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The effect of UV-light on the biological activity of silver nanoparticles

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UV radiation is of considerable interest for its negative effects on cells. At present, much attention is paid to the prospects of using nano materials with unique properties, one of which is photocatalytic ability. One of the most widely used nanoparticles whose properties have not yet been sufficiently investigated is silver. Here, silver nanoparticles were used as a test substance on recombinant strains of *E. coli* K12 MG1655, exposed to different times of UV light (from 0 to 30 minutes). Our results showed an increase in toxic effects in relation to the test strain on the time of UV irradiation. When exposed from 1 to 5 minutes, there was an increase in bactericidal action compared with samples that were not irradiated. At 10, 15 and 20 minutes of UV exposure, the level of luminescence of the test strain with silver nanoparticles differed slightly from the control sample (0 min UV). The difference was 14.8% in a concentration range of 0.0006–0.000025 M at UV exposure times of 25 and 30 minutes, with a decrease of toxic effect, in comparison with the samples before irradiation. At the same time, there was a decrease in bactericidal action of the substance at lower concentrations. The difference was greater than 60% of the control level. The data were confirmed by the results of evaluation of bactericidal action.

Keywords: Ultraviolet light, phototoxicity, bioluminescence, silver nanoparticles, E. coli K12 MG1655.

INTRODUCTION

Ultraviolet (UV) radiation is part of solar radiation reaching the Earth's surface. The decrease in ozone content in the stratosphere as a result of anthropogenic pollution has led to an increase in the presence of radiation of this region of the spectrum in the biosphere (Petrova, 2015). Recently, the scope of medical use of UV radiation has been expanding; in particular, it is used to accelerate the healing of wounds and trophic skin lesions. Extracorporeal UV irradiation of blood, which has been used successfully in the complex treatment of pneumonia, sepsis and other surgical infections, as well as for the correction of dyslipidaemia and rheological disorders, is also widely used (Sidorova, 2005; Baitakov, 2011). At the same time, in addition to positive applications, we are aware of the harmful effects of UV radiation that contribute to the development of skin cancer, phototoxicity, photoallergy, keratoconjunctivitis, etc. (Narayanan, 2010; Moren, 2010; Majdi, 2014; Overmans, 2018). Thus, a comprehensive study of the negative effects of UV radiation on cells, as well as the study of ways to modify it and possible protection from it, is of significant interest.

In the era of nanotechnology, much attention has been paid to the prospect of using nano scale

materials to create medicines and cosmetics to tackle this problem, due to a number of unique properties of nanoparticles, one of which is photocatalytic ability (Sudha, 2013; Roy, 2015). Photocatalytic activity is the capture of electrons of nearby molecules under the action of light. If nanoparticles are in aqueous solution, this process leads to the formation of reactive oxygen species, mainly hydroxyl radicals (Mamonova, 2015). These properties determine the antiseptic properties of nanoparticles, and can also be used for directional modification of the surface of nanoparticles or molecules on their surface. Such effects are described for a small number of nanoparticles, including Zn O and TiO2. Due to these properties, these nanoparticles are actively used in various industries, especially cosmetic and food.

However, the possible photo activating effects of nanoparticles may include functionalphysiological, destructive or modifying natures (Sohrabi, 2008; Kansal, 2008; Wang, 2013). Recently, there have been an increasing number of works, which show that photocatalytic activity can have toxic effects on cells and tissues, causing the appearance of new properties. In this case, the description exists for a very limited number of nanoparticles (Li, 2011; Ilan, 2012).

In this regard, a very important area of study are the mechanisms of biological activity of nanoparticles at the direction of change in their photocatalytic, physical and chemical properties, the study of the mechanisms of action of biological cells at different levels of organization and prediction of regularities of interaction of new nanoscale materials with biological systems.

One of the most widely used nanoparticles at present is silver nanoparticles. Silver has a pronounced antimicrobial effect, stronger than penicillin and other antibiotics (Zhang, 2009). At the same time, the main mechanism of antibacterial action of silver is universal, since damages the cell structures of the bacterium (Tran, 2013). This property has led to wide application in medical and cosmetic industries. It was also found that action is also due to the shape of nanoparticles. The antibacterial effect of silver nanoparticles on E. coli strains, depending on the shape of the nanoparticle was analysed and it was shown that silver nanoparticles with a truncated triangular shape have a stronger bactericidal effect, compared to spherical and rodshaped (Satyavani, 2011).

As the increase of knowledge of new properties has provided an expansion of the field,

not only are more in-depth studies of the mechanisms of action of the previously studied photoactive nanoparticles required, but also obtaining data for new nanomaterials.

MATERIALS AND METHODS

2.1 Physical and chemical characteristics of silver nanoparticles

Silver nanoparticles obtained by chemical method were the substance under study. According to the data stated by the manufacturer, the size of the nano-preparation was 70 nm. The chemical and phase composition of 99.99% was metallic silver, adsorbed gases up to 0.01 % - CH₄, CO₂, Ar, N₂. The specific surface area was $6.5 \text{ m}^2/\text{g}$.

To conduct a study on the measurement of the zeta-potential and the size of nanoparticle aggregates by the method of dynamic light scattering on analytical scales, nanoparticles were prepared and suspended in distilled water in an ultrasonic disperser UZDN-2T (Sapfir, Russia), under the conditions of f-35 kHz, N-300 W, a-10 µa, for 30 min. The suspensions were kept at room temperature for 10 minutes to stabilize the nanoparticles in the solution, after which the zetapotential and the average hydrodynamic particle size were measured on a nanoparticle analyser (Fotokor, Russia). The particle size distribution, average particle radius and zeta-potential were determined.

To visualize nanoparticles, we used an atomic force microscope; Certus Light (Nanoscan technology, Russia) equipped with NSG 10 cantilevers with a beam stiffness of 37.6 N/m and a probe radius of < 10 nm (Tips Nano, Estonia). Scanning was performed in the air in the mode of intermittent contact.

Preparation of the preparation of silver nanoparticles was carried out in isotonic solution on an ultrasonic disperser (f-35 kHz, N-300 W, A-10 µa) for 30 minutes.

2.2 Preparation of strain

We used a recombinant strain of *E. coli* K12 MG1655 (pXen7), obtained by transformation of the host strain with a hybrid plasmid pUC18 with a built-in EcoRI-DNA fragment with a size of about 7000 bp, containing the structural genes of the bioluminescence soil microorganism *Photorhabdus luminescens* ZM1 (Manuhov, 1996). Previously, this strain was used in the study of carbon nanotube toxicity (Chesnokova, 2006). The strains carried constitutive pACXen promoters that allowed the evaluation of the toxic effects of the studied factors by the nature of bioluminescence.

2.3 Bioluminescence method

The bacterial strain was cultured during the day on LB-agar (according to Muller) at 37 °C, with 100 μ g/ml of ampicillin added to the medium, which is a selective factor.

Then the cells were suspended in 0.9 % NaCl solution to an optical density of 0.5 at 450 nm, while measurements were performed in transparent plastic plates on the photometer StatFax 303+ ("Awareness", USA).

To study the effects of silver nanoparticles exposed to radiation, dilutions of the analysed substances in the final concentration from 1M to $2.4*10^{-4}$ M, in the volume of 50 µl, were prepared in white opaque plates of the tablet. Next, UV irradiation was carried out at time intervals 0, 1, 2, 5, 10, 15, 20, 25 and 30 minutes. For this purpose, the system of UV-dosed irradiation "Bio-Link" (Viber) with a wavelength of 254 nm was used. The radiation source was 5 lamps of 8 watts

Then 50 μ I of the prepared suspension of microorganisms and 100 ml LB-broth were added to the experimental microplane. In the control wells, instead of the test substance, 50 μ I of deionized water was added

The microplane was then placed in the measurement unit of the luminometer PROF Infinite 200 (TECAN, Austria). The measurement was carried out in the kinetic mode for 120 minutes. The experiments were performed with at least three repetitions.

The data were analysed using the software provided with the instrument. Quantification of the bioluminescence inhibition index (I) toxicity was calculated by the algorithm (1).

$$I_{normal} = \frac{I_{120}^{experience} \times I_0^{control}}{I_0^{experience} \times I_{120}^{control'}},$$

Where $I_{120}^{experience}$ – index of the illumination in the experimental sample in the 120 minute of the experiment;

(1)

 $I_0^{experience}$ - Index of the illumination in the experimental sample in the 0 minute of the experiment;

 $I_{120}^{control}$ – Luminescence index in the control sample at 120 minutes of the experiment;

 $I_0^{conrtol}$ – Luminescence index in the control sample at 0 minutes of the experiment

2.4 Method of evaluation of bactericidal action

For the evaluation of bactericidal activity, serial dilutions were carried out and then the density was measured to determine the dynamics of destruction of microorganisms in the environment with the toxicant. For this purpose, E. coli K12 MG1655 (pXen7) was cultured for 24 hours on LB-agar (Muller) at 37 °C, with 100 µg/ml ampicillin. Then the cells were suspended in a physiological solution (0.9% NaCl) to an optical density of 0.5 at 450 nm, the measurements were performed in transparent plastic wells on the photometer StatFax 303+ ("Awareness", USA).

Then, the dilutions of the studied substance were prepared and UV irradiation was carried out according to the method described above. Breeding of silver nanoparticles was carried out in white opaque plate wells in the volume of 50 µl, 50 µl of water was added to the control wells, then 50 µl of bacterial suspension and 100 µl of LBbroth were added to all wells. After that, optical density was measured at 630 nm in plastic transparent holes on a StatFax 303+ photometer ("Awareness", USA). Next, the plate was incubated in a thermostat at 37 °C for 120 minutes, after which the optical density was measured every 60 minutes.

2.5 Statistical processing

All tests were carried out in 2-3 independent experiments with 3-4 repetitions per sample and subjected to statistical processing. The data obtained was processed using a variety of statistical methods with Statistica V10 (StatSoft Inc., USA).

In processing the results, the arithmetic mean (x) of the two controls and repeats of the experiment, the standard deviation (Sx) and the mean error (S) were calculated.

RESULTS

3.1 Physical and chemical certification of silver nanoparticles

According to the data of dynamic light scattering, the preparation of silver nanoparticles in aqueous solution formed 3 types of fractions, differing in size of agglomerates (Table 1).

It was found that the majority of Ag nanoparticles (58%) were dispersed in aqueous suspension up to the size of 86 ± 6.5 nm, while 30% formed complexes with diameters of 324 ± 43.3 nm. About 12% formed large conglomerates

with a diameter of > 1500 nm. In turn, the zeta potential of the silver nano-preparation was characterized by a negative value, not exceeding 15.5 mV.

Table 1: Main physical characteristics ofnanoparticles preparations

Sample	Fractions, %	Size, nm*	Zeta potential , mV		
	58	86 ± 6.5			
Ag	30	324 ± 43.3	-15.53 ± 0.22		
	12	> 1500			

* dynamic light scattering data

These studies were carried out to evaluate the real parameters of silver nanoparticles in aqueous suspension with subsequent evaluation of antibacterial effects on the cell.

3.2 The results of the bioluminescent test

Before assessing the effect of UV irradiation on the biological activity of silver nanoparticles, a study was conducted showing their initial properties. Thus, the analysis of bioluminescence (A)

> 1.2 100000 1.1 10000 1 **Bioluminescence**, **RLU** Normalized bioluminescence, RLU 0.9 1000 0.8 100 0.7 0.6 10 0.5 1 0.4 0 60 120 0.3 Time, min 0.2 Control 0.1 → 0,0001 M → 0,0003M **★** 0,0006 M **●** 0,0012 M 0 +-0,0025 M ---0,005 M 0 M 0,0003M 0,0025 0,02 M Μ -0,01 M → 0,02 M **Concentration**, M <u>→</u>0,1 M

Figure 1 – a) The kinetics of the luminescence of a strain of E. coli K12 MG1655 pACXen when exposed to silver nanoparticles; b) the dependence of the normalized bioluminescent strains of *E. coli* K12 MG1655 pACXen from the concentration of the NPs Ag

demonstrated effect the toxic of silver nanoparticles on E. coli K12 MG1655 pACXen (Fig. 1A). High concentrations of the active substance provided inhibition of luminosity from the first minutes of contact (0.1-0.0025 M), and throughout the time period. This confirmed the high toxicity of this concentration range. Further dilutions of the test substance showed no toxic effect and corresponded to the background glow of the test strain, and after 15 minutes, the induction of strain glow occurred, which may indicate the absence of a negative effect.

The dose-effect results showed that the most toxic concentrations, characterized by EC50, were 0.005 M (Fig 1B). The maximum suppression of luminosity was fixed at 0.1 M of silver nanoparticles.

At the second stage of this study, the effect of silver nanoparticles was evaluated at different times of UV light irradiation (1, 2, 5, 10, 15, 20, 25 and 30 minutes) for biological activity in relation to the test strain (Fig 2).

(B)



Concentration, M

Figure 2 : The dependence of the normalized luminescence index on the concentration of molecules of silver nanoparticles at different times of UV light irradiation.

The analysis of the results showed an increase in toxic effects relative to the test strain on the time of UV irradiation. Thus, at 1-5 minutes of UV irradiation, an increase in bactericidal action was observed in comparison with samples that were not exposed to this physical factor. It should be noted that high concentrations of the substance (0.1 M to 0.005 M) before and after irradiation were equally lethal for microorganisms, but before irradiation, a decrease in concentration resulted in a decrease in toxic effect, the exposure time of 1, 2 and 5 minutes showed approximately the same negative effect. The difference in the level of light was more than 50%.

At 10, 15 and 20 minutes of irradiation of silver nanoparticles, the level of the test strain luminescence differed slightly from the control sample (0 min UV). The difference was 14.8% in a concentration range of from about 0.0006–0.000025 M. However, at higher exposure time, high concentration of the substance had reduced toxic effects against the test strain. Thus, the difference in the level of luminescence when compared with samples without UV treatment was from 42.8% at a concentration of 0.1 M to 63.6% at a concentration of 0.05 M.

The exposure time of UV light for 25 and 30 minutes showed a decrease in toxic effect, in comparison with samples before irradiation. At the same time, high concentrations of silver nanoparticles (0.1 M) led to differences in the level of luminescence of 23%, then with a

decrease in the concentration, there was a decrease in the bactericidal effect of the substance under study, and the difference was already more than 60% of the control level.

The results of the evaluation of survival of the test strain under the action of the silver nanoparticles

In order to compare the results of exposure of the test substance on the luminosity of the *E. coli* K12 MG1655 pACXen strains with their survival, we studied the effect of silver nanoparticles with different UV light exposure times on the growth of the microorganisms.

Dates for assessment of survival data confirmed the bioluminescent test (Table 2). Thus, the number of cells in contact with silver without irradiation varied from 11.2% at high concentrations to 90.4% ($P \le 0.05$) at a minimum concentration, in comparison with the control. It should be noted that the EC50 value corresponded to 0.005 M (53,2%, $P \le 0.05$).

Small doses of irradiation of silver nanoparticles (1, 2 and 5 minutes) inhibited the viability of microbial cells throughout the incubation period. When detecting the results, it was found that at the maximum concentration of silver nanoparticles, the number of surviving cells was 0-4% ($P \le 0.05$), and at the minimum, 36%. The number of surviving cells increased with increasing exposure time. The maximum values were reached at 25-30 minutes of irradiation.

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Concentration,	ion, Time of exposure, min										
M	0	1	2	5	10	15	20	25	30		
0,00005	90,4±3,4*	35,4±2,2	36,3±2,01	35,5±1,2	89,5±0,12	93,0±0,85	89,5±0,30	+37,3±0,22	+45,5±0,13		
0,0001	89,5±2,8	35,7±1,4	34,8±1,25	35,9±2,5	86,5±0,87	87,7±0,3	81,5±0,24	+16,9±0,66	+32,7±0,77		
0,0003	88,6±0,5	34,5±0,09	34,9±0,3	34,7±0,01	84,2±0,05	85,0±0,51	75,3±1,08	97,4±0,10*	106,1±0,03		
0,0006	83,3±1,2	32,7±1,0	27,1±1,1	27,4±1,8	86,8±0,98	79,7±0,56	73,1±0,45	84,2±0,72	93,8±0,81		
0,0012	75,3±0,8	30,1±0,13	25,2±1,02	25,3±1,1	73,4±0,40	69,1±0,12	69,9±0,19	81,5±0,37	89,8±0,9		
0,0025	62,0±1,6	24,8±0,03*	23,9±0,15	25,5±0,58	63,8±0,27	60,2±0,36	67,3±0,63	77,1±0,12	82,4±0,8		
0,005	53,2±0,2*	17,7±0,4	19,5±0,89	20,1±1,02	52,3±0,02	55,7±0,18	62,0±1,02	65,2±0,38	70,1±0,65		
0,01	46,1±0,08	14,2±0,26	15,9±0,12	17,0±0,39	45,2±0,12	40,9±0,28	53,2±0,33	57,6±0,11	62,3±0,25		
0,02	38,9±1,07	6,7±0,7	13,3±0,32	16,4±0,04	37,2±0,02	35,0±0,23	46,1±0,01	48,8±0,2	52,0±0,40		
0,05	28,3±0,62	0	4,3±0,110*	3,2±0,7	27,0±1,36	29,2±0,03	35,9±0,43	31,0±0,05	42,7±1,07		
0,1	11,2±0,02	0	1,8±0,20	3,9±0,04*	13,3±0,02	15,5±0,32	24,4±0,31	25,7±0,37	33,1±0,29		
Control	100	100	100	100	100	100	100	100	100		

Table 2 : Percentage of surviving cells

Note: *P ≤ 0.05

In comparison with the control, this percentage was increased by 37 and 45.5%, by 25 and 30 minutes, respectively. It should be noted that the increase in cells was observed in the entire range of concentrations, in comparison with other minutes of irradiation.

Thus, as a result of the study, we found that time of UV dose determined the increase or decrease of the bactericidal action of the silver nanoparticles.

DISCUSSION

researchers, in explaining Some the bactericidal effect of silver on the cell, attached particular importance to intracellular physical and chemical processes. In particular, the oxidation of the protoplasm of bacteria and its destruction by oxygen dissolved in water, with silver acting as a catalyst for this process (Chahine, 2011; Díaz-Visurraga, 2011). During this process, silver ions are absorbed by the cell membrane, which performs a protective function. Further, the particles penetrate into the cell, inhibiting the enzymes of the respiratory chain, which separates the processes of respiration and oxidative phosphorylation in microbial cells, resulting in cell death (i.e., a bactericidal effect) (Soni, 2004; Hmel, 2009; Arora, 2012). It is assumed that one of the reasons for the broad antimicrobial action of silver ions was the interaction with ribosomes and subsequent inhibition of the expression of enzymes and proteins necessary for the production of ATP (Yamanaka, 2005). Summing up all possible variants of actions, it is possible to observe a pronounced destructive oxidative action of silver.

The mechanism of phototoxic action of nanoscale silver with a small UV exposure time may be as follows. Activation of metal under UV light leads to the formation of electron-hole pairs (eh+). This causes the destruction of water molecules present in the surrounding cell environment to OH and H+. Dissolved oxygen molecules become converted to superoxide anion radicals (O₂). In the interaction of O₂ with H+ HO₂ radicals are formed and then peroxidation. They are then able to react with H+ to form hydrogen peroxide molecules H₂O₂, toxic to bacterial cells.

The increase in cell viability with longer exposure time to light can be explained by several possibilities. From the physical point of view, as the exposure time increases, it leads to the aggregation of nanoparticles into larger complexes, in which there is an accelerated redistribution of electrons, which reduces the possibility of electronic holes. It also does not exclude the possibility of the effects of absorption and dispersion of UV radiation. In addition, a small scattering of incident radiation occurs on the particles themselves as a catalyst. As a result, there would be no hyperproduction of superoxide anions, which reduces the toxic effect of the substance.

From a biological point of view, the cells under stress are characterized by the restructuring of metabolic and physiological processes that lead to the adaptation of the body to changing conditions.

Currently, a number of nanomaterials and their derivatives are known which are activated by the action of various radiation sources. For example, gold nanoparticles become good catalysts for chemical reactions, and also directly participate in them. In the human body, these nanoparticles can lead to a whole range of responses of the body tissues, for example, to the activation of cells, their death, generation of reactive oxygen species, inflammatory processes in different tissues and organs (Zawrah, 2011).

The most interesting properties are the nanoparticles of zinc oxide and titanium dioxide (Petritskava, 2012). For example, TiO2 nanoparticles have a higher photocatalytic effect than TiO2 microparticles (Bermudez, 2002), forming under the influence of UV radiation active forms of oxygen, hydroxyl radicals, H2O2, etc. This effect of TiO2 has been widely used in the field of environmental engineering for purification of water and air from organic pollutants, for neutralization of industrial waste, for sterilization of various components and surfaces (Park, 2008).

CONCLUSION

This new knowledge will allow us to expand the range of applications, in particular, the use of biologically active surface modifiers, in medical practice or on the problem of interaction of microorganisms to the surface of nanoparticles and substrates. This will lead to advances in the biotechnology of material destruction, and the formation of fundamentally new approaches to increasing the bioavailability of biologically active substances.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

DBK designed and performed the experiments and also wrote the manuscript. EAR, VIK, performed the experiments on microorganisms. EVYa performed data analysis. ChSK reviewed the manuscript. All authors read and approved the final version.

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