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Nano drug (AgNPs capped with hydroxychloroquine): Synthesis, characterization, anti-covid-19 and healing the wound infected with S. aureus

Aisha H. Ali, Mustafa A. Alheety, Mahmood Hasen Alubaidy, Sushil Dohare

Department of Tikrit Education, Directorate of Salah El-din, Ministry of Education, Tikrit, Iraq
Department of Nursing, Al-Hadi University College, Baghdad, Iraq
Department of Dentistry, Al-Hadi University College, Baghdad, Iraq
Department of Epidemiology, Faculty of Public Health & Tropical Medicine, Jazan University, Jazan, Saudi Arabia

HIGHLIGHTS

- Silver nanoparticle (AgNP) was prepared by the green method.
- Silver nanoparticle was coated with the hydroxychloroquine to prepare AgNPs/HQ.
- AgNPs/HQ with antiviral activity against COVID-19 was prepared in aqueous medium using one-pot method.
- The nanodrug was also used for healing the wound infected with S. aureus.
- Histological results revealed that all of the disease symptoms improved, with the epidermal layer multiplying quickly.

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ABSTRACT

Almost existing anti-viral drugs are only organic molecules that are able to circumvent the system the virus works with, which leaves it facing the immune system of our bodies and then kills it. Unfortunately, this type of pharmacological fight did not succeed in a way to overcome this virus, so it became necessary to think outside the box, to find a drug that would kill the virus or alter its protein structure. This research aims to prepare silver nanoparticle (AgNPs) by the green method depending on the reaction of the silver nitrate (safe for humans) with the phoenix dactylifera extract (safe for humans) and then coated with the hydroxychloroquine (HQ, known antiviral drug). This substance will fight the virus with different mechanisms (i) silver will carry the drug to cells easily, and then (ii) nano silver will perform a physical inhibition of the virus and thus reduce its susceptibility to binding to host cells. In addition, (iii) silver nanoparticle is much smaller than the size of the virus which qualifies it to cross into the virus and change the structure of RNA. Furthermore, (iv) it is possible for silver to interact with the amino and carboxylic ends in the virus proteins. The results of TCID50 shows that the prepared nano drug is able to reduce the viability of covid-19 to about 22% using 400 mg/ml of AgNPs/HQ. The resulted nanodrug was also used for healing the wound infected with S. aureus and the histological results revealed that all of the disease symptoms improved, with the epidermal layer multiplying quickly and the infected wounds healing quickly.

1. Introduction

Since the end of 2019 to now, public health issues have been exacerbated primarily by the consequences of the sudden spread of a new infectious disease. Many names have been used to identify the new virus and however now the name covid-19 reflects the new virus as suggested by WHO [2]. While the Chinese health authorities took strict measures, the new infection spread rapidly in Wuhan on 30-Dec-2019 [1]. Scientific studies continued on the possibility of classifying this virus, as the first experiment involved identifying the genetic sequence of the virus. Furthermore, this study contributed in the identification of the virus very quickly by using reverse-transcription polymerase [3]. The risk of
this virus lies in the ability of spike proteins to interfere tightly with angiotensin converting enzyme receptors. Nevertheless, despite knowledge of the mechanism by which the virus works, it has caused more than a quarter of a million infections so far, with an increase in mortality rates, particularly in countries that are struggling in their health care system. However, at the beginning of 2020 the studies demonstrated that this virus belongs to the β-corona virus group and shares its origins with the bat corona [1] as it consists of 5′-UTR, 3′-UTR, spike proteins, E genes, N genes, orf1ab, nsps and unidentified non-structural open reading frames [3]. This virus is considered to have new properties, although it is classified under beta corona viruses, but it differs from MERS-COV and SARS-COV. However, current studies have shown that the virus is nearly 80% similar in its nucleotide identity to SARS-COV and more than 89% match SARS-COV genes [4,5]. This type of virus produces polypeptide, which is analyzed into 11 distinct sites by the protease enzymes (3CLpr0, PLpr0), and thus the production of non-structural proteins, which play the main role in viral replication [6], and therefore this type of enzyme can be considered the target in fighting these viruses. Almost existing anti-viral drugs are only organic compounds that are able to circumvent the system the virus works with, which leaves it facing the immune system and then kill. Unfortunately, this type of pharmacological fight did not succeed in a way to overcome this virus, so it became necessary to think outside the box, to find a drug that would kill the virus or alter its protein structure. Our thinking here is based on the use of silver nanoparticles, which are less than a hundred nanometers in diameter, and which take different nano shapes as it was proved by our previous works [7–9]. Because of silver nanoparticles has the benefit of inhibiting the respiratory enzyme [10], it is commonly used as an effective and safe antibacterial. Silver also has the advantage that it can be prepared using environmentally friendly biological methods and thus not to use many chemicals [8,9]. Silver nanoparticles were used as an anti-viral agent against species HIV-1, hepatitis B, and herpes simpex in addition to other types [11–15]. However, the mechanisms in which nanoscale silver destroys viruses are still not extensively studied, but it is believed to be a physical adsorption mechanism that relies mainly on physical inhibition of the virus’s ability to bind to host cells. We also believe that nanoscale silver is bind-able to the amino and carboxylic terminal groups of the virus’s proteins or to penetrate its walls and cause a significant change in RNA. The toxicity of silver nanoparticles is almost non-existent, and there have only one study that draws attention when using nanoparticle of silver, because the study demonstrated that the susceptibility of silver to the atonaligal stem cells of sperms in rats [16]. This can be overcome either by using polymeric materials carrying the nanosilver or by adding other drug compounds to it. Our work here mainly focuses on the study of the susceptibility of silver nanoparticles coated hydroxychloroquine to killing the covid-19 virus.

2. Experimental part

2.1. Materials

All chemicals and solvents were purchased from Sigma Aldrich and used without further purification.

2.2. Methods

2.2.1. Synthesis of silver nanoparticles (AgNPs)

This nanoparticle was prepared depending on our previous typical method [7,17–19]. One milliliter of freshly-prepared phoenix dactylifera extract, was added to the solution of 355 ppm of Ag1 under vigorous stirring. Thereafter, the mixture was heated to sixty °C for 15 min. The resulted clear red solution was saved under 10 °C and used in the next experiments.

2.2.2. Synthesis of silver nanoparticles coated hydroxychloroquine (AgNPs/HQ)

This nano drug was prepared by one-pot method. To the above prepared solution of AgNPs, 80 mg of HQ was added and well mixed under vigorous stirring for 3 h under atmosphere pressure and 40 °C. The resulted AgNPs/HQ solution was saved under 10 °C and used without further purification.

2.2.3. Anti-covid-19 assaying

Assaying the anticovid-19 activity of the AgNPs/HQ was assayed using the TCID50 protocol. Suspension of covid-19 in Phosphate-buffered saline was added to 250 μl AgNPs/HQ solution. The mixture of Covid-19 and AgNPs/HQ solution was well-mixed by stirring for 1 min and then, the mixture was left at 25 °C for half an hour. Thereafter, the mixture was centrifuged at 8000 rpm for 5 min to eradicate AgNPs/HQ nanoparticles. 50 μL of supernatant were divided for two-fold serial dilution in Phosphate-buffered saline until we full a 96-well plate. The plate was then incubated at 37 °C with CO2 (5%) for 1 h sown with MDCK cells. The cells were sustained by addition of 50 μl DMEM to each plate’s well immediately following infection and again five days post-infection. Week post-infection, the living cells were immediate with CH2OH and stained with a solution of Giemsa stain (5%). Using the Reed-Muench assay, the dose of fifty-percent tissue culture infectious was obtained from the number of infected wells. The anticovid-19 activity of the AgNPs/HQ was calculated as the TCID50 ratio of the treated supernatant to the untreated covid-19 suspension [20].

2.2.4. Treating of S.aureus wound infected

Diethylether was used to anesthetize the rats, after which a little section of the animals’ hair was cut and the identified area was cleansed (lateral area). After that, ethyl alcohol was used to sanitize the area. After that, a 2 cm incision was created.

1. S. aureus suspension (0.05 cm3) was injected into the muscle of the first group of animals.
2. The second group received an injection of S. aureus suspension (0.05 cm3) in physiological saline, followed by surgical closure of the incision.

The wounds infected with S. aureus were treated with nanodrug (1 mg/ml) and sterile cotton.

3. Results and discussion

3.1. The cytotoxicity of as-prepared AgNPs/HQ

The cytotoxicity of as-prepared AgNPs/HQ was studied before determining anti-covid-19 activity. The MNTD for AgNPs/HQ was examined by determining the cytopathic effect of nine different concentrations of plant AgNPs/HQ on kidney cells. From Fig. 1, the MNTD for AgNPs/HQ was found to be 320 μg/ml. The determined MNTD of AgNPs/HQ was chose for further in vitro anticovid-19 studies. The antiviral prospective of AgNPs/HQ was examined against covid-19 using kidney cells. The antiviral activities of AgNPs were measured as the dependence of concentration of AgNPs/HQ against COVID-19 virus. We notice that the anticovid-19 activity of the AgNPs/HQ increased with the increasing of concentration of anticovid-19 (100, 200, 400, 600 μg/ml). No significant increase in anticovid-19 activity was detected above 400 μg of AgNPs/HQ. The TCID50 was 55%, 41%, 22% and 21% using 100, 200, 400 and 600 μg/ml of AgNPs/HQ drug. In order to further investigate the mechanism of action of this AgNPs/HQ, SEM measurement of the virus was carried out before and after treatment with the AgNPs/HQ. As noted in the measurement, the drug caused a number of changes to the structure of the virus, the first of which was the destruction of spike glycoprotein (s) and the protein membrane as shown in Fig. 2.
3.2. Wound healing tests

Wound samples infected with opportunistic bacteria were taken to verify their presence by culturing them on nutrient agar media for periods of 1–10 days after infection. Samples were collected from the infected wound area using swabs for the same periods of the experiment and incubated at 37 °C for 24 h after culturing and then read the results as shown in Table 1. The results prove that after only five days, measurements showed that there are no bacteria on infected wounds treated with nanodrug (AgNPs/HQ), means that it can be used as an antiseptic for wounds during surgical operations, while the untreated wounds remained in a bad condition with a clear growth of bacteria.

The following observations on the outcomes of infected wounds were made:

On day 3 of the experiment, the untreated infected rats showed significant necrosis and inflammatory cell infiltration.

After two days, the granulation tissue infiltrated with multinucleated inflammatory cells multiplied in the dermis layer, with a proliferation in hair follicles with inflammatory cell infiltration after five days in the group of rats infected with S. aureus bacteria treated with AgNPs/HQ, it was observed that the granulation tissue infiltrated with multinucleated inflammatory cells multiply only after the fifth day of the damage in the epidermis and dermis layer does the healing process begin.

4. Conclusion

AgNPs/HQ with antiviral activity against COVID-19 was prepared in aqueous medium using one-pot method. The activity of anticovid-19 was demonstrated from the decreased of TCID50 ratio of covid-19 suspension after treatment with the AgNPs/HQ drug. Despite of the anticovid-19 mechanism still undiscovered, but the experiments showed a relationship between AgNPs/HQ drug activity and its concentration. These assignments suggest that this substance will fight the virus with different mechanisms (i) silver will carry the drug to cells easily, and then (ii) nano silver will perform a physical inhibition of the virus through tight binding of the silver nanoparticles to glycoproteins of covid-19 envelope thereby reduce its susceptibility to binding to host cells. In addition, (iii) silver nanoparticle is much smaller than the size of the virus which qualifies it to cross into the virus and change the structure of RNA. Furthermore, (iv) it is possible for silver to interact with the amino and carboxylic ends in the virus proteins. We can also theoretically demonstrate that when this drug is used in humans, the virus will have a much lower rate, since HQ drug works exclusively on ACE2 inside the body, which provides a greater opportunity for the immune system to completely eliminate the virus. The anti S.aureus

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Table 1

| Days | 1 | 3 | 5 | 7 | 10 |
|------|---|---|---|---|----|
| Treated with nanocomposite | + | + | + | + | - |
| Untreated with nanocomposite | + | + | + | + | + |

*(−) no bacterial growth, (+) presence of bacterial growth.*
results prove that after only five days, measurements showed that there are no bacteria on infected wounds treated with nanodrug (AgNPs/HQ).

CRediT authorship contribution statement

Aisha H. Ali: Conceptualization, Investigation, Validation, Writing – review & editing, Data curation, Investigation. Mustafa A. Alheety: Conceptualization, Investigation, Validation, Writing – review & editing, Data curation, Investigation. Mahmood Hasen Alubaidy: Methodology, Investigation, Formal analysis, Writing – review & editing. Sushil Dohare: Methodology, Investigation, Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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