https://doi.org/10.15407/microbiolj84.05.030

S.D. Zahorodnia*, K.S. Naumenko, O.V. Zaichenko, P.Yu. Zaremba, G.V. Baranova, A.V. Holovan

Zabolotny Institute of Microbiology and Virology, NAS of Ukraine,
154 Academika Zabolotnoho Str., Kyiv, 03143, Ukraine

*Author for correspondence; e-mail: svetazsilverorodnya@ukr.net

EFFECT OF METAL NANOPARTICLES ON EBV-ASSOCIATED CELL CULTURE

Today, one of the topical areas of research is the search for antiviral drugs to fight against virus-associated oncological manifestations. One of the viruses for which a role in the transformation of cells is proved is Epstein-Barr virus (EBV), which is associated with a variety of lymphoproliferative diseases. The use of drugs that not only inhibit the replication of the virus but also stimulate the elimination of tumor cells is important for the treatment of tumors associated with the viruses. ***The purpose*** *of this work was to investigate the ability of silver and gold nanoparticles to inhibit EBV replication under conditions of chronic infection.* ***Methods****. The objects of the study were 5 to 20 nm gold and silver nanoparticles stabilized with tryptophan, sodium dodecyl sulfate, and citrate. The investigations were performed in P3HR-1 (virus-productive) lymphoblastoid cells. MTT-assay, neutral red and trypan blue dyeing were used to study cell viability. Antiviral activity was estimated by the real-time polymerase chain reaction (RT-PCR). Transmissive electron microscopy was used to visualize nanoparticle-virus binding.* ***Results.*** *It was found that nanoparticles of silver and gold stabilized by tryptophan and citrate were low-toxic for the used cell cultures; the vitality of the cells was in the range of 65—100%. Silver nanoparticles in a citrate buffer were more effective against EBV because the used concentrations inhibited replication of the virus up to 70%. Gold nanoparticles reduced the amount of EBV DNA by a maximum of 16% at the lowest concentration of 0.00001 μg/mL, indicating a dose-dependent effect. The virucidal effect of gold nanoparticles against EBV was shown using transmissive electron microscopy. It was found that the interaction of the virus with 5 nm gold nanoparticles for 2 hr leads to damage of EBV virion, which indicates their virus-static effect.* ***Conclusions.*** *Thus, the cytotoxic and antiviral activity of silver and gold nanoparticles in different stabilizers was analyzed. Citrate buffer-stabilized silver and gold NPs were more effective against EBV.*

Keywords: *Epstein-Barr virus, silver nanoparticles, gold nanoparticles, cytotoxicity, antiviral activity.*

The emergence of a new coronavirus strain and the related COVID-19 pandemic necessitate the search and development of new classes of reagents with antiviral activity. Despite extensive research and scientifically substantiated data on bactericidal, fungicidal, and antiviral properties of gold and silver nanoparticles (NPs), there are no licensed preparations based on them. Thus,

Citation: Zahorodnia S.D, Naumenko K.S., Zaychenko O.V., Zaremba P.Yu., Baranova G.V., Holovan A.V. Effect of Metal Nanoparticles on EBV-Associated Cell Culture. *Microbiological journal.* 2022 (1). P. 30—37. https://doi.org/10.15407/microbiolj84.05.030

© Publisher PH «Akademperiodyka» of the NAS of Ukraine, 2022. This is an open access article under the CC BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0/)

the universal characterization of the biological properties of nanoparticles will allow us to approach the creation of a new drug with polyvalent properties, at the same time safe for human health. Potential applications of NPs of gold and silver in biomedicine are increasingly reported due to the ease of synthesis, characteristics of bioinert materials, optical properties, chemical stability, high biosumistence, and specificity. The spectrum of the use of such NPs in the current medical and biological research is extremely wide. In particular, it includes genomics, biosensors, immune analysis, clinical chemistry, detection and photothermolysis of microorganisms and cancer cells; targeted delivery of drugs, DNA and antigens; optical biovisualization, and monitoring of cells and tissues by means of modern registration systems [1].

Epstein-Barr virus (EBV) is one of the most widespread viruses in the human population. It is best known as the primary cause of infectious mononucleosis (or «lymphatic fever»). It is also indirectly associated with various malignant lymphoproliferative diseases: lymphogranulomatosis (Hodgkin's disease), certain non-Hodgkin's lymphomas (particularly Burkitt's lymphoma — Central African lymphoma), post-transplantation lymphoproliferative disease, and hemophagocytic lymphohistiocytosis. About 200 thousand cases of cancer per year are associated with EBV. It is known that infection with EBV can occur through oral plaque transmission or from the vsilverinal tract [2].

Thus, EBV infection is a problem in various areas of medicine, including infectiology, infectious neurology, transplantology, and hematology. In recent years, there has been an increase in the number of cases of EBV infections, which in some way reflects the general trend of an increase in the number of infectious diseases caused by viruses [3].

Now we know that gold and silver NPs exhibit their antiviral and antimicrobial activity by means of various mechanisms. First, the unique properties of NPs, such as their small size (which can facilitate the delivery of drugs to anatomically relevant places), large surface area and volume ratio (which guarantees the possibility of placing large corrosive substances (such as drugs), and regulated surface charge (for easier penetration into the cell through its negatively charged membrane). All these advantages make NPs attractive tools for the development of drugs for the treatment of diseases caused by viruses and microorganisms [4].

The size of NPs is very important for their activity and stability. For example, it was found that the smaller the size of silver NPs, the higher their toxicity. Silver NPs sizing up to 10 nm (especially 5 nm) demonstrate the highest bactericidal effect [4]. However, when the diameter exceeds 50 nm, NPs lose their efficacy completely due to poor penetration into the middle of the cell. The form of silver NPs also determines their ability to interact with pathogens; nanocrystals, nanoplates,and quantum dots are the most effective against bacteria [5].

The aim of this work was to investigate the ability of silver and gold NPs to inhibit EBV replication under conditions of chronic infection.

Materials and methods. ***Cell cultures and their cultivation.*** P3HR1 — human B3 lymphocytes producing EBV was obtained from the Tissue Bank of the Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine (Kyiv, Ukraine).

B95-8 — mammalian lymphocytes, which are transformed by EBV and chronically producing it, was loyally provided by the Institute of Virology of the Russian Academy of Medical Sciences (Moscow, Russia).

Lymphoblastoid cultures were grown on a cultural medium consisting of 90% RPMI-1640 medium with L-glutamine (2 mM) (Sigma, USA), 10% fetal bovine serum, and antibiotics (50 μg/mL gentamicin) in plastic flasks (Sarstedt, Germany), at 37 °C in a thermostat under a 5% CO_2 atmosphere. Every three days,

passages were carried out to the concentration of $5 \cdot 10^5$ cells/mL. Every two days, the proliferative activity of the cells was checked using a light microscope Micromed XS-3320 (Ukraine) at 20x magnification.

Studying samples of metal nanoparticles. NPs, gold and silver in sizes from 5 to 20 nm, which were synthesized at the Chuik Institute of Surface Chemistry of NAS of Ukraine and submitted for examination, were investigated. Tryptophan, sodium citrate, and dodecyl sulfate were used as stabilizing agents for NPs. The molar concentration of metals was for silver 10^{-4} mol/L = 0.0107 g/L (10.7 μg/mL) and for gold 10^{-4} mol/L = 0.0197 g/L (19.7 μg/mL).

Determination of cytotoxic action of NPs by the colorimetric method with MTT. The investigative method of the cell viability is based on the function of the mitochondrial dehydrogenase system of intact cells, which under normal conditions, MTT (3,(4,5-dimethyltriazol-2-yl)-2,5-diphenyltetrosolium bromide) substrate convert into formazan (BioFroxx, German). The reaction product can be determined by the quantitative spectrophotometric method. MTT conversion to formazan is dose-dependently decreased when the cells are inhibited by the studied toxic substances. Cells were seeded in 96-well plates with a density of 5000000 cells/mL in 100 μL per well. 100 μL of a medium containing the studied drugs in different concentrations was added to corresponding wells. Control cell wells were a medium not added with drugs. At least 3—4 wells were used for each drug concentration. The plates with cells were incubated in the thermostat at 37 °C in a 5% CO_2 atmosphere for 48 hr. Then 20 μL of MTT substrate diluted in a sterile phosphate buffer (pH 7.2) at room temperature to a concentration of 5 mg/mL was added to each well of the 96-well plate, and the cells were incubated for 2 hr at 37 °C. The medium was removed after incubation, and 150 μL of 96% ethanol was added to the wells to dissolve the formazan crystals. The optical density of the solutions was determined spectrophotometrically using a Multiscan FC plate reader (ThermoFisherScientific, USA) at a wavelength of 538 nm. The measured optical density of cells treated with investigated drugs was compared with that of the control cells, and the following formula was used to calculate% of cellular viability in the presence of different concentrations of drugs [6]:

$$\% \text{ viability capacity} = (A/B \times 100) \quad (1.1)$$

where A is the average value of the optical density of the test samples for the given concentration of drugs;

B is the average value of the optical density of the control cells.

Virus purification and transmissive electron microscopy. The EBV was produced using a suspension of B95-8 lymphoblastoid cells. For virus accumulation, cells-producers were cultured without medium replacement at a cell density of $1 \cdot 10^6$ cells/mL for 10 days with a TFA (12-O-tetradecanoylphorbol-13-acetate) inductor at a concentration of 20 mM (Sigma, USA), which was added according to the manufacturer's instructions. The first stage of centrifugation was performed to precipitate the virus from the supernatant (90,000 g, 2 hr, 4 °C). The obtained suspension was suspended in a TNB buffer (0.01M Tris-HCl, pH 7.2, 0.15M NaCl). The following centrifugation was at 76,000 g, 1 hr, 4 °C. The opalescent ring will be between the dextran fractions 15% and 30% and will contain only the virus. The extracted sample was diluted with an equal amount of the TNB buffer and subjected to another step of centrifugation for virus precipitation and dextran purification (60,000 g, 1 hr, 4 °C) The obtained sediment contained only the virus. Preparations containing EBV were stored in aliquots at —70 °C.

The purified virus in an amount of 5 μL was applied to prepared copper plates with a formvar coating and carbon fillings. The adsorption of the sample onto the plate lasted 1 hr. The nanoparticle samples were not contrasted or

disintegrated. The samples containing the virus were contrasted with a 2% aqueous uranyl acetate solution for 45—55 sec and discharged in sterile H_2O for 10 sec. Microscopy was performed on a TEM microscope Jeol 1400 (Jeol, Japan) at 80 V.

Study of antiviral activity of NPs. Cells were seeded in 12-well plates with a density of 5000000 cells/mL in 1000 μL per well. 1000 μL of medium containing the studied drugs of different concentrations was added to the corresponding wells. The control cell wells were a medium without the drug. At least 3—4 wells were used for each drug concentration. The plates with cells were incubated in the thermostat at 37 °C in a 5% CO_2 atmosphere for 48 hr. Then the cells in each well were counted, and 500,000 cells were selected for further PCR analyses.

Polymerase chain reaction. Virus DNA was detected using commercial kit C-8896 «RealBest Extraction 100» (Vector Best, Russia). A DS-11 FX+ spectrophotometer (DeNovix, USA) was used to determine DNA concentration. The polymerase chain reaction method was used to determine the level of reproduction of viruses in the studied cells. Detection of EBV DNA was carried out using test-systems D-2185 «RealBest DNA EBV» (kit 1) (Vector Best, Russia) according to the manufacturer's instructions for real-time detection with Quant Studio 3 biosystems, Life Technologies (Thermo Fisher Scientific, Singapore).

Statistical data processing. Statistical processing of the data was performed according to the standard approaches to the calculation of statistical errors (standard deviation). The results were expressed as the mean ± S.D. of the values obtained in three independent experiments. The indicator $p<0.05$ indicated a statistically significant difference between the control and the studied samples.

Results. A cytotoxic effect of silver and gold NPs with different stabilizers was tested by the MTT test, which evaluated the mitochondrial activity of the cells. The number of metabolic-active cells for each concentration of NPs was determined.

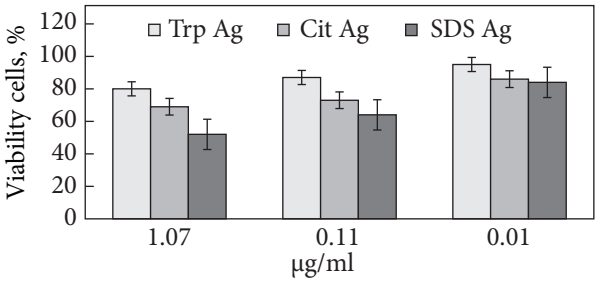

Fig. 1. Cytotoxic effect of silver NPs on the viability of P3HR1 cells

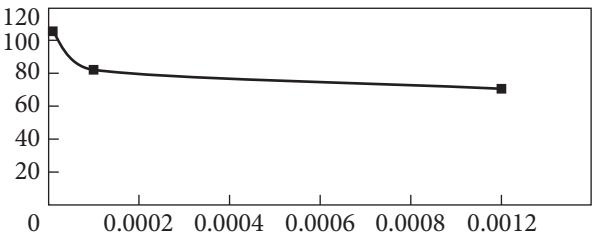

Fig. 2. Cytotoxicity of gold NPs against the viability of P3HR1 cells

The MTT test revealed that silver NPs stabilized with tryptophan and citrate solution showed lower toxicity compared to the silver NPs stabilized with sodium dodecyl sulfate (Fig. 1). The viability of cells treated with silver NPs in tryptophan was in the range of 80—87% at all studied concentrations, in citrate — 65 — 80%, whereas in SDS — 50—82%.

Gold NPs did not reveal marked toxicity toward P3HR1 cells (Fig. 2). The highest investigated concentration inhibited approximately 30% of cells.

The antiviral effect of NPs was studied. As seen in Fig. 3, silver NPs in all the used stabilizers showed high antiviral activity against EBV, which increased with the NPs dilution. At the same time, silver NPs in citrate buffer were more effective at low concentrations (from 1.07 to 0.0107 μg/mL). They inhibited virus DNA

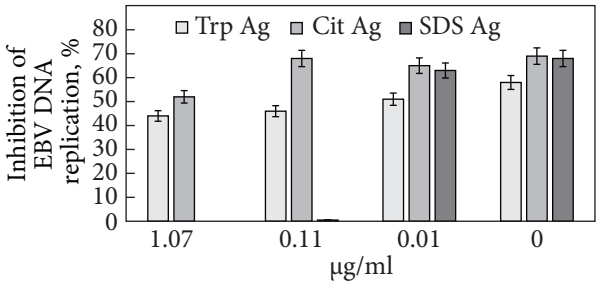

Fig. 3. Antiviral activity of silver NPs against Epstein-Barr virus

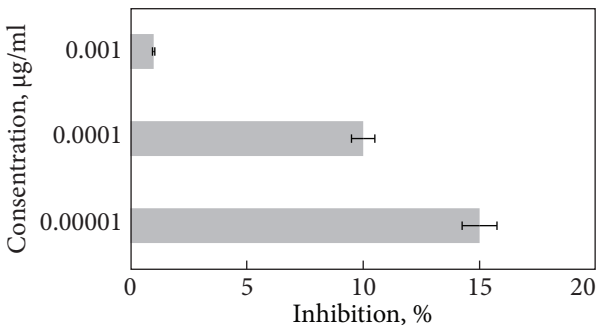

Fig. 4. Inhibition (%) of the level of expression of EBV DNA under the influence of different concentrations of 5 nm gold NPs

replication up to 70%. The application of SDS-stabilized NPs (0.0107 and 0.00107 μg/mL) led to a 62—68% decrease in the reproduction of EBV, whereas the antiviral effect of tryptophan-stabilized NPs was much lower, amounting to 45—59%.

Gold NPs in concentrations of 10^{-5}, 10^{-4}, and 10^{-3} μg/mL were added to the culture of cells, 48 hr later the samples were taken off, and DNA of EBV was determined using the PCR method (Fig. 4). The control of non-treated cells was taken as 100%, and the inhibition (%) was determined. Each sample was examined in three repetitions, and the average value was determined with the standard deviation not above 0.05.

So, gold NPs reduced the amount of EBV DNA by a maximum of 16% at the lowest concentration of 0.00001 μg/mL, indicating a reversed dose-dependent effect.

Transmission electron microscopy of samples of NPs and the virus was carried out. During incubation for 1 hr, there was a marked association of the virus with NPs. The association of the virus with 5 nm gold particles was more significant (more than 10 NPs per 1 virion) (Fig. 5A). The samples included both intact virions with NPs (viruses without damaged membrane covered with NPs) and virions with a damaged membrane (damage or loss of the membrane by the virus is regarded as loss of the ability to infect cells). Incubation of 5 nm gold NPs with the virus for 2 hr resulted in damage of EBV coronae. There were not only virions with a damaged lipid envelope but also virions that completely lost their spherical morphology (Fig. 5C). Such damage of virus leads to the loss of the ability to infect cells.

Discussion. Thus, cytotoxic and antiviral activities of silver and gold NPs in different stabilizers have been analyzed. Silver NPs in a citrate buffer stabilizer were more effective against EBV. It should be noted that the EBV model is a virus-associated B lymphoma. The results obtained testify to the high antiviral potential of gold and silver NPs as well as to the important role of stabilizers in their preparation, concentration, and size.

Nanomedicine is an extremely promising branch of biotechnology with a great number of new promising drugs. The main advantage of NPs is their size, which determines their unique chemical and physical properties, which can be used for visualization, diagnostics, and treatment [7]. There have been some systems that include NPs already subjected to clinical trials, in particular, drugs that contain liposomes and conjugated antigens.

Today there is a large enough number of works confirming their antitumor, bactericidal, fungicidal, virucidal, and antiviral activity [8, 9]. Nanosilver is an effective agent against bacteria, viruses, and other eukaryotic microorganisms due to its key mechanisms of action based on the release of silver ions, which enhance antimicrobial

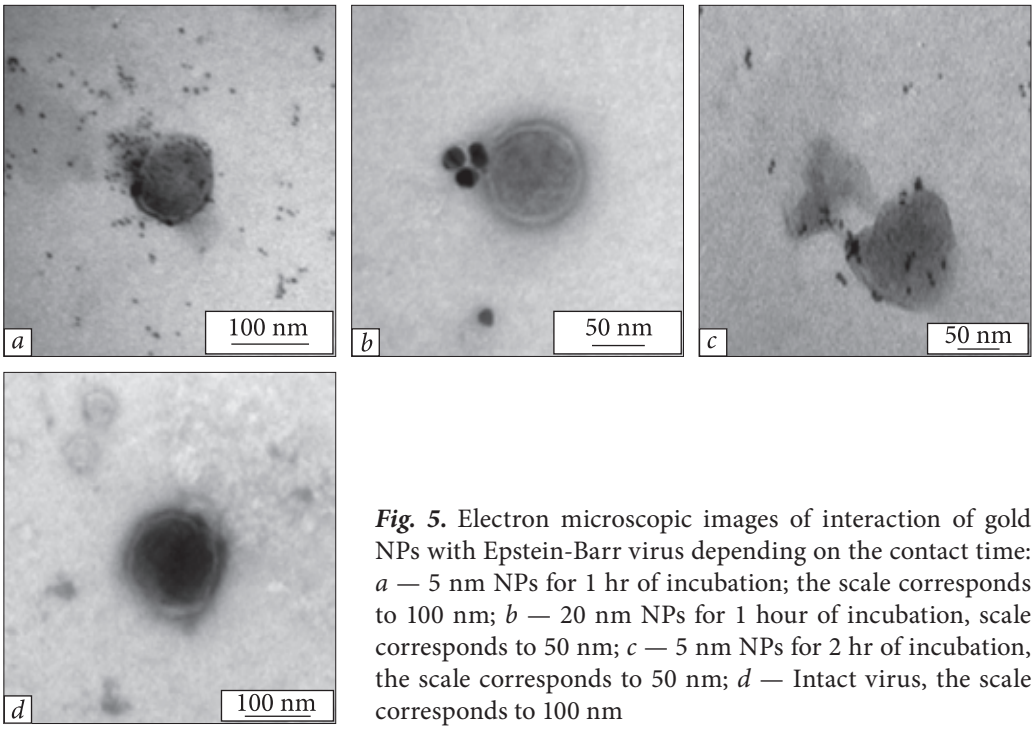

Fig. 5. Electron microscopic images of interaction of gold NPs with Epstein-Barr virus depending on the contact time: *a* — 5 nm NPs for 1 hr of incubation; the scale corresponds to 100 nm; *b* — 20 nm NPs for 1 hour of incubation, scale corresponds to 50 nm; *c* — 5 nm NPs for 2 hr of incubation, the scale corresponds to 50 nm; *d* — Intact virus, the scale corresponds to 100 nm

activity and lead to protein and DNA damage [4]. A possible mechanism of the silver NPs action in the cell which we can foresee could be the interaction of Ag+ with clathrin necessary for influenza virus penetration into the cytoplasmic space. It has been previously reported that nanosilver actively associates with proteins and influences their conformation and functions [10], therefore + can influence clathrin in the endosome, mobilizing it in the cytoplasm and thus blocking the transport of virions into the cell. Hu R.L. et al. [11] showed that PVP-coated 30—40 nm silver NPs inhibited HSV-2 at the early stages of viral replication. Their virucidal effect after 1 hr of contact with the virus was also shown. The work of Baram-Pinto D. et. al [12] demonstrated the antiviral effect of silver NPs coated with mercaptoethane sulfonate (MES), which effectively inhibited HSV1 in cells, implying that the antiviral effect of Ag-MES NPs is due to their polyvalent nature.

Gold NPs, due to their ultrafine sizes, can easily penetrate into tissues and cells. Vijayakumar S. [13] showed that gold NPs stabilized with polyline glycol inhibit the HIV-1 entry via linking gp120 and preventing virus attachment to CD4. These properties of gold NPs make them an effective inhibitor of the virus studied.

Thus, basing on the results of our data and literature sources, we can conclude that the effective inhibition of the EBV in P3HR1 cells with the studied NPs stabilized in different stabilizers under conditions of chronic infection is due to the interaction of NPs with biomolecules and DNA leading to the damage of virus proteins. Gold NPs were less effective; they probably block the viral penetration or viral entry for newly synthesized virions into cells.

Conclusions. It was found that silver and gold NPs stabilized by tryptophan and citrate were low-toxic for the used cell cultures (the vitality of the cells was in the range of 65—100%).

Silver NPs in citrate buffer were more effective against EBV: the investigated concentrations inhibited replication of the virus up to 70%.

Gold NPs reduced the amount of EBV DNA by a maximum of 16% at the lowest concentra-

tion of 0.00001 μg/ml, indicating a reverse dose-dependent effect.

The virucidal effect of gold NPs against EBV was shown using transmissive electron microscopy. It was found that the interaction of the virus with 5 nm gold NPs for 2 hr leads to the damage of EBV virions, which indicates their virus-static effect.

Acknowledgments. This work was supported by the National Research Foundation of Ukraine, Project 2020.02/0352.

REFERENCES

1. Dykman LA, Khlebtsov ng. Gold nanoparticles in biology and medicine: recent advances and prospects. Acta Nature. 2011; 3(2):34—55. DOI: 10.32607/20758251-2011-3-2-34-55
2. Maria K. Smatti, Duaa W. Al-Sadeq, Nadima H. Ali, Gianfranco Pintus, Haissam Abou-Saleh, Gheyath K. Nasrallah. Epstein—Barr Virus Epidemiology, Serology, and Genetic Variability of LMP-1 Oncogene Among Healthy Population: An Update. Front Oncol. 2018; 8:211. Published online 2018 Jun 13. doi: 10.3389/fonc.2018.00211
3. Luciana Cristina Fsilverundes Gequelin, Irina N. Riediger, Sueli M. Nakatani, Alexander W. Biondo, Carmem M. Bonfim. Epstein-Barr virus: general factors, virus-related diseases and measurement of viral load after transplant. Elservier. 2011; 33(5):383—388. DOI: 10.5581/1516-8484.20110103
4. Singh L, Kruger H, Msilveruire G, Govender T, Parboosing R. The role of nanotechnology in the treatment of viral infections. Ther Adv Infect Dis. 2017; 4(4):105—131. https://doi.org/10.1177/2049936117713593
5. Silvernihotri S, Mukherji S, Mukherji S. Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. RSC Adv. 2014; 4:3974–3983. DOI: 10.1039/c3ra44507k
6. Tonder A, Joubert M, Cromarty AD. Limitation of the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. BMC Res Notes. 2015; 8(47):1—10. DOI 10.1186/s13104-015-1000-8
7. Wei L, Lu J, Xu H, Patel A, Chen ZS, Chen G. Silver nanoparticles: synthesis, properties, and therapeutic applications. Drug discovery today. 2015; 20(5):595—601. DOI: 10.1016/j.drudis.2014.11.014
8. Dos Santos CA, Seckler mM, Ingle AP, Gupta I, Galdiero S, Galdiero M, Rai M. Silver Nanoparticles: Therapeutical Uses, Toxicity, and Safety Issues. Journal of Pharmaceutical Sciences. 2014; 103(7):1931—1944. DOI: 10.1002/jps.24001
9. Kavoosi F, Modaresi F, Sanaei M, Rezaei Z. Medical and dental applications of nanomedicines. Apmis. 2018; 126(10):795—803. https://doi.org/10.1111/apm.12890
10. Flores-Lopez LZ, Espinoza-Gomez H, Somanathan R. Silver nanoparticles: Electron transfer, reactive oxygen species, oxidative stress, beneficial and toxicological effects. Mini review. Journal of applied toxicology. 2019; 39(1):16—26. DOI: 10.1002/jat.3654
11. Hu RL, Li SR, Kong FJ, Hou RJ, Guan XL, Guo F. Inhibition effect of silver nanoparticles on herpes simplex virus 2. Gen Mol Res. 2014; 13(3):7022—7028. DOI http://dx.doi.org/10.4238/2014.
12. Baram-Pinto D, Shukla S, Perkas N, Sarid R. Inhibition of Herpes Simplex Virus Type 1 Infection by Silver Nanoparticles Capped with Mercaptoethane Sulfonate. Bioconjugate Chem. 2009; 20(8):1497—502. DOI: 10.1021/bc900215b
13. Vijayakumar S, Ganesan S. Gold nanoparticles as an HIV-1 entry inhibitor. Curr HIV Res. 2012; 10(8):643—6. Doi: 10.2174/157016212803901383.

Received 15.11.2021

С.Д. Загородня, К.С. Науменко, Ф.В. Зайченко, П.Ю. Заремба, Г.В. Баранова, А.В. Головань

Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України,
вул. Академіка Заболотного, 154, Київ, 03143,Україна

ВЛИВ НАНОЧАСТИНОК МЕТАЛІВ НА ВЕБ-АСОЦІЙОВАНУ КУЛЬТУРУ КЛІТИН

На сьогоднішній день одним з актуальних напрямків досліджень є пошук антивірусних препаратів для боротьби з вірусасоційованими онкологічними проявами. Одним із вірусів, для якого доведена роль в трансформації клітин, є вірус Епштейна-Барр (ВЕБ), який асоціюється з низкою лімфопроліферативних захворювань. Застосування препаратів, які не тільки пригнічують розмноження вірусу, але й стимулюють елімінацію пухлинних клітин, є важливим для лікування пухлин, асоційованих з вірусом. **Мета.** Дослідити здатність наночастинок срібла та золота інгібувати реплікацію ВЕБ в умовах хронічної інфекції. **Методи.** Об'єктом дослідження були наночастинки золота та срібла від 5 до 20 нм, стабілізовані за допомогою триптофану, додецилсульфату натрію та цитрату. Дослідження проводили в лімфобластоїдних клітинах P3HR-1 (продукуючих вірус). Для вивчення життєздатності клітин використовували МТТ-метод з нейтральним червоним та трипановим синім фарбуванням. Противірусну активність оцінювали за допомогою полімеразної ланцюгової реакції в реальному часі (RT-PCR). Візуалізацію зв›язування наночастинок з вірусом здійснювали за допомогою трансмісивної електронної мікроскопії. **Результати**. Виявлено, що наночастинки срібла та золота, стабілізовані триптофаном та цитратом, малотоксичні: життєздатність клітин була в межах 65—100%. Наночастинки срібла в цитратному буфері були більш ефективними проти ВЕБ: досліджувані концентрації пригнічували реплікацію вірусу до 70%. Наночастинки золота зменшували кількість ДНК ВЕБ максимум на 16% при найнижчій концентрації 0,00001 мкг/мл, що свідчить про дозозалежний ефект. Встановлено, що взаємодія вірусу з наночастинками золота 5 нм протягом 2 год приводить до пошкодження оболонки вірусу, що свідчить про їхній вірусстатичний ефект. **Висновки.** Таким чином, було проаналізовано цитотоксичну та противірусну активність наночастинок срібла та золота у різних стабілізаторах. Найвищу ефективність щодо ВЕБ вони мали за стабілізації цитратним буфером**.**

Ключові слова*: вірус Епштейна-Барр, наночастинки срібла, наночастинки золота, антивірусна активність.*