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NANOCERIA CAN INHIBIT THE REPRODUCTION OF TRANSMISSIBLE GASTROENTERITIS VIRUS: CONSIDERATION FOR USE TO PREVENT AND TREAT CORONAVIRUS DISEASE

S. Rybalko[1], O. Demchenko[2], D. Starosyla[1], O. Deriabin[1], L. Rudenko[1], O. Shcherbakov[2], L. Babenko[2], R. Bubnov[2], M. Spivak[2]

[1]SI "Gromashevsky Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine", 5 M. Amosova Str., Kyiv, 03038, Ukraine
[2]Zabolotny Institute of Microbiology and Virology, NAS of Ukraine, 154 Acad. Zabolotny Str., Kyiv, 03143, Ukraine
e-mail: n.spivak@ukr.net

Nanoceria (cerium dioxide nanoparticles, CeO_2) has a broad range of biological properties including antiviral activity. The hypothesis was that nanoceria can efficacy against coronavirus (coronavirus of porcine transmissible gastroenteritis) and potentially can target SARS-CoV-2. Transmissible gastroenteritis coronavirus (TGEV) is the etiologic agent of porcine transmissible gastroenteritis (PTG), a highly contagious pig intestinal disease. The ***aim*** *of the study was to determine the antiviral activity of CeO_2 nanoparticles on the model of porcine coronavirus – TGEV.* ***Methods.*** *We used a highly pathogenic virus strain $D_{52\text{-}5}$ (BRE_{79}), of TGEV. We evaluated antiviral activity of CeO_2 nanoparticles on the experimental model of porcine coronavirus (transmissible gastroenteritis virus) in transplantable line of porcine embryonic kidney cells (PEK) culture.* ***Results****. The criterion for evaluating the inhibitory activity of antiviral drugs in different in vitro systems is the selectivity index (SI) and the reduction of infectious titer by 1.5–2.0 lgTCD_{50}. Nanoceria effectively inhibited the reproduction of porcine coronavirus with SI index of 83.3.*

Keywords: coronavirus, antiviral activity, nanoparticles, nanoceria, transmissible gastroenteritis virus, transplantable line of porcine embryonic kidney cells.

Today severe acute respiratory syndrome (COVID-19) is one of the most studied infectious diseases, however, no specific antiviral drug against novel 2019 coronavirus (SARS-CoV2) has not been developed up to the date. The current management for COVID-19 included antiviral treatment and symptomatic pathogenesis-based treatment.

Nanoceria (cerium dioxide nanoparticles, CeO_2) potentially can be considered to fit both approaches to its various antiviral and a broad range of biological properties [1–9], in particular proven as follows: antiviral properties [2, 3]; antioxidant activity and the ability to reduce the development of cytokine storms [4, 5]; prebiotic activity [6].

Recent evidence indicates the similarities between various coronavirus [7] and possible parallels demonstrated are discussed to be used in development potential treatment targets.

Hypothesis. Nanoceria can efficacy against coronavirus (coronavirus of porcine transmissible gastroenteritis) and potentially can target SARS-CoV-2.

Aim of the study was to determine the antiviral activity of CeO_2 nanoparticles on the model of porcine coronavirus – transmissible gastroenteritis virus (TGEV).

Materials and methods

Virus strain and cell lines

TGEV – etiologic agent of porcine transmissible gastroenteritis (PTG), a highly contagious pig intestinal disease. The virus strain: $D_{52\text{-}5}$ (BRE_{79}) is a highly pathogenic virus strain for pigs of all ages at the level of 5 passages in transplantable monolayer of swine testicular cell line (ST). The tropism of virus to the gastrointestinal and respiratory tract was shown. The strain was provided by Dr. Hubert Laude from the Laboratory of Molecular Virology and Immunology of Biotechnology Center of the National Institute for Agronomic Researches, Juas-en-Josas, France.

Cell lines, obtained from the cell bank of RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv:

PEK – transplantable line of porcine embryonic kidney cells;

PK – transplantable line of porcine kidney cell;

ST – transplantable line of swine testicular cells;

PT – transplantable line of porcine thyroid cells.

Synthesis of ceria sol

Aqueous ceria sol was synthesized by the following method. 3.73 g of cerium (III) chloride heptahydrate and 2.0 g of citric acid were dissolved in 20 mL of distilled water. Under continuous stirring this solution was added rapidly to aqueous ammonia solution prepared by mixing 10 g of concentrated ammonia (Sigma, USA) and 100 mL of distilled water. The solution was stirred for 5 h, with further boiling aiming at producing 100 mL of 0.1 M ceria sol.

Analysis of ceria sols

Optical absorption spectra were recorded on an OceanOptics QE 65000 spectrometer using a one beam scheme. The radiation sources were a DH 2000 deuterium–halogen lamp and an HPX 2000 xenon lamp. Optical absorption spectra were further used to calculate the bandgap energy E_g of CeO_2 nanoparticles.

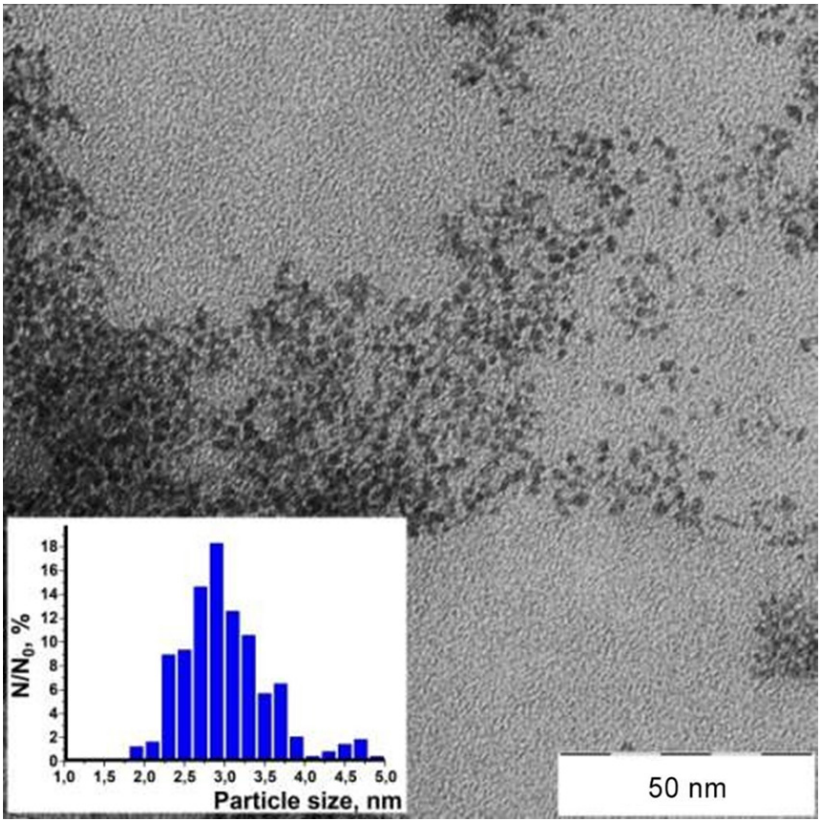
The size of ceria nanoparticles was determined by transmission electron microscopy (TEM) on a Leo 912 AB Omega electron microscope at 100 kV accelerating voltage.

The hydrodynamic diameter of citrate-coated CeO_2 nanoparticles was measured by the dynamic light scattering (DLS) method on a Malvern Zetasizer Nano ZS analyzer. Before measurements, the sol was diluted with distilled water.

Powder X-ray diffraction (XRD) analysis of ceria nanopowders prepared by sols centrifuging was carried out on a Rigaku D/MAX 2500 diffractometer (CuK_a radiation, instrumental broadening 0.10 ± 0.01°2q). The goniometer rotation speed was 2°2q/min. Crystallite size (D) of nanocrystalline ceria was calculated using Scherrer formula where coefficient of anisotropy was set to 1. Line profiles for (111) and (200) reflections were fitted to pseudo-Voigt functions.

Infectivity titration assay:

Titration of infectivity of viral materials on cell cultures was performed by two methods – by Kerber-Ashmarin method of



F i g. 1. Micrograph of CeO_2 nanoparticles in citrate-stabilized sol. Inset: particle size distribution according to TEM data

final dilutions according to cytopathic effect (CPE) the infectivity titer was determined and expressed in tissue cytopathogenic dose per 1 mL (TCD_{50}/mL); by the method of negative colonies (S-sign) under 1.35 % Difco Bacto Agar coating, the infectivity titer was expressed in plaque forming units per ml (PFU/mL). The results were counted after 120 hours of cultivation at 38 °C.

Determination of cytotoxic concentration (CC_{50}) of CeO_2

PEK cell culture was used to determine the CC50 of the preparation. At least ten rows of plate wells with cell culture for each dilution of CeO_2 in the culture medium were used in the experiments. Plates with cell culture were incubated at 37 ^{0}C with 5 % CO_2 for 5 days. Control and test samples were monitored daily to determine whether cerium nanoparticles have cytopathogenic effect (CPE). The degree of CPE was determined by the change in cell morphology (rounding, shrinkage of cells, rejection of degenerated cells from the surface of the wells) in a 4 plus system from + to

" – " – complete absence of cell degeneration;

"+" – no more than 25 % of the cell monolayer is affected (75 % of protection of cell monolayer from preparation);

"++" – no more than 50 % of the cell monolayer is affected; "+++" – no more than 75 % of the cell monolayer is affected; "++++" – complete degeneration of the cell monolayer.

The CC_{50} of the preparation was determined as the highest concentration that did not cause any cell degeneration.

MTT method of cell viability research

This method is based on the functioning of mitochondria dehydrogenase system of intact cells that under normal conditions are able to transform a water-soluble dye [3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide] (MTT) into an insoluble formazan. The reaction product can be quantified by spectrophotometric method. The transformation of MTT into formazan decreases with cell death under the action of toxic substances. A suspension of cells with a density of 5×10^5 μL/mL was cultured in growth medium with 10 % of fetal calf serum containing the test preparation at different concentrations in 96-well plates. Wells with control cells were not treated with the preparation. Each concentration of the preparation was checked in 3–4 repetitions. Plates with cells were kept in a thermostat at 37 ^{0}C in 5 % CO_2 atmosphere for 48 hours.

MTT substrate (Sigma, USA) was dissolved in phosphate buffer saline (PBS) at room temperature to the concentration of 5 mg/mL. The filtered MTT solution in a volume of 25 μL was added into wells containing 100 μL of cell suspension and incubated for 3 hours at 37 ^{0}C in 5 % CO_2 atmosphere. The plates were centrifuged at 1500 rpm for 10 min to precipitate the cells after incubation, and the supernatant was removed. 100 μL of 96 % ethanol was added to the wells with cell precipitate to dissolve crystalline formazan. After 10 min of thorough shaking at 37 ^{0}C, the optical density of the solutions was determined spectrophotometrically at a 540 nm wavelength on a Multiskan FC spectrophotometer for plates (ThermoScientific, US).

The percentage of cell viability inhibition by the action of CeO_2 in different concentrations was determined by the amount of formazan formed in the test samples compared with the control, which was taken as 100 %.

Determination of effective concentration (EC_{50})

The EC_{50} is the minimum concentration of the drug that inhibits the development of virus-specific CPE by 50 %. To determine the EC_{50} test virus at a dose of 100 TCD_{50}/0.1 mL was introduced into cell culture and incubated for 60 min at 37 ^{0}C. After virus adsorption on the cells, the remnants were removed, the cells were washed with nutrient medium, and then CeO_2 nanoparticles in different concentrations in the growth medium (RPMI-1640 + 2 % of fetal calf serum) were added to the cells. The absence of CPE in the wells with treated cells and the present of CPE in the control, as well as the reduction of infectious titer in the wells with treated cells in comparison with the control of the virus allowed to establish the EC_{50} of the preparation.

The criterion for drug antiviral activity evaluating in the *in vitro* systems

Cytotoxic concentration (CC_{50}) – the concentration of the drug that contributes to the reduction of cell culture viability by 50 % – was determined in the analysis of CeO_2 nanoparticles cytotoxic action in accordance with the regulatory guidelines for *in vitro* study of antiviral drugs. To determine the antiviral activity of CeO_2 nanoparticles, the effective concentration (EC_{50}) was determined, i.e. the concentration of the test substance at which the level of virus replication in the infected cell culture is inhibited by 50 %.

After determining the indicators of cytotoxic and antiviral activity, the selectivity index (SI) was calculated as the ratio of CC_{50} to EC_{50}. The substances that had SI ≥16 in the *in vitro* system are considered as active and promising for further animal studies.

RNA detection of D_{52} strain of transmissible gastroenteritis virus using reverse transcription polymerase chain reaction (RT-PCR) method

RNA isolation was performed using the "Ribo-Sorb" kit according to the manufacturer's instructions (AmpliSense, RF). The reverse transcription reaction was performed using the "RevertAid H Minus First Strand cDNA Synthesis Kit" according to the manufacturer's instructions (Thermo Fisher Scientific, Lithuania). Gene-specific nucleoprotein oligonucleotide primers of the following sequence were used for PCR: direct Uni_1 (5'-TGCACTGATCAATGTGCTAG-3') and reverse Uni_2 (5'- TGAAAACACTGTGGCACCCTT-3". The amplified fragment size was 309 bp. "100 bpPlus DNA Ladder" (Thermo Fisher Scientific, Lithuania) was used as marker. Biometra T-personal Combi Thermo Cycler was used.

Statistical analysis

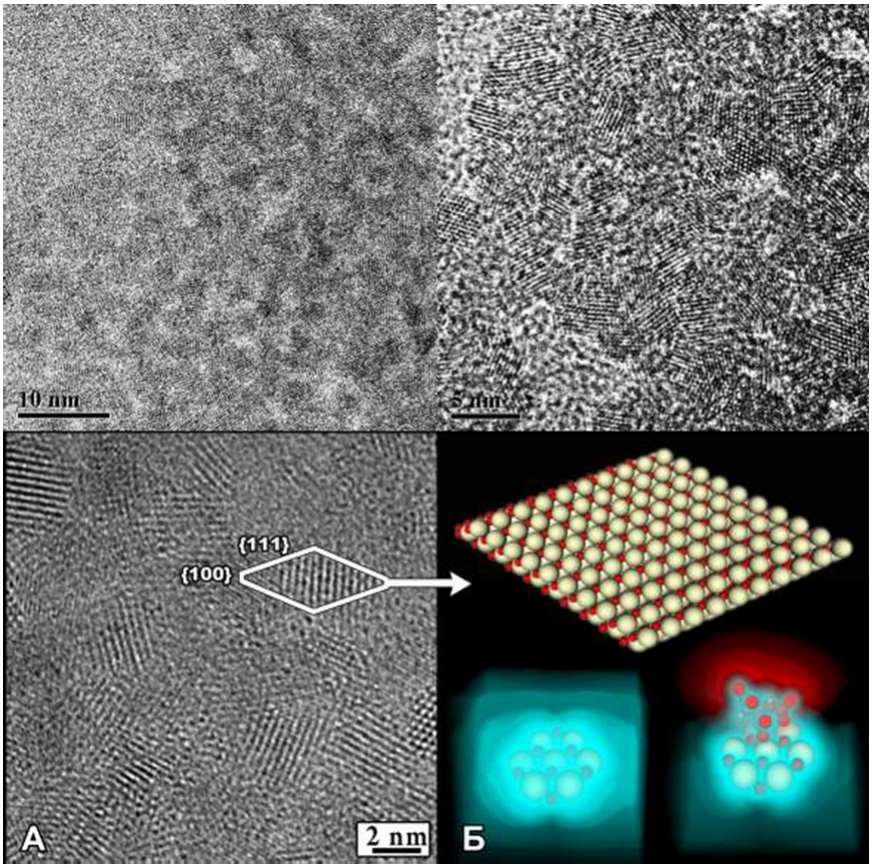
The digital material presented in the report is processed using variational methods of statistics. Statistical evaluation of the significance levels of the differences in the obtained digital indicators was performed using the Student's t-test in Microsoft Excel and Origin Version 8.0 programs. The differences were considered significant at $p<0.05$.

Results

Determination of CeO_2 parameters

XRD has shown that all the samples are single-phased and correspond to cubic CeO_2. Synthesis method proposed allowed us to obtain ceria samples with crystallite size about 3 nm.

According to TEM (see Fig. 1), the sol consists of weakly aggregated CeO_2 particles of nearly isotropic shape 2–5 nm in size. Dynamic light scattering data indicate that mean hydrodynamic diameter of CeO_2 particles is 4.9 nm. This is consistent with the TEM data and is evidence that the presence of a monomolecular (or submonomolecular) layer of citrate ions has no noticeable effect on the particle size. Upon long-term storage (for several months) mean



F i g. 2. HRTEM of CeO_2 nanoparticles in citrate-stabilized sol (top, bottom "А") and Virtual NanoLab model of citrate- stabilized nanoceria (bottom "Б")

hydrodynamic diameter of CeO_2 particles increases up to 40–60 nm due to aggregation processes.

According to UV-Vis spectroscopy data, the bandgap for ceria nanoparticles is 3.5 eV. Upon long-term storage the bandgap of CeO_2 stayed unchanged thus indicating that the particle size in ceria sols is constant.

Characteristic of the porcine coronavirus – transmissible gastroenteritis virus

Porcine coronavirus – TGEV was passaged on different cell lines and characterized by infectious titer. The results are presented in the Table 1.

Table 1

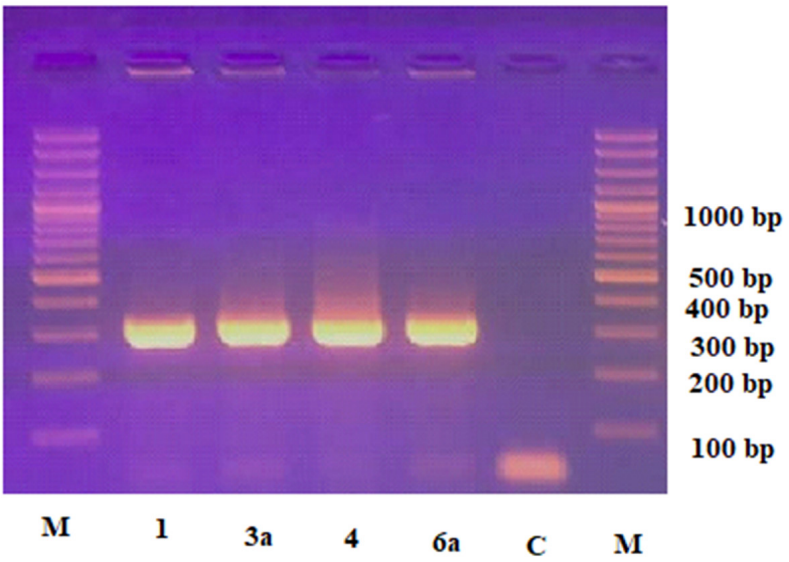
Infectious titer of TGEV in different cell lines

Cell line	Infectious titer, PFU/mL
ST_5	3.9×10^7
PEK_{100}	3.1×10^7
$PK100$	4.3×10^7
$PT100$	1.4×10^8

The virus neutralization reaction was performed in 96 well plates (Costar, USA) by the method of H. Laude using N6926 referent hyperimmune serum of the same author as a positive control [9].

RNA detection of D_{52} strain of transmissible gastroenteritis virus using reverse transcription polymerase chain reaction (RT-PCR) method

RNA isolation was performed using the "Ribo-Sorb" kit according to the manufacturer's instructions (AmpliSense, RF). The reverse transcription reaction was performed using the "RevertAid H Minus First Strand cDNA Synthesis Kit" according to the manufacturer's instructions (Thermo Fisher Scientific, Lithuania). Gene-specific nucleoprotein oligonucleotide primers of the following sequence were used for PCR: direct Uni_1 (5'-TGCACTGATCAATGTGCTAG-3') and reverse Uni_2 (5'-TGAAAACACTG-TGGCACCCTT-3". The amplified fragment size was 309 bp. "100 bpPlus DNA Ladder" (Thermo Fisher Scientific, Lithuania) was used as marker.



F i g. 3. Electrophoretic analysis of amplification products of TGEV with oligonucleotide primers of the following sequence were used for PCR: direct Uni_1 (5'- TGCACTGATCAATGTGCTAG-3') and reverse Uni_2 (5'-TGAAAACACTGTGGCACCCTT-3". The amplified fragment size is 309 bp. M "100 bpPlus DNA Ladder" marker ("ThermoFisherScientific", Lithuania): M – size marker of DNA fragments; # 1 – D_{52-5} strain of TGEV porcine coronavirus from PK cell line; # 3a – D_{52-5} strain of TGEV porcine coronavirus from PEK cell line; # 4 – D_{52-5} strain of TGEV porcine coronavirus from ST cell line; # 6a – D_{52-5} strain of TGEV porcine coronavirus from PT cell line; C – control.

The TGEV virus from different cultures was passaged in PEK cell culture and the analysis of the amplification products was carried out by distributing of DNA fragments in 1.5 % agarose gel.

Determination of cytotoxic action of CeO_2 nanoparticles

PEK cells sensitive to TGEV were used to determine the cytotoxic concentration of CeO_2 nanoparticles. PEK cells were grown in 96-well plates during 24 hours to form monolayer. Growth medium was removed from wells after cultivation, 100 μL of fresh medium with antibiotics was added to each well. CeO_2 nanoparticles were added to the wells with cell monolayer in different concentrations (0.25–2 mM), 3 repetitions for each concentration. After 24 hours of incubation, a visual evaluation of CeO_2 action on the cells was carried out, and MTT solution was added into the wells for colorimetric determination of cell viability. According to visual observations, the highest studied concentration of CeO_2 nanoparticles had toxic effect on the cells. Based on the obtained results, CC_{50} for CeO_2 nanoparticles is 0.5 mM.

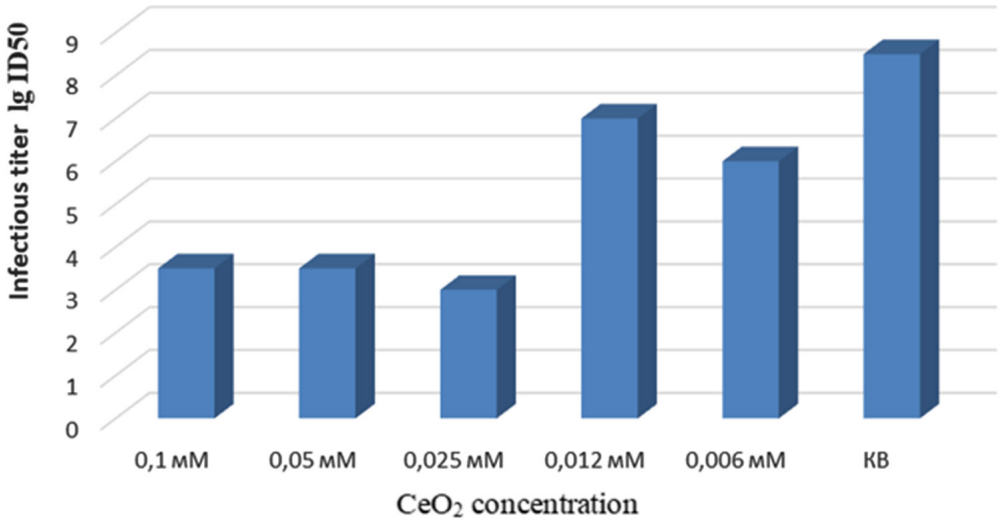
Investigation of antiviral activity of CeO_2 nanoparticles on the model of TGEV porcine coronavirus

The effect of CeO_2 nanoparticles on the reproduction of TGEV coronavirus was studied using the scheme of simultaneous introduction of TGEV and CeO_2 nanoparticles, i.e. during the adsorption of RPMI-1640 medium with 10 % of fetal calf serum on the cells. PEK transplantable line of porcine embryonic kidney cells culture was used to study the anticoronavirus activity of CeO_2 nanoparticles. Cells were grown in plates with RPMI-1640 medium + 10 % of fetal calf serum at 37 ^{0}C in thermostat in the presence of CO_2.

TGEV strain was used in infectious titer of 5.0–8.5 lg ID_{50}. Daily cultures of PEK cells were used to study the antiviral activity of CeO_2 nanoparticles. The culture medium was drained after cultivation, the monolayers of cells were treated with different concentrations of CeO_2 nanoparticles. After 1 hour of contact, the virus was injected at a dose of 100 TCD_{50}. The cultures were incubated in CO_2 atmosphere in thermostat for 2 days. Daily plates monitoring with a microscope was performed. Virus reproduction was detected by the cytopathogenic action of TGEV on PEK cells compared to control cultures where the monolayer was not treated. The cytopathogenic effect of TGEV coronavirus on cells is morphologically manifested in the formation of small cell degeneration. After 3 days, the culture medium was collected from the plate wells, the infectious titer of the virus was determined. The definition of CeO_2 anticoronavirus activity (EC_{50}) in PEK cell culture is presented in Fig. 2.

According to the obtained results, it was found that CeO_2 nanoparticles significantly inhibit the reproduction of TGEV coronavirus.

The criterion for evaluating the inhibitory activity of antiviral drugs in different *in vitro*



F i g. 4. Infectious titer of TGEV in wells with PEK cells monolayer treated with different concentrations of CeO_2 nanoparticles and TGEV in 100 ID_{50}

systems is the selectivity index (SI) and the reduction of infectious titer by 1.5-2.0 lgTCD$_{50}$. The Table 2 summarizes the results of studies on the determination of CC$_{50}$, EC$_{50}$, SI of CeO_2 nanoparticles on the *in vitro* TGEV coronavirus model.

Table 2
CC$_{50}$, EC$_{50}$, and SI indicators of CeO_2 nanoparticles antiviral activity in PEK cells culture on the model of TGEV coronavirus

Preparation	CC**50**	EC**50**	**SI**
CeO_2	0.5 mM	0.006 mM	83.3

Conclusions. The result of current study of antiviral activity of CeO_2 nanoparticles on the experimental model of porcine coronavirus (transmissible gastroenteritis virus) in PEK cell culture showed that CeO_2 nanoparticles effectively inhibited the reproduction of porcine coronavirus with SI index of 83.3.

Future prospects

The concerns regarding emergence of novel drugs are deeply justified therefore, severe requirements should be met before introducing to the clinical practice. During development should be carefully tested to avoid overestimation its activity and prescription to large group of patients. On the other hand the appropriate studies need to be conducted fast, and preserving good level of quality. Crucial is elucidating the mechanism of antiviral activity. The *in vitro* tests with a live culture of SARS-CoV2 should be conducted using the same protocols.

The next step includes *in vivo* tests using mouse models, e.g., as in [11]. Development of nanoceria-based drugs, and composites of nanoceria with effective substances (like fenugreek, curcumin, etc.) and drugs. Further clinical testing including focused interventional studies and randomized clinical trials (RCTs) to study the capability of nanoceria-based drugs to alleviate disease and can be included to protocols for treatment in particular groups and programs of rehabilitation and mild cases need to be conducted in a fast way and providing trustworthy results [12]. Placebo-controlled with a clear focus on individualized use providing extensive individualized profile of the patient [13, 14] including tests of immune system [15].

We suggest preliminary designs according to the testing of hypotheses:

1. Nanoceria-based drugs can prevent development COVID-19 having antiviral properties;

2. Nanoceria-based drugs can be effective at early stages for secondary prevention respiratory distress syndrome having ability to alleviate cytokine storm;

3. Nanoceria-based drugs can be a supportive treatment in severe forms, critically ill, terminal patients having antiviral and antioxidative activity;

4. Nanoceria-based drugs having prebiotic activity can be supportive treatments with probiotics for mild forms of disease and for rehabilitation programs after COVID-19.

The personalized medical approach in a agreement with the overall 3P attitude and strategies for evaluation of drug effects related to individual differences, including comorbid diseases and genetic backgrounds to avoid failed COVID-19 treatment or avoidable adverse events is strongly awaited in the field and should include utilizing pharmacogenetics with a systematic analysis of genetic differences for the safer use of COVID-19 drugs [16, 17]. The 373 pharmacogenes related to all 47 drugs were obtained from the different Genome Databases (PharmGKB https://www.pharmgkb.org/ and DrugBank https://www.drugbank.ca/ Genome Aggregation Database (http://gnomAD.broadinstitute.org/, version: 2.1.1) [17]. Thus, some drugs (ritonavir, daclatasvir, sofosbuvir, ribavirin, interferon alpha-2b, chloroquine, hydroxychloroquine (HCQ) and ceftriaxone had actionable pharmacogenomics (PGx) biomarkers among all ethnic groups, other drugs (ritonavir, daclatasvir, prednisone, dexamethasone, ribavirin, HCQ, ceftriaxone, zinc, interferon beta-1a, remdesivir, levofloxacin, lopinavir, human immunoglobulin G and losartan) showed significantly different pharmacogenomic characteristics in relation to the ethnic origin of the patient.

Finally, it can be implemented into the technological processes of the development specific substances, drugs, and vaccines effective at various stages of pathogenesis of coronavirus disease.

НАНОЦЕРІЙ ГАЛЬМУЄ РОЗМНОЖЕННЯ ВІРУСУ ТРАНСМІСИВНОГО ГАСТРОЕНТЕРИТУ: МІРКУВАННЯ ЩОДО ВИКОРИСТАННЯ ДЛЯ ПРОФІЛАКТИКИ ТА ЛІКУВАННЯ КОРОНАВІРУСНОЇ ХВОРОБИ

С. Рибалко[1], О. Демченко[2], Д. Старосила[1], О. Дерябін[1], Л. Руденко[1], О. Щербаков[1], Л. Бабенко[2], Р. Бубнов[2], М. Співак[2]

[1]ДІ «Інститут епідеміології та інфекційних хвороб ім. Л.В. Громашевського» НАМН України, вул. М. Амосова, 5, Київ, 03038, Україна
[2] Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, вул. Академіка Заболотного, 154, Київ, 03143, Україна

Резюме

Наноцерій (наночастки діоксиду церію, CeO_2) має широкий спектр біологічних властивостей, включаючи противірусну активність. Гіпотеза нашого дослідження полягала в тому, що наноцерій може бути ефективним проти коронавірусу (вірус трансмісивного гастроентериту свиней) і потенційно може мати антивірусну активність проти SARS-CoV-2. Коронавірус трансмісивного гастроентериту (TGEV) є етіологічним збудником трансмісивного гастроентериту свиней (ТГС), дуже заразного кишкового захворювання. **Метою** дослідження було визначення противірусної активності наночасток CeO_2 на моделі коронавірусу свиней TGEV. **Методи.** Ми використовували високопатогенний штам вірусу TGEV D_{52-5} (BRE_{79}). Була визначена противірусна активність наночасток CeO_2 на експериментальній моделі коронавірусу свиней у перещеплюваній культурі клітин нирки ембріона свині (СНЕВ). **Результати**. Критерієм оцінки інгібуючої активності противірусних препаратів у різних системах *in vitro* є індекс селективності (ІС) та зниження інфекційного титру на 1,5–2,0 $lgTCD_{50}$. Наноцерій ефективно пригнічував розмноження TGEV з ІС 83,3.

Ключові слова: коронавірус, противірусна активність, наночастки, наноцерій, вірус трансмісивного гастроентериту, перещеплювана культура клітин нирки ембріона свині.

1. Zholobak NM, Sherbakov OB, Babenko LP. et al. The perspectives of biomedical application of the nanoceria. EPMA Journal. 2014; 5:A136. https://doi.org/10.1186/1878-5085-5-S1-A136
2. Zholobak N, Sherbakov A, Ivanov V, Olevinskaya Z, Spivak N. Antiviral effectivity of ceria colloid solutions. Twenty-Fourth International Conference on Antiviral Research. Sofia, Bulgaria. 2011; 90 (2):A67
3. Zholobak NM, Mironenko AP, Shcherbakov AB, Shydlovska OA, Spivak MY, Radchenko LV et al. Cerium dioxide nanoparticles increase immunogenicity of the influenza vaccine. Antiviral Res. 2016; 127:1–9.
4. Hirst SM, Karakoti AS, Tyler RD, Sriranganathan N, Seal S, Reilly CM. Anti-inflammatory properties of cerium oxide nanoparticles. Small. 2009; 5(24):2848–56.
5. Zholobak NM, Ivanov VK, Shcherbakov AB, Shaporev AS, Polezhaeva OS, Baranchikov AYe, Spivak NYa, Tretyakov YuD: UV-shielding property, photocatalytic activity and photocytotoxicity of ceria colloid solutions. J Photochem Photobiol. B. 2011; 102:32–38. 10.1016/j.jphotobiol.2010.09.002.
6. Bubnov R, Babenko L, Lazarenko L, Kryvtsova M, Shcherbakov O, Zholobak N, Golubnitschaja O, Spivak M. Can tailored nanoceria act as a prebiotic? Report on improved lipid profile and gut microbiota in obese mice. EPMA Journal. 2019; 10:317–335. https://doi.org/10.1007/s13167-019-00190-1
7. Ahmed SF, Quadeer AA, McKay MR. Preliminary Identification of Potential Vaccine Targets for the COVID-19 Coronavirus (SARS-CoV-2) Based on SARS-CoV Immunological Studies. Viruses. 2020; 12:254.
8. Thakur N, Manna P, Das J. Synthesis and biomedical applications of nanoceria, a redox active nanoparticle. J Nanobiotechnology. 2019; 17(1):84. doi:10.1186/s12951-019-0516-9
9. Laude H, Chapsal JM, Gelfi J, Labiau S, Grosclaude J. Antigenic structure of transmissible gastroenteritis virus. I. Properties of monoclonal antibodies directed against virion proteins. J Gen

Virol. 1986; 67(1):119–130. doi:10.1099/0022-1317-67-1-119

10. Kobyliak NM, Falalyeyeva TM, Kuryk OG, et al. Antioxidative effects of cerium dioxide nanoparticles ameliorate age-related male infertility: optimistic results in rats and the review of clinical clues for integrative concept of men health and fertility. EPMA J. 2015; 6(1):12. doi:10.1186/s13167-015-0034-2
11. Arévalo AP, Pagotto R, et al. Ivermectin reduces in vivo coronavirus infection in a mouse experimental model. Sci Rep. 2021; 11(1):7132. doi: 10.1038/s41598-021-86679-0.
12. Bauchner H, Fontanarosa PB. Randomized Clinical Trials and COVID-19: Managing Expectations. JAMA. 2020; 323(22):2262–2263. doi:10.1001 /jama.2020.8115
13. Bubnov R, Spivak M. Towards individualized use of probiotics and prebiotics for metabolic syndrome and associated diseases treatment: does pathophysiology-based approach work and can anticipated evidence be completed? Preprints 2018; 2018090185. doi:10.20944/preprints201809.0185.v1.
14. Bubnov R, Spivak M. Individualized Short-term Probiotic Therapy of Metabolic Syndrome According to the Host's Phenotype. Journal of Clinical Gastroenterology. 2020; 54:25–26.
15. Jesenak M, Brndiarova M, Urbancikova I, et al. Immune Parameters and COVID-19 Infection – Associations with Clinical Severity and Disease Prognosis. Front Cell Infect Microbiol. 2020; 10:364. doi:10.3389/ fcimb.2020.00364
16. Golubnitschaja O, Baban B, Boniolo G, Wang W, Bubnov R, Kapalla M, et al. Medicine in the early twenty-first century: paradigm and anticipation. EPMA position paper 2016. EPMA J. 2016; 7:23.
17. Wang LY, Cui JJ, OuYang QY, et al. Complex analysis of the personalized pharmacotherapy in the management of COVID-19 patients and suggestions for applications of predictive, preventive, and personalized medicine attitude. EPMA J. 2021; 1–18. doi:10.1007/s13167-021-00247-0

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