Study of transmissible-gastroenteritis-virus-antigen-conjugated immunogenic properties of selenium nanoparticles and gold

Sergey A. Staroverov[1,2,3,] Alexei A. Volkov[1], Sergey V. Larionov[1], Pavel V. Mezhennyy[1], Sergey V. Kozlov[1], Alexander S. Fomin[1,2,3], Lev A. Dykman[2,3]

[1] Saratov State Agrarian University Named After N. I. Vavilov, Saratov, Russia
[2]The Department of the Russian Academy of Science, The Institute for Biochemistry and Physiology of Plants and Microorganisms, Saratov, Russia
[3]The Departament of the Russian Academy for Agricultural Sciences, State Institute Saratov Scientific and Research Veterinary Institute, Saratov, Russia

Abstract. There was performed the study of the transmissible-gastroenteritis-virus-antigen-conjugated immunogenic properties of selenium nanoparticles and gold. In comparative immunobiological studies there was found that immunization of guinea pigs driven by the colloid-selenium- as well as colloid-gold-conjugated transmissible gastroenteritis virus antigen of swine, leads to activation of the respiratory activity of lymphoid cells and peritoneal macrophages, which is directly related to increased activity of antibody-producing cells and activation of antibody generating. The obtained data suggest that the colloid particles promote antigen presentation to the reticuloendothelial system organs. In addition, there was established that these carriers stimulate production of proinflammatory cytokines, which leads to a complete and consistent immune response of both cellular and humoral components of immune system.
[Staroverov S.A., Volkov A.A., Larionov S.V., Mezhennyy P.V., Kozlov S.V., Fomin A.S., Dykman L.A. **Study of transmissible-gastroenteritis-virus-antigen-conjugated immunogenic properties of selenium nanoparticles and gold.** *Life Sci J* 2014;11(11):456-460] (ISSN:1097-8135). http://www.lifesciencesite.com. 78

Keywords: colloid particle, colloid selenium, colloid gold, nanoparticle, nanocarrier

List of abbreviations

TGS – transmissible gastroenteritis of swine
CS – colloid selenium
CG – colloid gold
IL-1β – interleukin-1 Beta
IL-6 – interleukin-6
INF-γ – interferon gamma

Introduction

Currently, the research groups pay much attention to the synthesis and properties of various nanomaterials [1, 2]. Undoubtedly, the nanoparticles have many positive attributes: they can be easily transported, attach to their surface diagnostic and therapeutic agents, as well as immunoactive biomolecules. These truly unique properties of nanoparticles make them suitable for diagnostic and for therapeutic usage while treating various diseases. Nanoparticles obtain reduced toxicity and short time for therapeutic agent release within the blood circulatory system because of their specific and target characteristic.

It is known that the nanomaterials may pass through biological membranes and access to the internal environment of the cells, tissues and organs. The protective proteins conjugation with nanoparticles for targeted delivery to immunocompetent cells and tissues provides opportunities to build a new generation of efficient and safe chemical (synthetic) vaccines [3, 4]. One of the positive aspects of nanoparticles application in vaccination is their active uptake of the reticuloendothelial system performed by their cells. However, yet to be explored a number of very important issues, in particular it is necessary to clarify the role of phagocytic immune cells in the immune response mechanisms using nanoparticle-conjugated antigens.

The use of gold nanoparticles as carriers of antigens and therapeutic agents and the use of data structures for immunological and therapeutic purposes have already been widely implemented in modern medicine and biology [5]. Of particular interest is the gold nanoparticles ability to cause humoral immune response to poorly immunogenic antigens and haptens [6]. There are data indicating the immunostimulatory effects of transmissible-gastroenteritis-virus-conjugated gold nanoparticles [7].

With regard to the colloid selenium (CS) in modern literature there are only few publications, which mainly refer to this structure as bioactive additive. However, in recent years, there have been obtained data providing evidence that nanoparticles of selenium may be advantageously used as a carrier of biologically active molecules [8]. There was also found that, unlike the colloid gold (CG), selenium nanoparticles themselves have a sufficiently high biological activity. It is proved that the selenium compound in the nanosized form with hyaluronic acid

exhibits an anti-tumor activity [9]. Application CS prevents the development of chrome-inducing hyperthyroidism in laboratory animals [10]. However, there are few publications on the usage of CS as nanoplatforms for antigen delivery to the target cells [11].

The purpose of this study was to evaluate the immunogenic properties of selenium and gold nanoparticles conjugated with the transmissible gastroenteritis swine virus antigen, as well as exploring the possibility of designing vaccines based on antigen of TGS virus using CG and CS as nanocarriers.

Methodology

In the present paper, as an antigen, there was used the capsid protein of the swine transmissible gastroenteritis virus (TGS) obtained in the virology laboratory of Saratov Research Veterinary Institute of Agricultural Sciences of Russian Academy of Agricultural Sciences.

TGS (in latin – Gastroenteritis infectiosa suum) is a highly contagious acute infectious swine disease of all age groups, which is characterized by vomiting, exhausting diarrhea, dehydration, high mortality, especially among piglets during the first 10 days of their life. The disease causes great economic losses of pig farms [12, 13].

The TGS causative agent is an RNA-containing virus belonging to the *Coronaviride* family, *Alphacoronavirus* genus, 1a group. This is a virion of spherical shape with a diameter of 75-160 nm [14]. Viral nucleocapsid is a flexible spiral, containing a single-stranded RNA molecules and a large number of nucleocapsid proteins [15]. The virus replicates in the cytoplasm of mature epithelial cells, which are located at the tips of the small intestine villi.

To release the antigen molecules from metal ions there was performed dialysis of an aqueous antigen solution in acetate buffer pH 4.0.

We synthesized the colloid selenium by the method [16]. The conjugation of the TGS virus agent with CS was carried out by the following scheme: to 2 ml of antigen solution, with total protein concentration of 5 mg/ml, there was added 250 µl of a 1 M solution of hydrazine hydrochloride and 62.5 µl of 1 M solution of sodium selenite. The total volume of the solution was adjusted to 5 ml with distilled water. The reaction was stopped by adjusting the pH solution to 7.2 by 1 M solution of sodium hydroxide (100 µl). The drug is released from low molecular weight compounds by dialysis against phosphate-buffered saline buffer of pH 7.2.

Colloid gold (CG) with a mean particle diameter (15 nm) was prepared using the reaction of chloroauric acid recovery by means of sodium citrate. The average CG particle size was controlled by the spectrophotometrical method [17, 18].

Preparation of TGS virus antigen's conjugate with CG was performed by selecting the optimal concentration of antigen that protects gold sol from salt aggregation.

For carrying out the immunobiological studies on the principle of analogues there were formed 4 groups of animals (guinea pigs) with 9 animals in each of them. Immunization of the animals was twice injected subcutaneously (along the spinal column), with an interval of 10 days.

The first group of guinea pigs was administered the TGS virus antigen solution at a dose of 1 ml. The second group received the TGS virus antigen's conjugate with CG at a dose of 1 ml. The third group was injected TGS virus antigen's conjugate with CS at a dose of 1 ml. The fourth group (control group) was administrated physiological saline at a dose of 1 ml.

Guinea pigs were euthanized at 10 days after the last injection. At the same time the blood serum was sampled for immunological studies. Furthermore, to evaluate the effect of drugs on cellular immunity, there was also determined the respiratory activity of peritoneal macrophages and spleen lymphocytes by the MTT assay.

Evolution and propagation of peritoneal macrophages and spleen cells were performed according to the standard procedures. Determination of respiratory activity was performed by the ability of cells to repair nitrotetrazolium blue bromide up to formazan level by the conventional method (MTT-assay) [19]. Measuring the recovered formazan amount was carried out on a spectrophotometer Genesys 10S UV Vis (Thermo Fisher Scientific, USA) at a wavelength of 490 nm. As a control measure, there was used formazan in concentrations of 0.002; 0.02; 0.2 and 2 mg/ml; these concentrations built a calibration curve.

To assess the effect of the TGS virus antigen's conjugates with colloid gold and selenium particles on the physiological parameters of the organism, there were performed biochemical studies of animal blood serum. To assess the functional state of the liver, there was determined the concentration of total protein and its fractions in the blood serum of guinea pigs. Enzyme spectrum included the determination of AST and ALT enzyme amplifiers activity. The kidneys functional activity was judged by the concentration of urea and creatinine in the blood serum of animals. Biochemical studies were performed using the Hospitex diagnostics kits at the biochemical analyzer Myndrey (PRC).

Maintenance and care of the animals, as well as their euthanasia were performed in accordance with

the requirements of the RF Ministry of Health to the work experimentally-biological clinics and the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes".

Determination of interleukin concentration in the blood serum was performed using immunoassay kits for determination of interleukin-1 beta, interleukin 6, and interferon gamma by the immunoenzymometric analyzer Plate Screen (Hospitex Diagnostics, Italy).

The main part (Study results)

Studying the efficiency of interaction of these colloid particles conjugates with the cells of the reticuloendothelial system was examined in the MTT assay. On the 10th day after immunization there was found an increase in respiratory activity of macrophage cells: under the immunization of antigens with CS by 66%, while under the immunization with CG by 46% compared to control and by 64% and 46% compared to the virus antigen, respectively (Fig. 1).

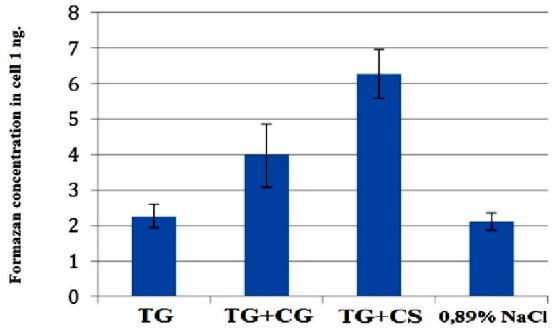

Figure 1. The results of MTT assay with peritoneal cells of guinea pigs (TGS + CS – antigen conjugate with CS; TGS + CG – antigen injected with CG)

When studying the respiratory activity of lymphoid spleen cells, there were obtained similar results. Thus, when animals were immunized with the antigen conjugates with CG, the respiratory activity of spleen cells was increased by 56%, while using the TGS virus antigen conjugates with CS it was increased by 78% (Fig. 2). Assessing the obtained data, we noted that, apparently, this is the stage of both activation for antigen-presenting cells and the proliferative activity increase of lymphoid (antibody) spleen cells. This finding may indicate that the colloid particles promote the antigen presentation to the organs of the reticuloendothelial system.

During the determining of the interferon-gamma (INF-γ) concentrations in the experimental animals, there was identified a significant increase in its concentration in the groups of animals treated with antigen conjugated with colloid carriers (Fig. 3).

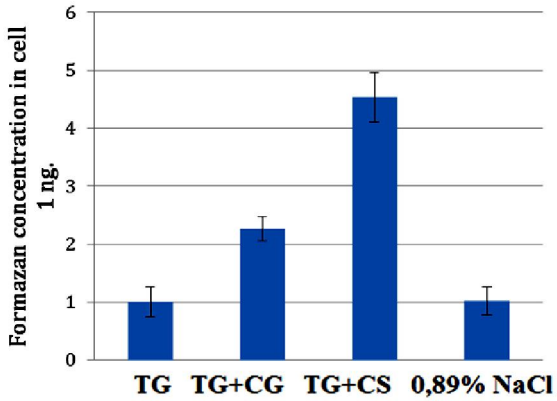

Figure 2. MTT assay results with lymphoid cells of the guinea pigs spleen

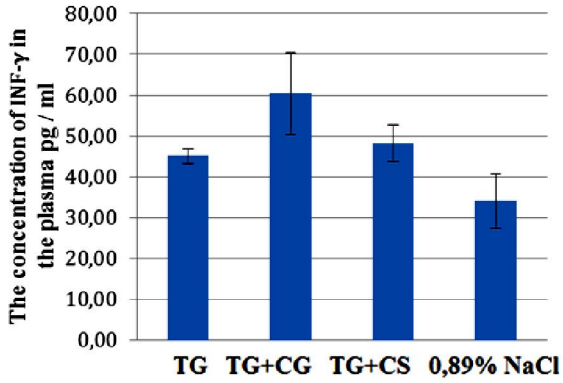

Figure 3. INF-γ concentration changes in the plasma of treated animals

From the obtained data we can draw the following information - the largest concentration of INF-γ is determined in animals immunized with an antigen-conjugated gold and selenium nanoparticles, and its concentration is 60.44 ± 9.91 pg/ml and 48.17 ± 4.43 pg/ml, respectively.

The obtained data, indicating the increasing activity of INF-γ, are directly correlated with the results indicating an increase in respiratory activity of splenocytes (10 days after the final injection), and indicating the ability of nanoparticles to stimulate the release of interferon gamma by T-cells, thereby enhancing the activity of immunity lymphoid link.

When studying the concentration of interleukin-1 beta (IL-1β) in the plasma of treated animals, we have found that the greatest increase in the concentration of IL-1β was observed in the group immunized with the antigen with CG and made 8.10 ± 0.74 pg/ml. In the group immunized with the antigen with CS and simple antigen, the concentration of interleukin-1 beta made 6.58 ± 1.06 pg/ml and 6.93 ± 0.91 pg/ml, respectively. However, in these groups there is also distinguished the increase if compared to intact animals, whose blood concentration of interleukin-1 beta was 4.93 ± 0.71 pg/ml (Fig. 4).

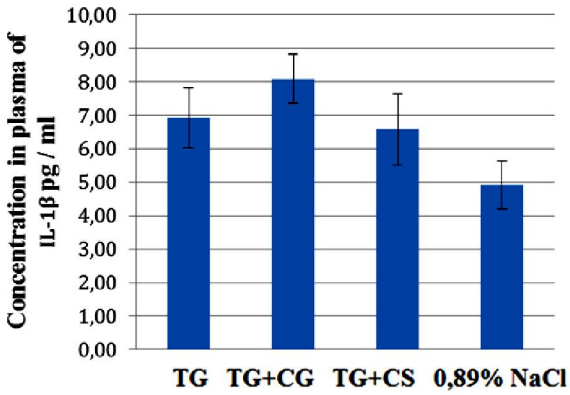

Figure 4. Changes in IL-1β concentration in the plasma of treated animals

It should be noted that the increased activity of IL-1β in the groups immunized both with an antigen conjugate with colloidal particles and the native antigen, directly correlates with the activity of macrophage cells and stimulated B-cells, which produce this cytokine while being activated.

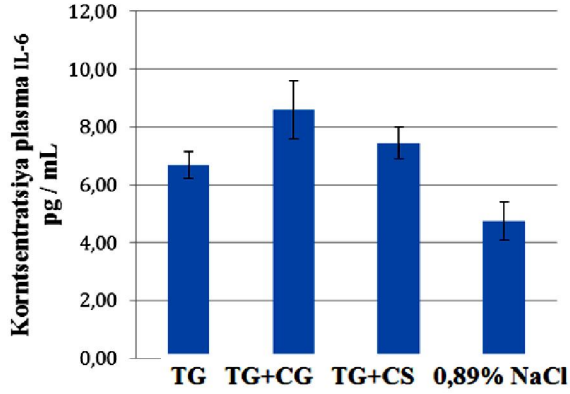

Figure 5. Changes in IL-6 concentration in the plasma of treated animals

The obtained data on the production level of interleukin-6 (IL-6) are comparable with those obtained during the study of IL-6 concentration in the plasma of treated animals (Fig. 5), indicating the stimulation of macrophages and lymphocytes. This correlates with the activity of macrophage kind cells and with stimulated lymphoid cells that produce this cytokine.

Analyzing the obtained antibody titers, there can be noted that during the immunization of animals with CG conjugates, the reverse logarithm (-lg) of the obtained antibody titer makes 13.9 ± 0.4, when during the CS immunization it makes 14.5 ± 0.4. What actually twice exceeds the antibody titers obtained in the control group (Table 1).

Table 1. Antiviral antibody titer (-lg)

	TGS virus antigen	Antigen conjugate+CG	Antigen conjugate+CS
Antibody titers	12.6±0.2 (1:8192)	13.9±0.4 (1:16384)	14.5±0.4 (1:32768)

Further, during the biochemical studies of blood serum of treated animals (Table 2), there was found that in all groups of animals the deviations from the physiological boundaries are absent. It may be an indirect evidence of the toxic properties absence for these drugs.

Table 2. Biochemical indices of the animal blood

Indicator	Unit	Group 1	Group 2	Group 3	Group 4 (control)
ALT	u/l	59.1±13.08	46.8±4.32	52.2±14.16	56±1.41
AST	u/l	117.0±18.49	126.8±28.38	142.8±36.35	168.5±2.12
LDH-L	u/l	432.9±46.54	570.9±93.07	489.0±77.01	554±8.49
Glucose	mmol/l	7.0±0.42	7.1±0.62	6.3±0.51	5.31±0.06
Crude protein	g/l	56.2±2.12	54.4±3.84	49.9±2.30	56.6±0.57
Albumine	g/l	34.1±2.79	33.6±1.93	33.1±1.29	33.35±1.77
Creatinine	mmol/l	60.8±7.47	57.1±5.97	50.7±2.90	54.55±1.06
Globulin	g/l	22.1±1.46	20.8±3.27	16.8±1.83	23.25±1.20
AST/ALT		2.0±0.16	2.7±0.60	2.8±0.71	3.02±0.06
A/G		3.0±4.29	1.7±0.25	2.0±0.23	1.4±0.14
Urea	mmol/l	9.9±1.15	10.9±1.11	8.8±1.30	8.4±0.28

Conclusion

Thus, on the basis of the present study, we can conclude that immunization of animals with the CS and SG-conjugated TGs virus antigen leads to the activation of the respiratory activity of lymphoid cells and peritoneal macrophages, which is directly linked to their transforming activity and activation of antibody production. In addition, these carriers stimulate the production of interferons and cytokines, which leads to a complete and consistent immune response of both cellular and humoral immunity components to the preventative immunization. These data suggest that the colloid particles, which serve as nanocarriers, promote the antigen expression enhancement on the surface of antigen-presenting cells and, in turn, provide an effective presentation of viral peptides to cytotoxic T-lymphocytes and natural killers.

Resume

1. Using the colloid particles (selenium and gold performing the role of nano-sized carriers) conjugated with the TGS virus antigens, there was confirmed the possibility of nano-modified vaccine production.

2. There was confirmed that the immunogenic effect of selenium and gold nanoparticles conjugated with TGS virus antigen. Besides, there was found that when administered to an

animal, they are able to generate an adequate immune response with minimal concentrations of viral antigen.

3. Immunization with the selenium and gold nanoparticles conjugated with TGS virus antigen leads to activation of respiratory activity of lymphoid cells and peritoneal macrophages, which is directly related to their transforming activity and activation of antibody production. Also, there was observed the stimulation of cytokine production, which leads to a complete and consistent immune response of both cellular and humoral immunity to preventative immunization.

Corresponding Author:
Dr.Sergey A. Staroverov
Saratov State Agrarian University Named After N. I. Vavilov, Saratov

References

1. Mout, R., D.F. Moyano, S. Rana and V.M. Rotello, 2012. Surface functionalization of nanoparticles for nanomedicine. Chem. Soc. Rev., 41: 2539-2544.
2. Bhattacharya, R. and P. Mukherjee, 2008. Biological properties of "naked" metal nanoparticles . Adv. Drug Del. Rev., 60: 1289-1306.
3. Mezhenny, P.V., S.A. Staroverov, A.A. Volkov, S.V. Kozlov, V.N. LAskavy, L.A. Dykman and A.J. Isaeva, 2013. Construction of influenza-virus-protein-conjugates of colloid gold and colloid selenium and the study of their immunogenic properties. Bulletin of the Saratov State Agricultural University named after Vavilov, N.I., 2: 29-32.
4. Dykman, L.A., S.A. Staroverov, V.A. Bogatyrev and S.J. Shchegolov, 2010. Adjuvant properties of gold nanoparticles. Russian Nanotechnology, 5: 58-68.
5. Dykman, L.A. and N.G. Khlebtsov, 2012. Gold nanoparticles in biomedical applications: recent advances and perspectives. Chem. Soc. Rev., 41: 2256-2282.
6. Dykman, L.A., S.A. Staroverov, V.A. Bogatyrev and S.Yu. Shchyogolev, 2010. Gold nanoparticles as an antigen carrier and an adjuvant. New York: Nova Science Publishers: 54.
7. Staroverov, S.A., I.V. Vidiasheva, K.P. Gabalov, O.A. Vaasilenko, V.N. Laskavy and L.A. Dykman, 2011. Study of the immunostimulatory action of gold nanoparticles conjugated with transmissible gastroenteritis virus. Bulletin of Experimental Biology and Medicine, 151: 418-421.
8. Kozlov, S.V., A.S. Fomin, V.S Stepanov and others, 2012. Construction of colloidal selenium complex with lactoferrin and study of its biodynamic properties. Actual problems of veterinary biology, 1: 27-32.
9. Ren, Y., T. Zhao, G. Mao, M. Zhang, F. Li, Y. Zou, L. Yang and X. Wu, 2013. Antitumor activity of hyaluronic acid-selenium nanoparticles in Heps tumor mice models. Int. J. Biol. Macromol, 57: 57-62.
10. Hassanin, K.M., S.H. Abd El-Kawi and K.S. Hashem, 2013. The prospective protective effect of selenium nanoparticles against chromium-induced oxidative and cellular damage in rat thyroid. Int. J. Nanomedicine, 8: 1713-1720.
11. Isaeva, A.J., S.A. Staroverov, A.A. Volkov and others, 2012. Construction of nanoscale structures based on colloid selenium. Veterinary Pathology, 3 (41): 114-117. Date Views 31.05.2014 http://elibrary.ru/item.asp?id=18047495.
12. Cheng, Q.H. and X.Y. Niu, 1992. Investigation on the porcine epidemic diarrhea prevalent on Qinhai. Vet. Sci., 22: 22-23.
13. Straw, B.E., S. D'Allaire, W.L. Mengeling and D.J. (Eds.) Taylor, 1986. Disease of Swine. Ames: Iowa State University Press, pp: 1209.
14. Delmas, B., J. Gelfi and H. Laude, 1986. Antigenic structure of transmissible gastroenteritis virus. II. Domains in the peplomer glycoprotein, J. Gen. Virol, 67: 1405-1418.
15. Moxley, R.A. and L.D. Olson, 1989. Clinical evaluation of transmissible gastroenteritis virus vaccines and vaccination procedures for inducing lactogenic immunity in sows. Am. J. Vet. Res., 50: 111-118.
16. Bo Huang, Zhang Jinsong, Hou Jingwu and Chang Chen, 2003. Free radical scavenging efficiency of nano-Se in vitro. Free Radical Biology & Medicine, 7(35): 805-813.
17. Martins, A.M.C.R.P.F., J.G. Bersano, R. Ogata, G. Amante, B.D.B. Nastari and M.H.B. Catroxo, 2013. Diagnosis to detect porcine transmissible gastroenteritis virus (TGEV) by optical and transmission electron microscopy techniques. Int. J. Morphol., 31: 706-715.
18. Frens, G., 1973. Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. Nature Phys. Sci., 241: 20–22.
19. Bernas, T. and J.W. Dobrucki, 2000. The role of plasma membrane in bioreduction of two tetrazolium salts, MTT, and CTC. Arch. Biochem. Biophys., 380: 108-116.

7/2/2014