPs solution was topically administered. The evolution of the wound healing during treatment was documented by photography, which clearly showed that DFU disappeared or significantly reduce its area without developing infection after treatment with AgNPs.

4. Material and Methods

4.1. Study Design and Subjects

The series of clinical cases presented here belongs to a pilot study. Data collection was performed at the Hospital of the Institute of Security and Social Service of Government and Counties Workers of Baja California State Hospital in Baja California, Mexico. Patients were included once the medical protocol was approved by the ethics committee of the hospital. The objectives and methods of the study were explained to the patients using documentation. Also it was explained that no disadvantage would be incurred by refusing to participate and that personal information would not be disclosed.

Patient selection was independent of age and sex. However, the inclusion criterion was to be a DM type 2 patients under glycemic control and the presence of a DFU of Wagner stages 1 to 3. Importantly was that patients must present a non responsive behavior of necrotic ulcer tissue to conventional antibiotic treatment. The treatment of DFUs with AgNPs started only after the patient signed an informed consent letter giving their approval to be included in the study.

4.2. Ethical Considerations

The medical protocol followed in this study was approved by the Ethics Committee of the General Hospital, Tijuana, Baja California, Mexico. The method for the topical administration of AgNPs for DFU treatment was developed.

4.3. Silver Nanoparticles

After comparison of different AgNPs formulations commercially available, we concluded that only Argovit preparation resulted to have multiple certificates for their usage in veterinary and human applications [23]. Argovit AgNPs (Scientific and Production Center Vector-Vita, Russia) is a preparation of highly dispersed silver nanoparticles with an overall concentration of 200 mg/mL (20%) of PVP-coated AgNPs in water. The content of metallic silver in Argovit preparation is 12 mg/mL, stabilized with 188 mg/mL of Poly Vinylpyrrolidone (PVP). AgNPs dilutions were calculated according to the metallic silver content in Argovit preparation. Solutions of AgNPs were prepared with distilled and sterile water and were kept at 4°C in darkness.

5. Results

5.1. Silver Nanoparticles

Physicochemical characteristics of AgNPs have been recently reported by our group [24]. A TEM micrograph showing the spheroidal morphology and size of AgNPs used in this study is shown in (Figure 1).

5.2. Patients

In the case of patient 1, besides inflammation, there was no evidence of infection prior to debridement and treatment with AgNPs. While in case of patients 2 and 3, due to previous infections, a conventional antibiotic administration schedule continued on a regular basis during treatment with AgNPs. Nevertheless necrosis, inflammation and fetid odor were observed during debridement, evidencing an active infection. Contrary to conventional treatment of DFU, in this study the topical application of AgNPs solution

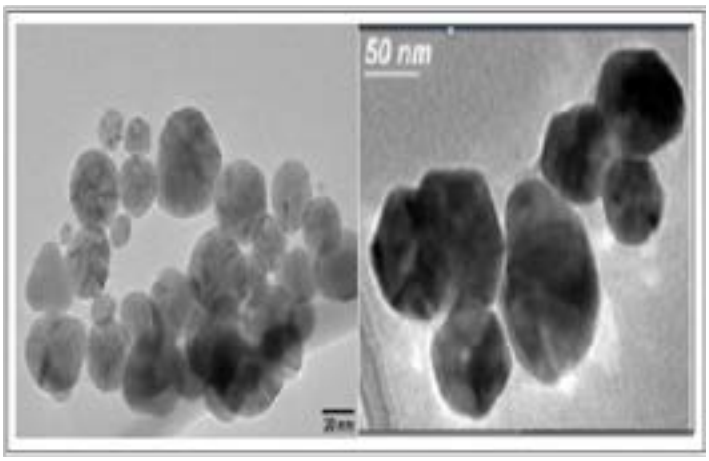

Figure 1: TEM image of AgNPs showing their spheroidal morphology and size.

(with a metallic silver concentration of 1.8 mg/mL) was performed daily. To record the evolution of the wound healing process, photographs were taken before and after the treatment with AgNPs. Here it is presented three clinical cases of diabetic patients with different grades of DFU according with Wagner classification (Figure 2).

First patient (P1, Figure 2a) is a 77 years old female with controlled type 2 diabetes, with a plantar Wagner-2 ulcer. The wound is located at the metatarsal region of the first toe of the left foot. As observed in Figure 2a the ulcer was restricted to an area of 4 cm x 4 cm and exhibits hyperkeratosis in the affected and surrounding tissue, associated with desquamation at the metatarsal region and fetid odor perception. Some grade of inflammation in forefoot

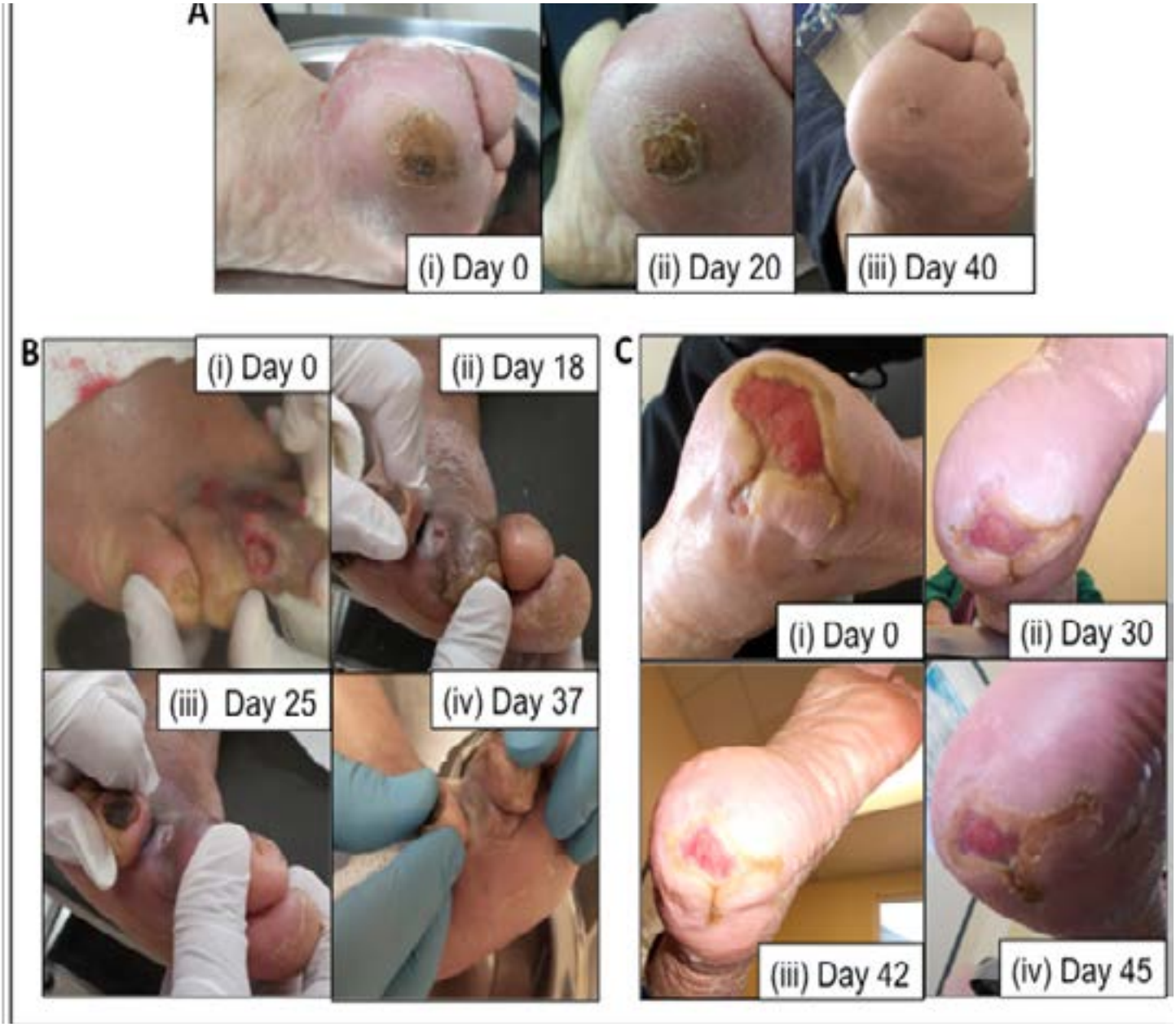

Figure 2: Chronological evolution of different diabetic foot ulcers treated with topical administration of AgNPs.

Table 1. Comparison of Herberprot-P (Cuba) and Nagsil Dermo (Mexico-Russia) products available on the market for the effective treatment of diabetic foot ulcers.

Features	Heberprot-P (Cuba)	Nagsil Dermo (Mexico-Russia)
On the market since	2007	2015
International distribution	15 countries	10 countries
Registration as	Medicine	Cosmetic
Origin	Recombinant protein	Silver nanoparticles
Number of treated patients	More than 100,000	More than 420
Treatment duration	8 - 11 weeks	3 - 6 weeks (in average)
Treatment cost in Mexico	136,000 - 187,000 MEX pesos (7,555 – 10,390 USD)	1,000 MEX pesos (56 USD)
Necessary to administer along with antibiotics	Yes	No
Hospitalization for treatment and product administration	Required	No Only for initial debridement
Exclussion criteria of patients	This product is not recommended for patients with: High blood pressure or hypertenssion Risk of brain hemorrages Risk of thrombosis Blood glucose levels ≥ 160 Ulcers with necrotic tissue	None
Stages of Wagner ulcer classification for an effective treatment	1 and 2	1, 2 and 3
Route of administration	Intra and perilesional zones	Topically with spray
Shelf-life of the product	1 year	2-4 years
Experitation days beyond first use	3 days	1 year

and toe was observed. Daily treatment of topical administration of AgNPs solution started after the debridement of the lesion. After 20 days of AgNPs administration, the ulcer showed a decrease in hyperkeratosis, noticeable on the lateral side of the first toe, while the metatarsal region surrounding the injury regained its normal appearance. It is shown a decrease of edema of the forefoot and toes and a peripheral improved of the pigmentation indicating an active edge of healing. No fetid odor was perceived. Forty days after the beginning of treatment the hyperkeratosis was minimal, the surrounding skin has regained its normal characteristics and desquamation was only noticeable at the fold of the first toe. The necrotic tissue disappeared and normal re-epithelialization process and a normalization of the pigmentation were evident.

The second patient (P2, Figure 2b) is a 63 years old male with controlled type 2-diabetes, who exhibited an ulcer grade 3 according to Wagner classification. Ulcer exhibits a pitting morphology with raised edges located on the lateral side of the third toe of the right foot. As observed in Figure 2b changes in pigmentation are evident.

After 18 days of a daily topical administration of AgNPs, the DFU showed reduction of the wound area in more than 50%, with evident re-epithelialization, active edges and presence of granulation tissue in the center of the ulcer. At the 25th day of treatment, an imminent closure of the ulcer is shown by secondary intention. Finally, after 37 days post-AgNPs treatment, the ulcer edges have been addressed, culminating in the closure of the injury where no necrosis or inflammation was observed.

The third patient (P3, Figure 2c) is a 59 years old female patient with controlled type 2 diabetes, exhibited a plantar ulcer in the left hindfoot without vascular involvement. After 4 weeks under a conventional antibiotics schedule no improvement in the evolution of the ulcer was observed. Chronological evolution of DFU from

P3 before and after treatment with AgNPs was observed in Figure 2c. It is shown the initial appearance of a Wagner 2 ulcer after the debridement process. The DFU was located at the hindfoot and superimposed on the tarsal region of the left foot, with irregular contours and edges with hyperkeratosis. After 30 days of treatment with AgNPs solution, there was an improvement of the ulcer, clearly observed as approximately 20% reduction of the wound surface area. The evolution of the healing process after 42 and 45 days of AgNPs administration showed a progressive diminishment of the lesion extent. The outer contour of the ulcer showed a progressive decrease of hyperkeratosis. An evident improvement of the coping was observed along the edges of the DFU, while granulation tissue is depicted at the center of the ulcer. The central zone of the ulcer showed a diminishment of its diameter with an evident improvement in pigmentation, re-epithelialization process and the presence of active edges that favoring closure of the injury.

6. Discussion

DFU has been recognized as a worldwide health problem, especially in developing countries and as the number of diabetic patients continues to rise, so will be the number of DFU [25]. These types of ulcers are multifactorial, besides the presence of MDR microorganisms on it increases the risk for a chronic infection, limb amputation and morbidity [26,27]. The microbicidal properties of AgNPs are broader than ones of common antibiotics, which is suitable to 1 treat wounds with polymicrobial infections including MDR microorganisms also found in DFU [12]. The capability of AgNPs to accomplish successful DFU healing, could be exerted by several processes, indeed, the most important is their efficient wide-spectra antimicrobial properties [15,27]. Because the precise microbicidal mechanism of AgNPs has not been well established, it is considered to be multifactorial [28,29]. For example, AgNPs can interact with peptidoglycans present in both bacterial cell wall and membrane, damaging their integrity and causing a leakage of intracellular contents [16,30]. AgNPs also interact with intracellular proteins, interfering with microbial DNA duplication and inducing the generation of reactive oxygen species that finally leads to bacterial cell death by oxidative stress [27]. Once the infection is eradicated, the anti-inflammatory properties of AgNPs promote wound healing by decreasing the expression of pro-inflammatory cytokines and by reducing the infiltration of lymphocytes and mast cells. Causing with this a diminishment of local and systemic inflammatory response [16,22]. Although AgNPs in wound healing has been studied, their role in curing diabetic foot ulcers has been never addressed, thus limited information concerning this is available. Moreover, AgNPs used in other studies have produced genotoxic effects. Therefore, in attempts to compare the efficacy of AgNPs treatment for DFU, we searched products on the market capable to cure DFU. In this sense, we found a Cuban product named Heberprot-P [6]. To address the efficacy of both products to heal DFU we present the following comparison (Table 1).

Heberprot-P is based on the recombinant human Epidermal Growth Factor (rhEGF), a cytokine that stimulates cell growth and differentiation. It has been associated the poor ability of DFU to heal with the low levels of growth factors in the wound site [31]. Thus, the injection of Heberprot-P in the intra and perilesional zones of DFU facilitates the healing process in the wound. It has been reported that AgNPs promote wound healing due to several processes, such as the expression of fibrogenic cytokines, migration of keratinocytes and induction of fibroblast differentiation to myofibroblasts. These allowed to promote wound contraction and epidermal re-epithelialization [19, 22, 32].

It is worth mention that we recently reported that Argovit AgNPs have non genotoxic effects on cell lines at concentrations closer to the IC50 (3.5μg/mL of metallic silver) [24]. Moreover, Argovit AgNPs harbors many advantages over other silver formulations available on the market, because it has been approved by international instances as cosmetic and has been applied in medical devices for human use [23]. Argovit AgNPs is available on the market and distributed as cosmetic by the company Bionag S.A.P.I. de C.V. According with the supplier, this product cures more than 90% of patients with DFU with Wagner ulceration of stages 1 to 3 in approximately 2 months. The remaining patients are cured slower because they do not follow the directions given by the supplier.

Thus, to the best of our knowledge, this work represents the first nanomedicine approach study performed for the successfully treatment of DFU of Wagner classification degrees 2 and 3 by a daily topical administration of AgNPs solution with metallic silver concentration of 1.8 mg/mL. AgNPs administered caused an improvement of the wound healing in average in less than 60 days of treatment.

The implementation of this nanotechnology for the rapid healing of diabetic foot ulcers could help to drastically reduce the cost related to the care and treatment of DFU in the public health system. Therefore, this study lays the 1st bases for further systematic clinical and basic research on the usage of AgNPs for the treatment of chronic ulcers such as DFU.

7. Conclusions

To the best of our knowledge, this work reports the first nanomedicine approach study performed for the successfully treatment of DFU of Wagner classification degrees 2 and 3, where daily topical administration of AgNPs solution with metallic silver concentration of 1.8 mg/mL causes an improvement of the wound healing in average in less than 60 days of treatment. Therefore, this study lays the bases for further systematic clinical and basic research on the usage of AgNPs for the treatment of chronic ulcers such as DFU.

8. Acknowledgements

The authors thank the funding through CONACYT projects No. 279889, 269071, 1073, DGAPA PAPIIT-UNAM IT200114 and RFBR project No 18-29-24037. Medical support was given by Dr. Isis Cabrera, Carmen Enriques, Willfred Cardenas Acosta, Michel Natay Peraza Perales, Alejandra Rembao Hernandez and Nora Ines Carriquiry Chequer. We are grateful to Dr. Leonel Cota (CNyN-UNAM) for the studentships given through the CONACYT-SNI 3 researcher fund and to Dr. V. Burmistrov for the kindly donation of Argovit AgNPs.

9. Declaration of Conflicting Interests

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

10. Author Contributions: Almonaci-Hernandez CA, Luna-Vazquez-Gomez R

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