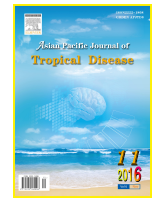




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Synthesis and evaluation of antiviral activities of novel sonochemical silver nanorods against HIV and HSV viruses

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ABSTRACT

Objective: To evaluate the effect of novel sonochemical silver nanorods on HIV and herpes simplex virus type 1 (HSV-1) viruses in human cervical cancer HeLa cells.

Methods: The formation of silver nanorods conjugated with sodium 2-mercaptoethane sulfonate (Ag-MES) was characterized by scanning electron microscopy, Fourier transform infrared spectroscopy and thermal gravimetric analysis. The antiviral activity of this Ag-MES was examined against HIV and HSV-1 virus replication.

Results: The characterizations of Ag-MES and physicochemical structure were determined by scanning electron microscopy, Fourier transform infrared spectroscopy and thermal gravimetric analysis. Approximately entire viral replication was inhibited by Ag-MES at 10 $\mu\text{mol/mL}$ concentration. About 90% of HSV virions failed to replicate in the present of this concentration of nanorods. However, HIV showed more sensitivity to Ag-MES than HSV-1.

Conclusions: According to the obtained data, the synthesized sonochemical silver nanorod in this study is a promising candidate for further drug discovery investigation.

1. Introduction

Herpes simplex virus (HSV) and HIV are common infectious pathogen worldwide and these viruses can infect humans of all ages[1,2]. Infections of HSV-1 result in a wide range of clinical presentations from oral cold sores to life threatening encephalitis or even with asymptomatic infection. Oral, topical or intravenous medicines like nucleoside derivatives (*e.g.*, acyclovir) are applied to cure HSV infections[3-5]. With advent of resistant strains,

mainly after repeatedly treatment in immune compromised patients, researchers are encouraged to investigate new antiviral agents which are able to prevent infection that caused by wild-type viruses and drug-resistant strains[6]. Synthesizing antiviral drugs that are able to inhibit living process of viruses and retain in host cells turns into a challenge to researchers[7,8]. The present problems of many antiviral drugs are linked to their limited solubility in aqueous media, short biological half-life and incomplete absorption by cells. For example, acyclovir, a high effective drug against herpes viruses, faces that challenge and the body needs to obtain a relatively constant drug level[9]. Lately, researchers have developed antiviral agents which are able to limit the spread of viral infections. Some significant antiviral agents against HIV are tenofovir, dapivirine, enfuvirtide and zidovudine that target viruses as a nucleotide reverse transcriptase inhibitor. Some important antiviral agents against HSV are acyclovir,

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penciclovir, famciclovir, brivudin, valaciclovir, idoxuridine and trifluridine which inhibit virus activity mostly as a DNA elongation inhibitor[10]. The use of cocktail drugs is known as a highly active anti-retroviral therapy against HIV infection which has significantly reduced morbidity and mortality among AIDS patients[11-14]. Owing to advent of resistance HIV strains and having toxicity, success of highly active anti-retroviral therapy as an efficient drug is insufficient[15]. Nevertheless, researchers have made efforts to combat viral infection in HIV/AIDS patients. However, formulated drugs must be efficient against viruses[16]. Recently it is confirmed that silver nanoparticles (AgNPs) block key steps in viral replication cycle. To restrain bonding molecule-3-grabbing non-integrin-mediated HIV transfection to dendritic cell-specific intercellular, different oligomannosides on AgNPs have been applied[17]. Using sulfonated nanorods as a nanocarrier to improve the efficacy of antiviral agents has still not developed[18]. The main goal of this study is to develop synthesis of sonochemical silver nanorods and to evaluate their potential antiviral activities.

2. Materials and methods

2.1. Silver nanorods synthesis and purification

Sonochemical method was used for the synthesis of silver nanorods. Briefly, 0.5 g of $\text{AgNO}_3 \cdot \text{H}_2\text{O}$ (1.2 mmol) was dissolved in 150 mL of ethanol, then 50 mL of mercaptoethane sulfonate (3.0 mmol, S/Au = 2.5 molar ratio) methanol aqueous solution (1:1, v/v) was added. A freshly prepared 0.4 mol/L sodium borohydride aqueous solution ($\text{NaBH}_4/\text{Au} = 10$) was added quickly into the solution with vigorously shaking. The reaction was completed after more than 1 h shaking and then the solution was kept in a refrigerator for further use[19,20]. The solution was poured into opening balloon of ultrasonic device which equipped with an inlet and an outlet hole. The balloon was filled with argon in order to discharge whole oxygen (60 min). After 15 min from the start of ultrasonic, 0.3 mL ammoniac of 0.25% solution was added by needle through a balloon hole. Temperature of container was tried to maintain between 10 and 15 °C. The solution was shaken well to dissolve all sediment, and then it was dialyzed against deionized water using membrane (pore size of dialysis bag was 10 kDa) for 24 h to separate untreated materials and water. After a severe stirring at room temperature, the bag was taken out from deionized water and the sediment was put with the rest of solution in a new pot. Then it was dried and purified using Leo freezing instrument and phase was prepared to produce purified sodium 2-mercaptoethane sulfonate conjugated silver nanorods (Ag-MES).

2.2. Characterization of silver nanorods

The morphology and size of silver nanorods were represented

by using scanning electron microscopy. Fourier transforms infrared spectra was also used to determine chemical structure. Thermogravimetric analysis measurement on Ag-MES nanorods was performed in a temperature range between 25 and 700 °C.

2.3. Plasmids

Single cycle replicable (SCR) retroviral system (HIV-1) was used in this study[21,22]. Vesicular stomatitis virus glycoprotein pseudotyped SCR HIV-1 virions were produced using pSPAX.2, pMD2G and pmzNL4-3 plasmids[23]. The plasmid pmzNL4-3 contains HIV-1 (NL4-3). This wild type has been proved to be safe due to large mutation in a two histidines and two cysteines motif which blocked replication of HIV-1[24].

2.4. Cell culture and virus production

HeLa cell lines were maintained in Dulbecco's modified Eagle medium (Gibco, Scotland) containing 12% heat-inactivated fetal bovine serum and appropriate concentrations of L-glutamine and sodium pyruvate (Thermo Fisher Scientific, Waltham, USA). HIV virus stocks were prepared by co-transfection of the Vero cells with PolyFect transfection reagent (Qiagen, Valencia, USA) according to the manufacturer's instructions. Then the cells supernatants were harvested at 24, 48 and 72 h after transfection, and afterward pooled and stored at 4–8 °C. Pooled supernatants were clarified by centrifugation at 10^4 gravity for 10 min and filtered through 0.45 µm filters. Viruses were stored at –70 °C for long-term use and infectious titer was determined by replication assay[25,26]. HSV-1 (KOS) virions were propagated in 6-wells plates and Vero cells were infected by 80 percent confluent with 1 mL of virus supernatants in each well. The supernatants were harvested and pooled every day until 96 h post-infection. To clarify the virus supernatants, they were filtered through 0.45 µm filters and then stored at –70 °C.

2.5. Antiviral assay

HIV replication assay was applied using SCR vesicular stomatitis virus glycoprotein HIV-1 (NL4-3) virions and HeLa cells[27]. The cells were placed in each well of 96-wells plates (7×10^3 /well) and infected with 100 ng P24 of SCR virions in the presence of diverse silver nanorods concentrations. After 20 h, cells were washed twice with pre-warmed Dulbecco's modified Eagle medium (to eliminate any unattached virions) and then the fresh medium with nanorods was added into the wells. Cells supernatants were evaluated for P24 load 72 h after infection. The level of P24 was evaluated by quantitative P24 sandwich ELISA technique (Zeptometrix, Buffalo, USA) according to manufacturer's protocol. Phosphate buffered saline and nevirapine (extracted from commercial tablets) were applied as the negative and positive controls respectively.

2.6. Statistical analysis

Statistical data analyses were performed using SPSS 22 and Excel software. For quantitative data analysis, unpaired *t*-test was used. A $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Synthesis and characterization of silver nanorods

Scanning electron microscopy result showed that the molecular size and morphology have shaped following Ag-MES conjugation. Fourier transforms infrared spectra spectroscopy was applied to determine the existence of the Ag-MES conjugated in the region of 400–4000 cm^{-1} . Peak at 2571 cm^{-1} due to S-H was omitted when MES molecules attached on the surface of Ag nanoparticle (Figure 1). Thermogravimetric analysis measurement on Ag-MES nanorods was performed in a range of 25–700 $^{\circ}\text{C}$ to measure the quantity of the MES on the surface of the silver. Weight loss (%) as an action of temperature is depicted in Figure 2. An amount of 1.74% was seen in the beginning. Weight loss was more a range of 30–270 $^{\circ}\text{C}$ which attributed to the loss of the physically adsorbed organic solvents, water and nanorods molecules. A more weight loss about 29.81% was observed in the temperature range of 260–498 $^{\circ}\text{C}$. This loss

happened in two key points: first, 19.09% weight was lost in the range of 263–377 $^{\circ}\text{C}$ and the second one was 10% between 377 and 500 $^{\circ}\text{C}$. The observed huge weight loss was contributed to bound between MES molecules and silver nanorods where was stable even over 600 $^{\circ}\text{C}$.

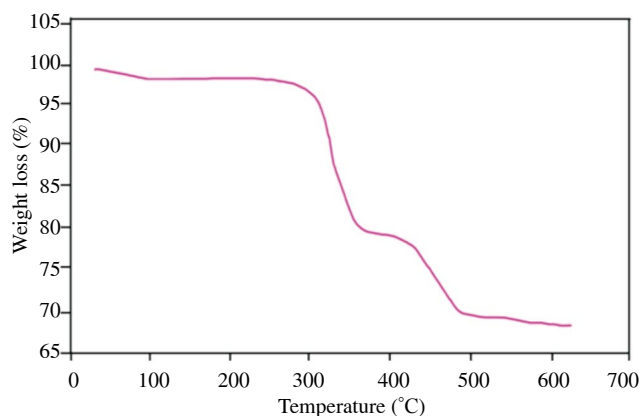


Figure 2. Thermogravimetric analysis data of Ag-MES nanorods.

3.2. Antiviral activity of silver nanorods

The overall anti-HIV and HSV activity of silver nanorods were appraised in this study. HIV and HSV virus multiplication were significantly decreased in presence of ω -sulfonated nanorods in different extents (Figure 3). Silver nanorods inhibited 50% of HIV-1

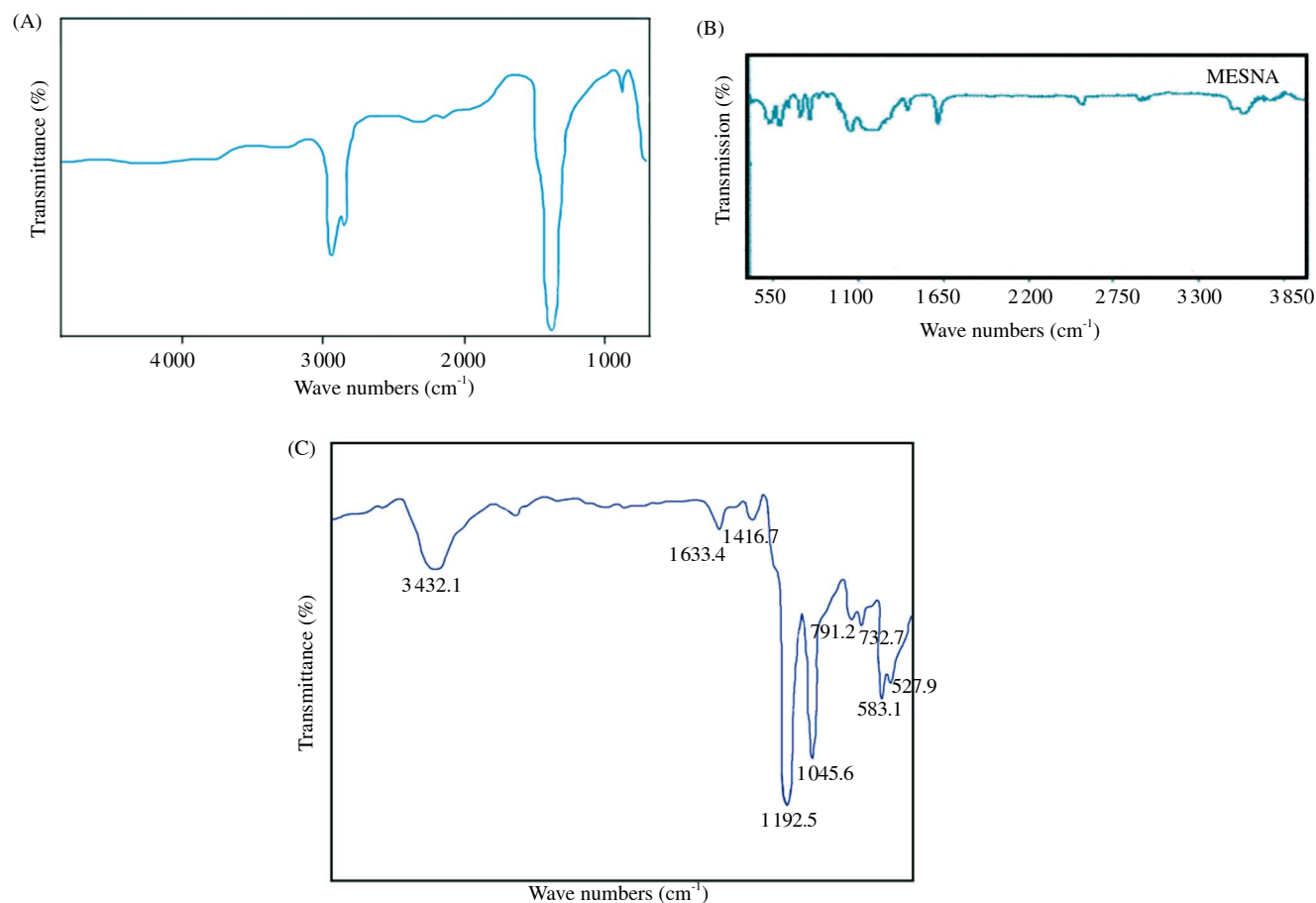


Figure 1. Fourier transforms infrared spectra spectrum for (A): AgNO₃; (B): Na-MES; (C): Ag-MES nanorods.

virions replication at 5 $\mu\text{g}/\text{mL}$ concentration. This result emphasized the significant anti-HIV and anti-HSV potential of Ag-MES.

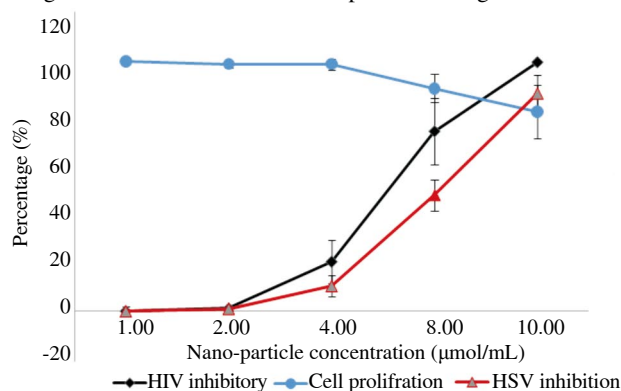


Figure 3. The antiviral activities of sodium 2-mercaptoethane sulfonate on HeLa cells conjugated silver nanoprods were shown against HIV and HSV-1 viruses. In this figure, almost entire viral replication were inhibited at 10 $\mu\text{mol}/\text{mL}$. However, HIV showed more sensitivity to synthesized nanorods.

4. Discussion

Recently, metal nanoparticles have shown considerable promise as novel antiviral therapies. Different proposed applications of nano materials such as drug delivery, biosensors, antimicrobials, cancer therapeutics and imaging have been reported[28].

There are a number of previously reported approaches in relation to nanoparticles based on therapies against viruses[29]. It has been proposed that AgNPs can be applied for inhibition of HIV virions[30], or utilized as virucidal agents in condom. Furthermore, functionalized gold nanoparticles have been constructed with inhibitory effects against HIV. Moreover, there are other groups which have been reported using modified AgNPs to inhibit various viruses such as HSV, hepatitis B, and monkey pox[31]. Also, the antiviral activity of ω -sulfonated nanorods was previously reported in different studies[27,31,32]. To evaluate the antiviral function of the wild-type HSV-1 McIntyre strain, the mercaptoethane sulfonate conjugated with AgNPs and mercaptoethane sulfonate conjugated with gold nanoparticles were applied. Silver and gold nanoparticles conjugated with MES blocking adhesion between HSV-1 to the host cell prevents viral entry into cells.

It is proved that AgNP poly (N-vinyl-2-pyrrolidone) coating which has 1–10 nm size acts against HIV through two main mechanisms. The first one is interaction with glycoprotein 120 and the other one is bind with viral envelope glycoprotein. Mercaptobenzoic acid coating has 2–20 nm size inhibiting HIV activity through TAK-779 receptor. Both AgNP and gold nanoparticle with MES coating have 4 nm size act against HSV via competition for the attachment of the virus to the cell[33]. Mori *et al.*[34] demonstrated AgNP/chitosan composites with antiviral activity against influenza A (H1N1) virus. Size dependence of the AgNPs on antiviral activity was also observed. Antiviral function was mainly stronger with smaller AgNPs in the composites. By adding 1 mg chitosan to amount of less than 100

μg of AgNPs, this size was dramatically effective[34]. Recently, Hu *et al.*[35] illustrated that 100 $\mu\text{g}/\text{mL}$ AgNPs could utterly restrain HSV-2 reproduction. AgNPs at nontoxic concentrations were able to prevent HSV-2 replication before viral infection or at the beginning of virus exposure while we demonstrated that approximately entire viral replication was inhibited by Ag-MES at 10 $\mu\text{mol}/\text{mL}$ concentration[35]. Orłowski and his colleagues demonstrated that tannic acid modified AgNPs sized 13 nm, 33 nm and 46 nm are able to decrease HSV-2 infectivity both *in vitro* and *in vivo*[36]. Price *et al.*[37] showed that SPL7013 as a dendrimer functions as a microbicide against HIV and HSV-2 *in vitro*. The anti-infection character of SPL7013 has been proved in animal mode studies[37]. Inhabitation of both HIV and HSV virus' replication with low concentration and high efficiency is the most notable feature of Ag-MES. Regarding to the result of this study illustrated that this functional nanoparticles has potential to block viruses entering into the cells[35] and therefore, has been known as one of the most optimistic plans that is able to positively impact the spread of viral infections. Some suggestion focuses on using the nanorods with antibodies, fluorescence materials and biological pigments as labeled materials which are helpful in therapy and diagnostics[22-24].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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