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Authors Maryna Chaika, Krystyna Naumenko, Yuliia Pankivska, Olga Povnitsa, Iuliia Mukha, Nadiya Vityuk, Anatoliy Dorovskih, Valeri Lozovski, Volodymyr Lysenko, Mykhailo Lokshyn, Natalia M. Rusinchuk and Svitlana Zagorodnya

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ORCID® iDs Valeri Lozovski - https://orcid.org/0000-0002-9142-0427; Natalia M. Rusinchuk - https://orcid.org/0000-0002-0361-1961

Size- and concentration-dependence of antiviral and virucidal activity of Au nanoparticles against adenoviruses and influenza viruses H1N1

Maryna Chaika[1], Krystyna Naumenko[1], Yuliia Pankivska[1], Olga Povnitsa[1], Iuliia Mukha[2], Nadiya Vityuk[2], Anatoliy Dorovskih[3], Valeri Lozovski[4,5], Volodymyr Lysenko[5], Mykhailo Lokshyn[5], Natalia Rusinchuk[4*], Svitlana Zagorodnya[1]

[1)] Zabolotny Institute of Microbiology and Virology NAS of Ukraine, Zabolotnogo str., 154, Kyiv, 03143, Ukraine

[2)] O.O. Chuiko Institute of Surface Chemistry, National Academy of Sciences of Ukraine, Ghenerala Naumova str., 17, Kyiv, 03164, Ukraine

[3)] LLC Scientific-Production Enterprise «International Medical Center», Luteranska str., 16, Kyiv, 01024, Ukraine

[4)] Institute of High Technologies, Taras Shevchenko National University of Kyiv, Volodymyrska str. 60, Kyiv, Ukraine

[5)] Lashkariov Institute of Semiconductor Physics, National Academy of Sciences of Ukraine, Nauki ave. 45, Kyiv, Ukraine

* Corresponding author: Natalia Rusinchuk, n.m.rusinchuk@gmail.com

Abstract

Over the past 10 years, many scientific groups have experimentally shown that non-functionalized nanoparticles show a pronounced antiviral and antimicrobial action against different pathogens. In order to understand the mechanism of nanoparticles action it is important to know its peculiarities, i.e. dependences on different nanoparticles and pathogen properties.

In this work we studied how Au nanoparticles act on the viruses outside and inside the cell, and compare this action for two sizes of nanoparticles and two types of the viruses. The study has been conducted for adenovirus and H1N1 influenza virus, and nanoparticles of 5 nm and 20 nm diameter. Virucidal and antiviral actions were observed experimentally for both types of nanoparticles against both viruses. It has been shown that intensity of virucidal action depends on the nanoparticles concentration non-monotonically for adenovirus. It has also been shown with electron microscopy that the viruses are destructed after 5 nm nanoparticles adsorption on their surface; and that the viruses change their shape after 20 nm nanoparticles adsorption on their surface. The model of physical adsorption of nanoparticles on the virus surface due to near-field interaction proposed in previous works may explain observed results on virucidal action of nanoparticles.

Keywords: Au nanoparticles; antiviral action; virucidal action; adenovirus; influenza virus H1N1.

Introduction

The development of our civilization leading to globalization opened Pandora's Box for the epidemic/pandemic of the viruses. The last examples of the outbreaks of viral diseases are the Ebola epidemic and COVID-19 pandemic [1,2]. The problems in current state of combating viral infections are the reason for the need for new methods of the virus inhibition. One of the directions of this search is investigations of antiviral action of nanoparticles, which are actively held last decades, e.g. [3-7]. The wide range of the types of nanoparticles which demonstrate effect of the virus inhibition against wide range of the viruses allowed us to suppose that the mechanism of such inhibition does not directly depend on the material of the nanoparticle and type of the virus. Consequently, the mechanism of antiviral properties of nanoparticles may be based not on the chemical interactions but on physical ones, probably, field interactions. This idea was applied for simulation of interaction between the nanoparticle and the virus, and for explanation of experimental results of different research groups in [8]. One of the features of field action of the nanoparticles on the virus is the local field enhancement effect [9,10], which depends much on the nanoparticles properties: size, shape, stabilizer, etc. So, in [8] the authors analyzed the works, in which experimental results on virucidal activity, differed in one parameter, were reported. Why just virucidal? Virucidal action on nanoparticles is due to the nanoparticle action on the extracellular virus. In this case there are no biochemical processes in the virus, so the simplest model of two nanoparticles (the nanoparticle and the virus) may be applied. Indeed, it was shown in [8] that difference in virucidal action of different-sized nanoparticles, the difference in virucidal action of nanoparticles on two different viruses, and the weakening of virucidal action of high-concentrated nanoparticles samples can be explained in the frame of the proposed idea. However, the existed experimental results give fragmentary view on the phenomenon of nanoparticles inhibition of the viruses. If we want to understand the mechanisms of interaction and apply them for disinfection and/or antiviral therapy, there is a need of complex study of this phenomenon. One of the relevant questions in this field, from our point view, is the

comparison of virucidal and antiviral action of nanoparticles. Also it would be useful to "see" how the nanoparticles act on the virus, if there is the nanoparticles adsorption on the virus surface, and what the consequences of such adsorption are.

Hence, the main aim of the work is to study how the different-sized Au nanoparticles (as one of the most widely used ones) effect on the human viruses (both RNA and DNA viruses) and to compare their effect in case of virucidal and antiviral action.

Methods

Design of the study

In order to achieve the aim of the study several experimental methods have been used:

- Transmission electron microscopy of the viruses samples mixed with nanoparticles in order to study how the nanoparticles interact with the extracellular virus, and to reveal if the nanoparticles adsorb on the virus surface. In case of the nanoparticles adsorption it is also importantly to know at what place the nanoparticles mainly adsorb and what the consequences of these adsorption are.
- Cytotoxicity study of nanoparticles on the model cell lines which are used for study of antiviral action. This is the necessary stage of the study as before introduction of the nanoparticles in the cell culture we need to be sure that there will be no effects caused by nanoparticles on the living cells.
- Study of virucidal action of nanoparticles. In these experiments the nanoparticles are added to the virus sample before the cells infection. After that the cells are incubated with and without the viruses (intact cells, control) and the cell viability and the virus reproduction rate are evaluated. Virucidal studies allow drawing attention mainly on the effects of nanoparticles directly on the virus (but we do not exclude other types of interactions).
- Study of antiviral action of nanoparticles. In these experiments the cells were infected with the viruses and after some time of infection (when the viruses, probably, are inside the cells)

the nanoparticles are added. After the incubation the cells viability and the virus reproduction rate are evaluated. Antiviral action of nanoparticles is the result of the set of interactions, which may include of the nanoparticles interaction with cells, nanoparticles interaction with cell organelles, nanoparticles interaction with the viral DNA/RNA and inhibition of its replication (which was shown in [3,11]), nanoparticles interaction with the extracellular viruses, etc.

It was decided to check two possible modes of action of nanoparticles: antiviral action (inhibition of the virus replication in already infected cells) and virucidal action (destroying the virus out of the cell). The choice was made ground on literature data and findings [12-14].

For experiments Au nanoparticles stabilized with citrate were used. In order to study the size-dependence (which is now widely studied by many research groups) two sizes of nanoparticles were synthesized. In order to reveal if the effects differ for the RNA and DNA virus, and to reveal if the surface structure of the virus influences on the effects adenovirus and Influenza A virus H1N1 were used.

Viruses

The experiments have been conducted for two viruses: adenovirus and Influenza A virus H1N1. Adenovirus is the DNA virus, it has capsid of icosahedral shape, it is about 60-80 nm in diameter and has spikes on the tops of the icosahedron. The spikes are 8 - 30 nm long with and have a 4 nm head at the end [15]. Influenza A virus H1N1 is the quasi-spherical RNA virus of 80–120 nanometers in diameter. Influenza A virus H1N1 is the enveloped virus with the spikes on its surface [16].

Nanoparticles

Colloidal solutions of Au nanoparticles were synthesized via chemical reduction of tetrachlorauric acid ($HAuCl_4$, Merck, Germany) with trisodium citrate (NaCitr, $Na_3C_6H_9O_9$, ACROS, Germany) in aqueous solutions according to Turkevich method [17,18]. Au NPs with an average size of 20 nm were obtained via citrate reduction using the molar ratio of components as ν(NaCitr):ν(Au) = 2:1. The

initial pH of water before adding the reagents was adjusted to pH = 5.3 with 0.01N HCl. $HAuCl_4$ was inserted into boiling water, then NaCitr was added, stirred while boiling for 5 min, then cooled at room temperature.

Au NPs with an average size of 5 nm were prepared in the presence of NaCitr with the same quantity (ν(NaCitr):ν(Au) = 2:1) and using an additional reducer, namely $NaBH_4$ in a molar ratio ν($NaBH_4$):ν(Au) = 10:1. The redox reaction proceeded at room temperature.

Colloids were brought to final concentrations: $C_{Au} = 1.5 \cdot 10^{-4}$ M, $C_{NaCitr} = 9 \cdot 10^{-4}$ M. The citrate was used as the nanoparticle stabilizer.

The particle size distribution function was studied by a laser correlation spectrometer Zeta Sizer Nano S (Malvern, UK) equipped with a correlator (Multi Computing Correlator Type 7032 CE) by the dynamic light scattering (DLS). The helium-neon laser LGN–111 was used with the output power of 25 mW and wavelength of 633 nm to irradiate the suspension. The registration and statistical processing of the scattered laser light at 173° from the suspension were performed three times during 120 seconds at 25°C. The resulting autocorrelation function was treated with standard computer programs PCS–Size mode v.1.61.

The absorption spectra of Au colloids were recorded in the UV-visible region by a spectrophotometer Lambda 35 (Perkin-Elmer, United States) in 1 cm quartz cells.

The samples were also characterized by transmission electron microscopy (TEM) using a JEOL-1200 EX (JEOL, Tokyo, Japan) at an accelerating voltage of 120 kV. The NP dispersions were diluted in DI water, dropped onto a carbon coated copper grid, and dried at room temperature. Particle size analysis was performed from TEM images manually via the image analysis software ImageJ.

Cells

The cell culture Hep-2 was used for virucidal and antiviral studies against adenovirus. The suspension of the cells was putted in 96-well plate: 200 μl per well. The cells concentration was $2 \cdot 10^5$ per one ml. Then the cell culture was incubated for 24 hours at 37° C and 5% CO_2. In 24 hours the cells formed a monolayer.

The cell culture MDCK was used for virucidal and antiviral studies against influenza virus H1N1. MDCK cells were seeded in 96-wells plates the day before study at concentration $3 \cdot 10^4$ per well. Then the cell culture was incubated for 24 hours at 37° C and 5% CO_2. In 24 hours the cells formed a monolayer.

Before the study, the monolayer was checked to be 80-90%.

Cytotoxicity study

Reproduction of the viruses inside the cells causes the difficulties for development of antivirals which would suppress the virus reproduction inside the cell but do not influence on the cell viability and the whole organism. The high toxicity restricts the number of antivirals which are used in the clinical practice. In this connection, we study the influence of Au nanoparticles on proliferation and vitality of the cell culture used MTT test [19] and neutral red uptake [20]. The determination of Au nanoparticles cytotoxicity on MDCK cell culture via indirect measuring of mitochondrial activity was conducted.

Virucidal studies

For virucidal studies the nanoparticles were added to the virus samples in 30 minutes before the cells infection.

For adenovirus studies 50 µl of the virus sample were mixed with 450 µl of maintenance medium, and then the dilutions from 10^{-1} to 10^{-3} for definition of the virus titre were prepared. Then 50 µl of the virus sample were added to the well. For each virus sample were used three wells in order to compare results. After that the plate was kept at 37°C and 5% CO_2 athmosphere for 1.5 hours for virus adsorption. After the incubation the virus samples were not removed from the wells. Then 150 µl of the maintenance medium were added to the wells. After 72 hours of incubation 20 µl of MTT were added to the wells. Then the plate was kept in thermostate for 4 hours. After that 150 µl of 96% ethanol were added to flush the dye from the cells, and the optical densities of the wells were measured at 540 nm wavelength using Multiskan FC (Thermo Scientific, USA).

For influenza virus studies 50 µl of the virus sample were mixed with 450 µl of maintenance medium, and then the dilutions from 10^{-1} to 10^{-4} for definition of the virus titre were prepared. Then 50 µl of the virus sample were added to the well. For each virus sample were used three wells in order to compare results. After that the plate was kept at 37ºC and 5% CO_2 athmosphere for 1.5 hours for the virus adsorption. After the incubation the virus samples were not removed from the wells. Then 150 µl of the maintenance medium were added to the wells. After 48 hours of incubation 20 µl of crystal violet were added to the wells. Incubation was performed at room temperature on a bench rocker with a frequency of 20 oscillations per minute for 20 minutes. Next, the wells were washed in water, dried at room temperature and 200 µl of 96% ethanol was added to flush the dye from the cells. After that the optical densities of the wells were measured at 540 nm wavelength using Multiskan FC (Thermo Scientific, USA).

Antiviral studies

For antiadenoviral activity studies the cell monolayer was infected by 50 µl of the virus sample per a well. The plate was incubated at 37ºC and 5% CO_2 athmosphere for 1.5 hours for the viruses adsorption. After finish of incubation the viruses were removed from the wells. Then, 200 µl of nanoparticles samples in 10 order dilutions (from 10^{-1} to 10^{-7}) were added to the wells. For each nanoparticles sample were used three wells in order to compare results. In 72 hours after that 20 µl of MTT were added to each well. After that the plate was kept for 4 hours in thermostate. After that 150 µl of 96% ethanol were added to flush the dye from the cells, and the optical densities of the wells were measured at 540 nm wavelength using Multiskan FC (Thermo Scientific, USA).

For studies of antiviral action of nanoparticles against influenza virus H1N1 before infection the cells were washed with cold phosphate buffered saline PBS and then the viruses and fetal bovine serum (FBS) were added and the plate were incubated at 37ºC and 5% CO_2 for 1.5 hours. After that the cells were washed with PBS solution to eliminate the non-adsorbed virus. Then cells were supplemented with maintenance medium: 45% DMEM ((Dulbecco's Modified Eagle Medium), 45% RPMI 1640 (Roswell Park Memorial Institute medium), 4% FBS inactivated by heating, penicillin (100µg/ml),

streptomycin (100µg/ml) and trypsin (0.5 µg/ml). The monolayer was checked for cytopathic effect (CPE) daily in order to obtain significant CPE occurred in the virus control wells. So the results were registered after 48 hours of incubation. Then 20 µl of crystal violet were added to the wells. Incubation was performed at room temperature on a bench rocker with a frequency of 20 oscillations per minute for 20 minutes. Next, the wells were washed in water, dried at room temperature and 200µl of 96% ethanol were added to flush the dye from the cells. the optical densities of the wells were measured at 540 nm wavelength using Multiskan FC (Thermo Scientific, USA).

The infection inhibition rate was calculated as:

$$\mathrm{IIR} = \frac{(\mathrm{ODexp} - \mathrm{ODcv})}{(\mathrm{ODcc} - \mathrm{ODcv})}) \times 100\%, \quad (1)$$

where ODcc is the average value of the optical density of the cell control samples, ODcv is the average value of the optical density of the virus control samples, ODexp is the average value of the optical density of the test samples for a defined concentration of the substance.

Electron microscopy

Influenza virus purification was performed due to the standard protocol with minor changes [21]. MDCK cells were infected at multiplicity of infection 0.1 to obtain the homogeneous virus culture. To obtain clear supernatant cells were centrifugated at 1000 g for 15 minutes. The sediment was discarded. The supernatant was centrifuged for the first step at 112000 g for 90 minutes at +4ºC in Beckmann centrifuge (USA). The supernatant was discarded. Sediment was dissolved in NTC buffer (100 mM NaCl, 20 mM Tris-HCl, 5 mM $CaCl_2$). Obtained sediment was overlayed on sucrose gradient 30%, 40%, 50%, and 60% and centrifugated at 209000 g for 150 minutes at +4˚C. As a result, opalescent circle in the middle of the tube (between 40% and 50% sucrose solution) was obtained and extract. The extraction was diluted in NTC buffer and centrifuged at 154 000 g for 60 minutes at +4˚C. The virus was obtained in sediment, dissolved in NTC buffer, aliquoted and stored at -20˚C.

For the transmission electron microscopy (TEM) samples were adsorbed at formvar overlaid copper grids for 1 hour and washed in sterile distilled water. Then samples were stained with 1% aqueous

solution of uranyl acetate for 30 seconds – 1 minute and also washed in sterile distilled water. Examining samples were performed at 80 V with Jeol JEM-1230 (Japan) or Jeol JEM-1400 (Japan).

Description of interactions between the virus and the nanoparticle

The main idea of the mechanism of nanoparticle action on the virus is the formation of inhomogeneous local field at the surface of the virus. This effect may prevail over chemical interactions as the physical adsorption of the nanoparticle on the virus is at the distance at least the radius of the nanoparticle. The mechanism of such adsorption is described in [22]. The inhomogeneous local field (electromagnetic field) is formed due to the local field enhancement effect in the nanostructured systems and depends much on the system geometry. This effect leads to the appearance of the areas with high intensity local field and rather high local field gradients as it was shown in [8]. Here we may suppose at least three ways for inhibition of the virus activity:

1. The virus loses its ability to infect the cell due to the nanoparticle adsorption on the spikes. We suppose that the nanoparticles should adsorb mainly on the virus spikes as the adsorption force is greater near the edges of spiked than near the surface (theoretical description of this effect can be found in [23]). As the virus "open" the cell with the molecules in the spikes, the adsorbed nanoparticle may prevent this.
2. The molecules in the virus spikes have polar sites; hence the molecule has some polarization, which may be described as the spatial distribution of dipolar moment. In the non-homogeneous field the dipole is under the action of ponderomotive forces, which may lead to the change of molecules conformation. In this case the virus loses its ability to "open" the cell as the molecules are in wrong conformation.
3. The forces described in previous paragraph may be too great to deform of even to broke the virus.

Consequently, we suppose that in experiments the nanoparticles adsorption on the virus spikes should be observed, and that the smaller nanoparticles should adsorb more effectively as the adsorption force is higher for smaller nanoparticles [8]. However, three mechanism described above can be applied

well for virucidal action but need to be supplemented with other mechanism occurred in cells at the antiviral action.

Results and discussions

Nanoparticles size

The distributions of the particles over the sizes are shown in Fig.1.

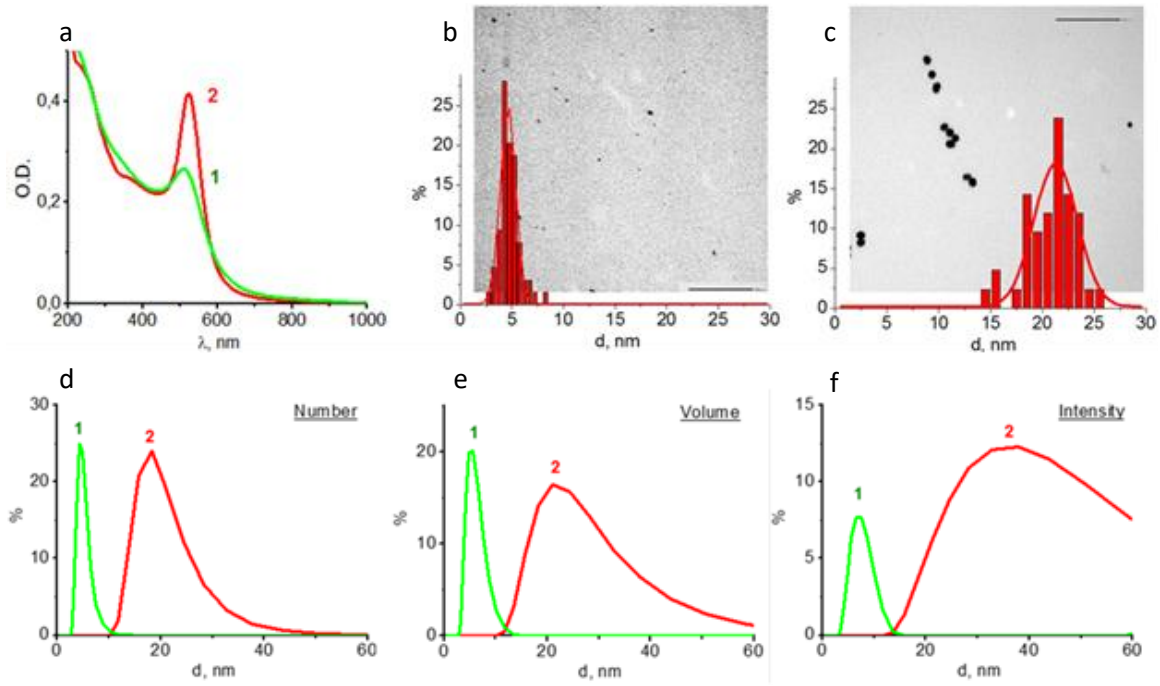

Figure 1. (a) - Absorption spectra (a) of nanoparticles; (b) and (c) - TEM with the size distribution of 5 nm and 20 nm diameter respectively; (d)-(f) results of DLS measurements of solutions with the nanoparticles of 5 nm (1) and 20 nm (2) diameter.

Nanoparticles cytotoxicity

The results of the measurements of nanoparticles cytotoxicity are shown in Table 1. Similar results were obtained for the other cell culture (Hep-2) used in the present work. When the antiviral actions of the nanoparticles were studied the Au nanoparticles toxicity was taken into account. The highest

concentration for NPs was used due to the fact, that these concentrations didn't decrease cell viability for more than 40%.

Table 1. Influence of Au nanoparticles to proliferation and vitality of the MDCK cell culture

Concentration, µg/ml	Au nanoparticles 5 nm	Au nanoparticles 20 nm	Au nanoparticles 5 nm	Au nanoparticles 20 nm
	MTT test, % mitochondrial activity ± SE		NR uptake test, % lysosomal activity ± SE	
0.03	91±4.5	107±5.3	116±5.8	130±6.5
0.061	107±5	114±5.7	118±5.9	118±5.9
0.123	100±5	110±5.52	121±6	122±6.1
0.246	94±4.7	99±4.9	116±5.8	118±5.9
0.492	104±5	111±5.5	121±6	114±5.7
0.985	91±4.5	103±5.1	111±5.5	112±5.6
1.97	59±2.9	76±3.8	100±5	92±4.6

Virucidal action of nanoparticles against adenovirus

Virucidal action of nanoparticles against adenoviruses was studied by TEM and by evaluation of the virus titer. TEM images of the studies samples are shown in Fig. 2.

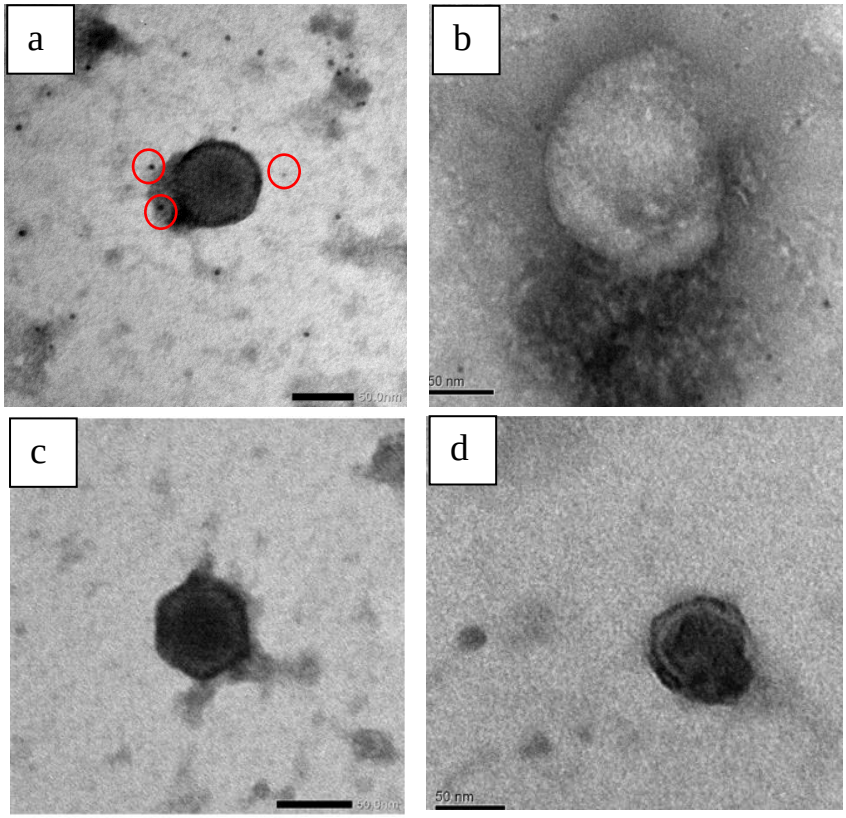

Figure 2. TEM images of adenovirus: a – in 1 hour of incubation with 5 nm gold nanoparticles, b - in 2 hours of incubation with 5 nm gold nanoparticles, c – intact virus, d – in 2 hours of incubation with 20 nm gold nanoparticles.

It can be seen, that in 2 hours after adding of the nanoparticles to the viruses, the virus is destructed. However, in Fig. 2, a, it may seem that there is no nanoparticle adsorption on the virus. But, long and thin adenovirus spikes also cannot be seen in the micrograph. But if we compare the micrograph with the real adenovirus structure, we can state that some nanoparticles (rounded by red) are adsorbed at the edges of the spikes. The virus destruction observed in the micrograph in Fig. 2, b, cannot take place without a reason. As there was no virus destruction in the intact sample (Fig. 2, c), it is obviously should be connected with the interaction with nanoparticles. As outside the cell the virus can be described as some non-living object, we can suppose that indeed when nanoparticles are adding to the viruses the near field interaction and the ponderomotive forces occur. In Fig. 2, d it can be seen that interaction on the virus with bigger nanoparticles leads to the change of the virus shape.

This is in good agreement to the model of near-field interaction and ponderomotive forces, which are less intensive for bigger nanoparticles.

Results of the study of the adenovirus titer are shown in Fig.3. It can be seen that the decrease in adenovirus titer is observed equally well for both nanoparticles. However in average nanoparticles concentrations from 0.197 to 19.7 ng/ml smaller nanoparticles demonstrate better effect. This indicates that the virus destruction is not necessary for the viral inhibition, and as it was supposed above the viral re-shape may be enough for this. Also in Fig. 3 interesting concentration-dependence is observed. It can be seen that with the increase of 5 nm Au nanoparticles concentration the viral inhibition rate raises and then falls. So we have non-linear dependence of virucidal action on the nanoparticles concentration (similar results were observed in [3] for Ag against Hepatitis B virus, and in [24] for Au nanoparticles coated with SiO_2 shell against adenovirus). This effect was also descripted in [8] and is caused by the nonlinear effect when the nanoparticles located rather close one to another "screen" the effect of each one [25]. Also this effect indicates that the optimal nanoparticles concentration may be chosen for the maximum adenovirus inhibition in virucidal studies.

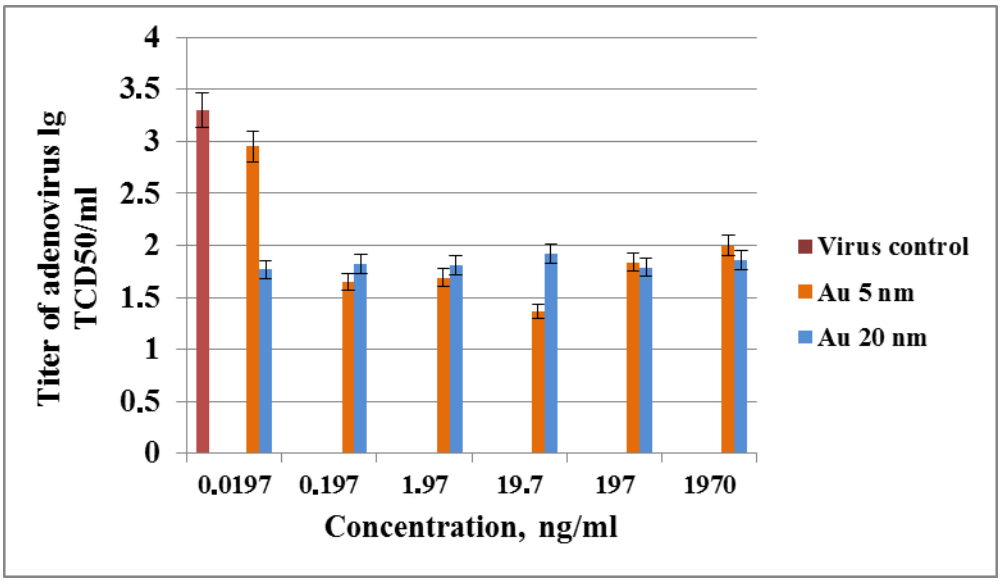

Figure 3. Adenovirus titer after interaction with nanoparticles for Au nanoparticles of diameters of 5 nm (blue) and 20 nm (pink) of different concentrations.

Antiviral activity of Au nanoparticles against adenovirus

Dependence of inhibition of adenovirus reproduction on the concentration of Au nanoparticles of 5 and 20 nm diameters is shown in Fig. 4. We can see that smaller nanoparticles demonstrate better antiviral activity which was expected and can be explained by local-field enhancement effect inside the system. However, nanoparticles with a diameter of 5 nm demonstrate their effectiveness even at the smallest concentration of 0.00197 ng/ml; in the case of nanoparticles with a larger diameter (20 nm) no effect on the reproduction of adenovirus was observed at this concentration.

Compare of these results with the previous ones allows concluding that the nanoparticles effect no only on the virus as it was observed in virucidal studies, but also act as cytoprotectors. In the case of antiviral action there is no nonlinear dependence of the virus inhibition rate on the nanoparticles concentration. It can be easily explained, as for the described previously effect the small distance between the nanoparticles is needed. However, in the case of antiviral studies the nanoparticles are added to the cell monolayer, which has bigger volume that the viruses sample in virucidal studies. Also in virucidal studies the nanoparticles interact only with the viruses, whereas in antiviral studies the nanoparticles may interact with the cell membrane, may penetrate into the cell and interact with its organelles, viral DNA/RNA, newly produced viruses, etc. Consequently, the case when two or more nanoparticles adsorb on the surface of the virus (or other object) to close one to another is the unlikely one.

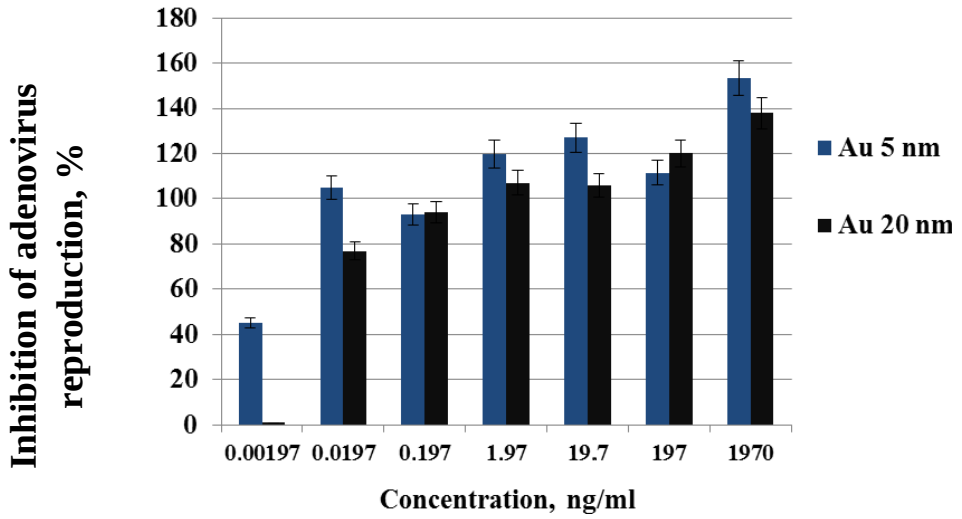

Figure 4. Inhibition of adenovirus reproduction by Au nanoparticles with different sizes and concentrations

Virucidal action of nanoparticles against adenovirus

Obtained TEM images (Fig. 5) revealed interaction between influenza virus H1N1 and nanoparticles similarly to their interaction with adenovirus. It can be seen in Figs. 5, a-b, that 5 nm Au nanoparticles adsorb evenly on the viruses surface (as influenza virus has short spikes located rather close one to another we can see black dots on the virus, which corresponds nanoparticles adsorbed on the spikes) and in 2 hours of incubation the virus is destructed. 20 nm Au nanoparticles (Figs. 5, d-e) also directly interact with a viral lipid membrane, causing disruption of lipid membrane in some viral particles in a sample, compared to control samples (Fig. 5, c). Treated with 20 nm Au nanoparticles viruses have an irregular shape, rupture of the lipid membrane and capsid, incorporation of nanoparticles in the viral membrane.

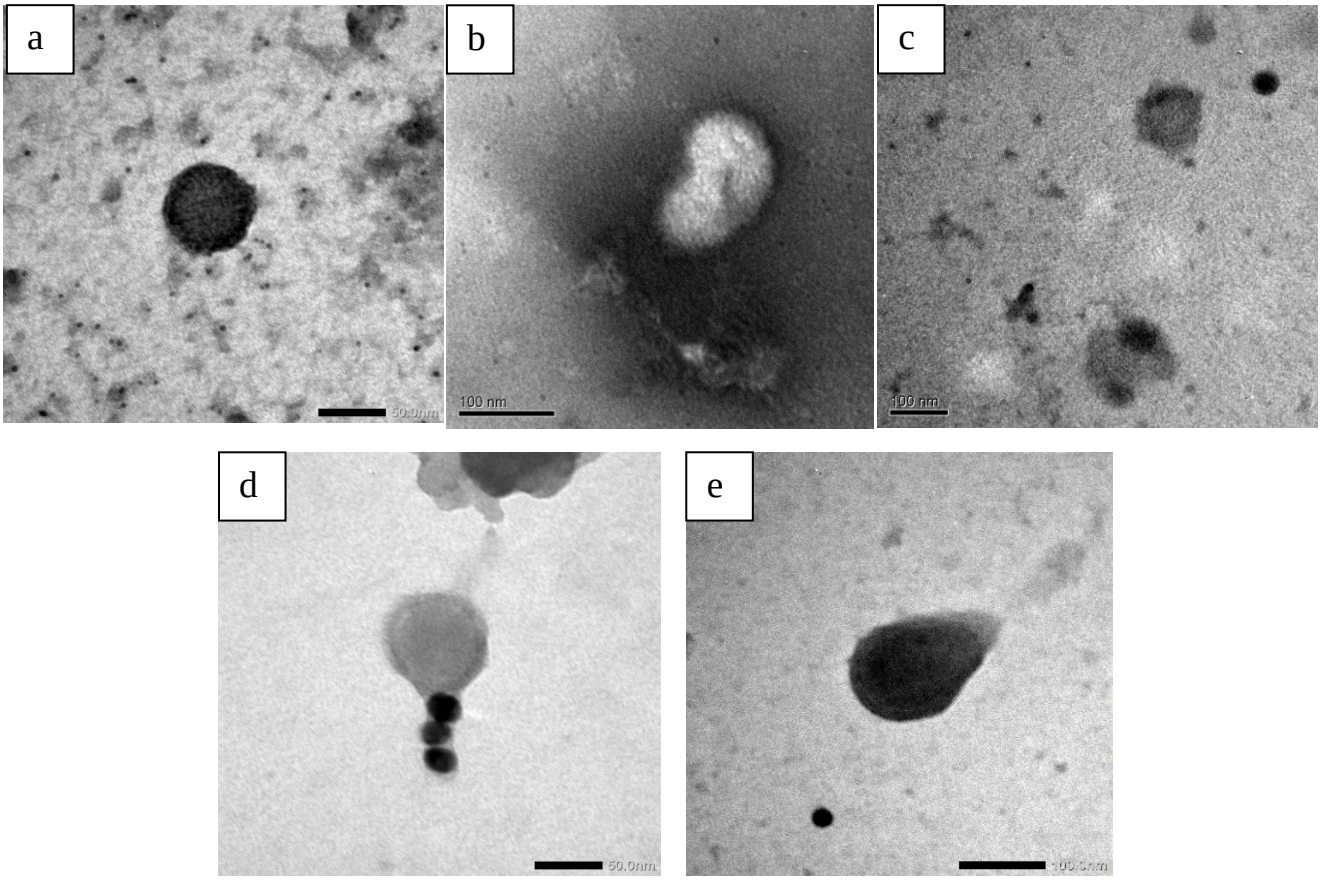

Figure 5. TEM images of influenza A virus H1N1: a – in 1 hour of incubation with 5 nm gold nanoparticles, b - in 2 hours of incubation with 5 nm gold nanoparticles, c –intact virus, d – in 1 hour of incubation with 20 nm gold nanoparticles, e – in 2 hours of incubation with 20 nm gold nanoparticles.

Results of study of influenza virus inhibition at virucidal action of nanoparticles are shown in Fig. 6. It can be seen that there is high infection inhibition rate for 5 and 20 nm Au nanoparticles up to 78% for highest nanoparticles concentration in experiments (1.97 μg/ml). Also higher inhibition rate for rather high concentrations of nanoparticles is observed for 5 nm Au nanoparticles compared to bigger 20 nm Au nanoparticles. However in this case (compared to experiments with adenovirus) we do not observe the nonlinear dependence of inhibition rate on the nanoparticles concentration. This may be due to the bigger size of influenza virus compared to adenovirus and due to the bigger amount of spikes where nanoparticles can adsorb.

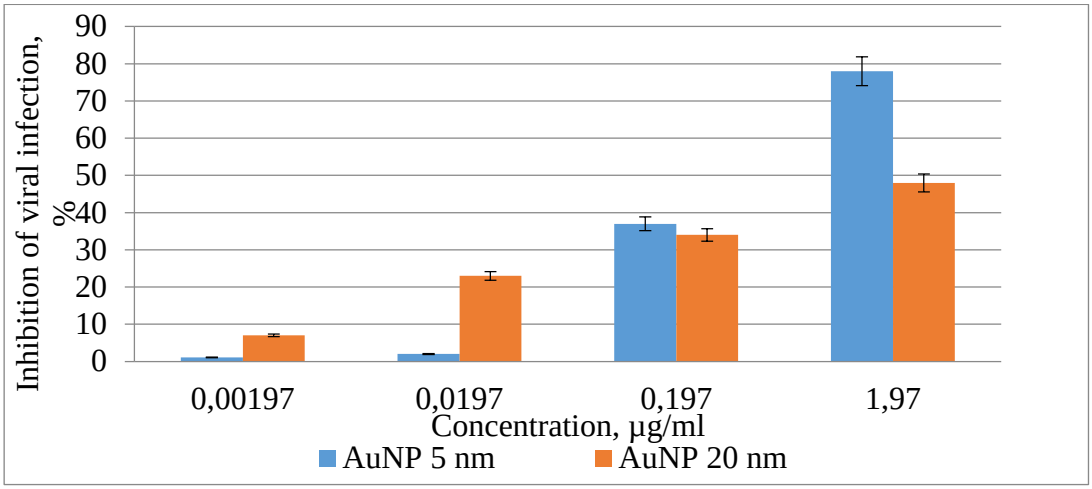

Figure 6. Virucidal activity of Au nanoparticles (diameter 5 nm and 20 nm)

Antiviral activity of Au nanoparticles against influenza virus H1N1

Based on previous cytotoxicity study, the range concentration 1.97 – 0.00197 µg/ml for Au nanoparticles with 10-fold dilutions were chosen.

To investigate the antiviral activity of Au nanoparticles, MDCK cells were firstly infected with 8 $TCID_{50}$/ml of influenza A virus H1N1 and then infected cells were treated with mentioned above concentrations of Au nanoparticles diluted in maintenance medium. Assessment of infection inhibition rate was carried out when significant CPE was observed in the control sample with the intact virus, usually it was in 48 hours after infection. Results showed small dose-response infection inhibition rate (Fig. 7) for 5 nm Au nanoparticles: maximum was observed for 1.97 µg/ml and was equal to 11%. Slightly bigger infection inhibition effect was observed for 20 nm Au nanoparticles: up to 30% for 1.97 µg/ml concentration. The results clearly indicate a small inhibition effect of Au nanoparticles on H1N1 replication in already infected cells with greater efficiency of 20 nm Au nanoparticles.

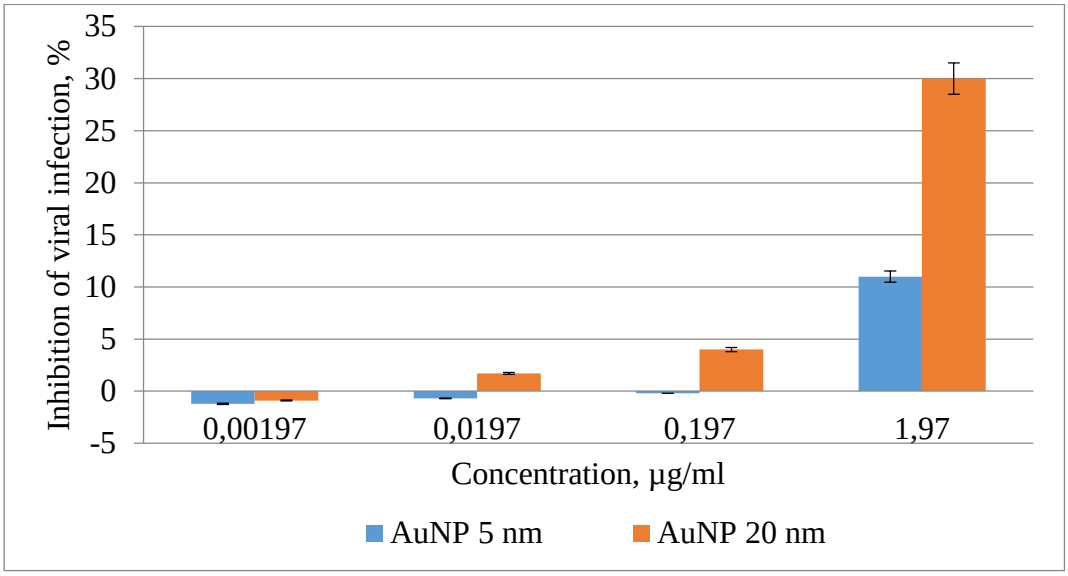

Figure 7. Antiviral activity of Au nanoparticles against influenza virus

In order to define if Au nanoparticles have impact on infectivity of the newly synthesized virus, MDCK cells were infected with 8 $TCID_{50}$/ml of influenza A virus H1N1 and then infected cells were treated with Au nanoparticles. After development of significant CPE in samples of the virus control, usually 48 hours after infection, samples of *de novo* synthesized virus were collected and immediately titrated to identify $TCID_{50}$/ml. $TCID_{50}$/ml was calculated with classic Spearman-Karber formula and conventional microscopy technique was used for detection of CPE. The results (Fig. 8) indicate that the treatment with Au nanoparticles during the virus replication leaded to the dose-dependent decrease of viral infectivity of *de novo* synthesized influenza A virus.

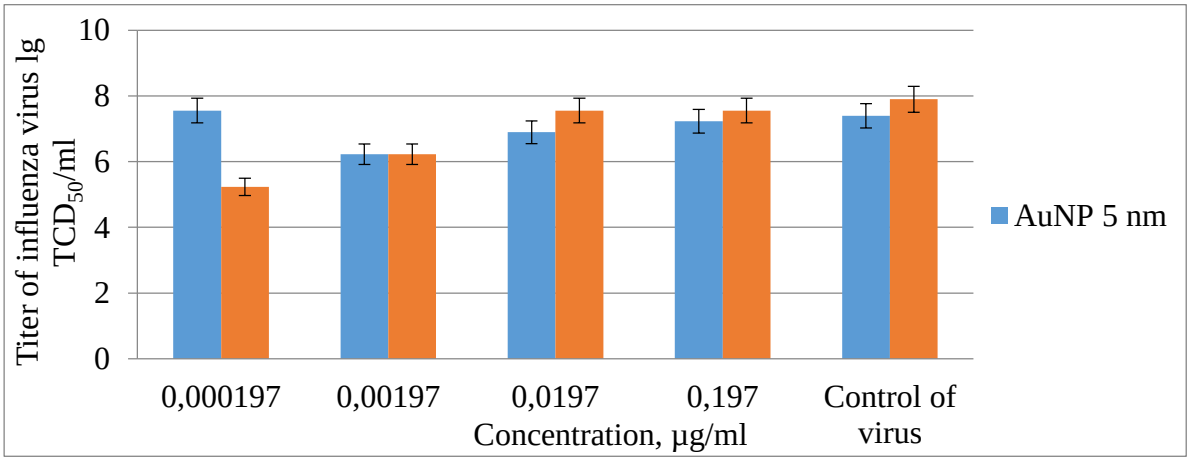

Figure 8. The titer of *de novo* synthesized influenza virus after interaction with Au nanoparticle (5 nm and 20 nm in diameter)

Particularly, the most effective concentration of 5 nm Au nanoparticles (Fig. 8) was 0.00197 μg/ml and caused the decrease of the virus titer for more than ~145 fold. The significant decrease of viral infectivity for more than ~ 450 fold was caused by 20 nm Au nanoparticles (Fig. 8) in concentration 0.197 ng/ml. The result stated a prolonged antiviral effect of both Au nanoparticles on newly synthesized virus.

Discussion

Comparison of experimental results for two types of viruses gives similarity in interaction of Au nanoparticles with two types of viruses. Namely, these similar points are: nanoparticles adsorption on the viral spikes, viral destruction in two hours of incubation with 5 nm Au nanoparticles, viral reshape in two hours of incubation with 20 nm Au nanoparticles, brighter virucidal action of smaller nanoparticles. Taking into account that similar results were previously obtained by our and other research groups [8] and were expected to obtain in this work, we may conclude that physical interaction between the extracellular virus and the nanoparticles indeed may lie at the origin of the virucidal action of nanoparticles. Also obtained results open the directions for management of nanoparticles virucidal activity by changing their size and concentration.

Concerning antiviral activity of Au nanoparticles, they differ for two studied viruses. At the present stage of study it is hard to reveal the reason for such differences and this question needs for further studies. However, based on results for adenovirus we may suppose that Au nanoparticles may be potential antivirals against adenoviruses and that their antiviral effectivity may be changed by changing the nanoparticles parameters.

For authors idea of physical reason for viral destruction is seemed to be attractive one. This mechanism of action may be the base of new type of antivirals that act due to mainly physical

interactions. It is well known that the viruses can easily adapt to drugs action (biochemical action) due to mutations. We hope that physical action on the viruses may open the way for overcoming the problem of acquired drug resistance.

Conclusions

1. Virucidal and antiviral actions were observed experimentally for both types of nanoparticles against both viruses.
2. Intensity of virucidal action depends non-monotonically on the nanoparticles concentration for adenovirus.
3. Virucidal action of smaller nanoparticles of 5 nm in diameter is mostly higher compared to bigger nanoparticles for both viruses.
4. Both adenovirus and influenza A virus H1N1 are destructed after 5 nm nanoparticles adsorption on their surface, and change their shape after 20 nm nanoparticles adsorption on their surface.
5. The model of physical adsorption of nanoparticles on the virus surface due to near-field interaction proposed in previous works may explain observed results on virucidal action of nanoparticles.
6. Antiviral activity of nanoparticles differs for two types of the viruses: Au nanoparticles are more effective against adenoviruses in Hep-2 cells compared to influenza A viruses H1N1 in MDBK cells.

Outlook

Au nanoparticles of 5 nm in diameter stabilized with citrate potentially may be universal virucidal agents and may destroy various viruses after adsorption on their surface. As observed effects may be explained by the proposed physical mechanism of action, the authors suppose that such virucidals may be the way for overcoming the problem of the viral drug resistance.

Antiviral properties of Au nanoparticles differ for different viruses and cells, but may be changed by choosing their size and concentration. Based on other studies of the nanoparticles it may be supposed that antiviral properties of nanoparticles may be improved by changing the nanoparticles material, stabilizer, shape, etc. Study of the possibilities of control of the nanoparticles antiviral properties connected with development of intracellular nanoparticles interactions are the tasks for further studies.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Most experimental results abtained and analysed during this study are included in this published article.

Code availability

Not applicable.

Authors' contributions

VLys, AD, VLoz and SZ designed the study; MC, KN, YP, OP and SZ conducted experiments on study virucidal and antiviral properties of nanoparticles; IM and NV developed the nanoparticles synthesis protocols and synthesized the nanoparticles; ML characterized the nanoparticles; NR and SZ analyzed experimental results and interpreted experimental data. All the authors drafted the manuscript.

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