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DETERMINATION OF IMMUNIZING DOSES OF THE COWPOX VACCINE CANDIDATE: PRELIMINARY RESULTS

Kuandyk Zhugunissov, Nurayim A. Sarsenkulova, Shalkar T. Tabys, Dias I. Muzarap, Marzhan K. Kenzhebaeva, Kaynar B. Barakabaev, Dinara B. Aidarbekova, Alina B. Alieva, Sanat S. Kilibaev, Muratbay A. Mambetaliev

Research Institute for Biological Safety Problems, Gvardeisky, Republic of Kazakhstan

Correspondent-author: Kuandyk Zhugunissov, e-mail: kuandyk_83@mail.ru
Co-authors: Nurayim A. Sarsenkulova, e-mail: nuraiym.sarsenkulova@mail.ru, Shalkar T. Tabys, e-mail: shalkar.tabys@bk.ru, Dias I. Muzarap, e-mail: m.dias00@mail.ru, Marzhan K. Kenzhebaeva, e-mail: kenzhebaeva80k@mail.ru, Kaynar B. Barakabaev, e-mail: kainar7@mai.ru, Dinara B. Aidarbekova, e-mail: dinka_bolatovna86@mail.ru, Alina B. Alieva, e-mail: alina_beisenovna2@mail.ru, Sanat S. Kilibaev, e-mail: sanat.kilibaev@mail.ru, Muratbay A. Mambetaliev, e-mail: m.mambetalyev@mail.ru

Abstract

Determining the effective immunizing dose of a vaccine is one of the important pillars of a vaccination strategy. In this regard, this article presents the results of studies to determine the effective immunizing dose of a candidate cowpox vaccine prepared from the BIEMG-51 strain of the vaccinia virus. At determining a immunizing dose of the vaccine, it was found that in all animals vaccinated at doses of 10^3 $TCID_{50}$, 10^4 $TCID_{50}$, 10^5 $TCID_{50}$, antibodies were not detected in the serum neutralization test (SNT). However, these vaccinated calves at the indicated doses were resistant to the challenge, while the unvaccinated group responded to the challenge and fell ill with the characteristic clinical signs of cowpox. The obtained primary results give grounds for improving the technology of vaccine preparation and in-depth study of the factors of cellular immunity of vaccinated animals in case of poxvirus infections.

Key words: cowpox; minimum immunizing dose; safety; vaccine; virus.

Basic position and Introduction

Cowpox is a contagious disease characterized by intoxication of the body, fever and nodular-pustular rash on the skin and mucous membranes. The causative agent of this disease is a viral agent that belongs to the genus *Orthopoxvirus* of the *Poxviridae* family [1]. Many *Orthopoxviruses* have an animal reservoir and can be transmitted by contact from an infected animal to a person. The main reservoirs and carriers of the cowpox virus (CPXV) are wild and predatory rodents [2].

Until the 70s of the XX century, it was believed that CPXV causes outbreaks of the disease only in the population of cattle, the clinical picture of which is more often manifested in the form of local (lesions on the skin of the udder and on the nipples), less often generalized form infection (more typical for calves). Later, it was found out that a much wider range of animals are susceptible to the virus, in addition, CPXV is pathogenic to humans and can cause generalized infection in people with weakened immunity [3-5]. In people with weak immunity, exposure to *Orthopoxvirus* can lead to severe forms of the disease or even death [6].

Recently, human infection with cowpox associated with domestic rats has been reported in Europe, with usually mild and self-healing lesions [7, 8]. In many countries, the reappearance or emergence of other orthopoxviruses in human and animal populations is an urgent global health and veterinary problem. There are literature data on human infections and diseases caused by zoonotic *Orthopoxviruses*, such as Monkeypox virus (MPXV) [9], CPXV [10], vaccinia virus (VACV) [11] and Ahmet virus [12]. Also, Research Institute for Biological Safety Problems (RIBSP) employees found seropositive animals to cowpox virus during monitoring studies on the territory of Zhambyl region (data not published). These facts raise concerns about the habitats and distribution of orthopoxviruses, as well as their potential to cause outbreaks among animals and humans, thereby having a further impact on the health of animals and the population. This indicates the need for special efforts to develop modern means of rapid diagnosis of the etiological agent of this disease, the search for antiviral drugs and vaccination of susceptible animals. So, vaccination is one of the most important achievements of science, and is an effective, safe, economical means of controlling and eliminating life-threatening infectious diseases. Taking into account the above situations, research on the creation of a live vaccine against cowpox based on the VACV was started at the RIBSP.

Therefore, the aim of the study was to determine the immunizing dose of an experimental cowpox vaccine for target animals.

Materials and Methods

2.1 Virus strains

The BIEMG-51 strain from the VACV obtained from the Moscow Research Institute of Viral Preparations in 1996 was used as an object of research. The virus was adapted by three consecutive passages on the chorion-allantois membrane (CAM) of embryonated chicken eggs (ECE). It is stored in vials under vacuum at a temperature of minus 40 ° C. There is no information about its genetic characteristics. The lyophilized CowPOX strain obtained from All-

Russian Research Institute of Veterinary Virology and Microbiology, which passed 12 passages on the CAM of ECE, was also used in the work.

2.2 laboratory animals

To study the immunogenicity of the vaccine against CPXV, local breeds of one-year-old twelve calves were used in the experiment, and fifteen white mice weighing 18-21 g, ten guinea pigs weighing 700-800 g were used to determine the safety of the vaccine and ten rabbits weighing 1.5-2 kg. Maintenance and feeding of laboratory and target animals was carried out in accordance with the instructions [13].

2.3 Virus propagation in cell culture and determination of infectious activity

The CPXV growth in lamb kidney cell culture was carried out according to the method [14]. Further, microscopy, collection, preparation of viral suspensions and determination of their infectivity titers in cell culture were carried out according to the above method. The titer of the virus was considered to be its greatest dilution, causing CPE in 50% of infected vials with cell culture. The virus titer was calculated using the method of Reed L.J. and Muench H.A. [15].

2.4 Preparation of the protective environment and formulation of the vaccine

As a protective environment for the CPXV strain, we used 5% peptone and 3% sucrose in final concentrations with double sterilization by liquid steam at (100±1) °C for 30 minutes (the interval between sterilizations is 18-20 h.). A sterile protective environment was combined with a viral suspension before lyophilization in a ratio of 1:1. The resulting mixture was poured into vials of 1 mL. Then mixture frozen at minus 60°C for (12±4) hours and dried in a lyophilic apparatus “Usifrua” under the following mode: freezing temperature – minus (55-60)°C; pressure in the chamber - from 3 to 7 Pa; the heating temperature of the shelves is from 10 to 40°C; the final temperature of the viral suspension is (22±2)°C. After drying the material, the vials were sealed under vacuum on a carousel-collector apparatus at a residual pressure of 25 to 30 Pa.

2.5 Determination of the safety of the vaccine in laboratory animals

To determine the safety of the developed CPXV vaccine, laboratory white mice weighing 16-18 grams, guinea pigs 1,5 months old, and rabbits 3 months old weighing 4,5-5 kg were used. The lyophilized vaccine was diluted with saline solution to the initial volume and injected into each animal, subcutaneously in the area of the hairless area of the axillary region at the appropriate dose: 10 white mice, 5 guinea pigs at doses of 0,1 cm^3/head and 0,3 cm^3/head and 8 rabbits at a dose of 0,5 cm^3/head. As a control, 5 mice, 2 guinea pigs and 2 rabbits were left unvaccinated. The observation period for vaccinated animals was 10-14 days. According to the results of the research, the animals should be alive and clinically healthy.

2.6 Determination of the immunizing dose of the vaccine

The immunizing dose of the CPXV vaccine was determined on one-year-old calves, on a cut, shaved and treated with ethyl spirit area of the skin in the neck by intradermal immunization.

To determine the immunizing dose, the following vaccine doses were tested: group I (n=3) – 1000 $TCID_{50}$, group II (n=3) – 10000 $TCID_{50}$, group III (n=3) – 100000 $TCID_{50}$, which were applied to test calves by intradermal immunization at different points in a volume of 1,0 cm3. The immunized calves were monitored daily in case of the appearance of characteristic clinical signs of smallpox disease and body temperature was measured. Blood serums were taken from vaccinated animals on days 7, 14, 21 after immunization to determine virus neutralizing antibodies to the cowpox virus in the SNT.

2.7 Determination of the titer of virus neutralizing antibodies

The activity of virus neutralizing antibodies in blood sera was determined in the SNT with a constant dose of virus and different serum dilutions according to the method [16]. The titer of antibodies of the serum under study was taken to be the largest dilution of serum that inhibits the development of the CPE virus in at least 50% of the infected cell culture.

Results

3.1. Determination of the safety of the developed vaccine on laboratory animals

In order to determine the safety of the developed vaccine against cowpox from the strain Biemg-51, experiments were carried out on white mice weighing 16-18 grams, guinea pigs 1,5 months of age and rabbits weighing 2,5-3 kg, which were injected subcutaneously with an experimental vaccine against cowpox in the amount of 0,1, 0,3 and 0,5 cm^3/head, respectively. The experimental animals were clinically observed with daily thermometry for 14 days, paying special attention to the general condition of the animals and the local reaction at the injection site. At the same time, the general reaction (according to the presence and severity of hyperthermia) and the local reaction in terms of the size and nature of seals at the site of vaccine administration were taken into account. Before and during the experiment, the animals were kept under standard vivarium conditions under natural light, on a balanced diet with free access to water. The results of the conducted studies are presented in table 1.

Table 1 - Evaluation of the safety of an experimental sample of the vaccine against cowpox in mice, guinea pigs and rabbits

Type of animals	Number of animals (head)	Animal reaction	
		local reaction	general reaction
White mice	15	absent	absent
Guinea pigs	6	absent	absent
Rabbits	6	slight swelling	absent
Control (saline solution)	2 heads from each type of animal	absent	absent

The results of the experiments and the data in Table 2 showed that no signs of deviation from the physiological norm were observed in all laboratory animals when the vaccine was administered subcutaneously. At the end of time, the animals remained alive and healthy.

3.2. Determination of the immunizing dose of the vaccine

To determine the immunizing dose of the experimental vaccine, we tested three doses of the strain BIEMG-51 of the vaccinia virus: 10^3 $TCID_{50}$, 10^4 $TCID_{50}$, 10^5 $TCID_{50}$. At the same time, the animals were immunized intradermally in a volume of 1 cm^3.

According to the results of the experiment, it was found that all vaccinated animals didn't have a temperature reaction and any post-vaccination complications to the introduction of the BIEMG-51 strain of the vaccinia virus.

Further, when studying the humoral immune response to the CPXV in SNT, it was found that in all animals vaccinated with vaccinia in different doses on days 7, 14, and 21, virus neutralizing antibodies was practically absent in the blood sera.

Table 2 – Results of determining the immunizing dose of the vaccine on calves

Group and doses of the vaccine	Animal number	SNT results, log2			Results of the control infection		
		7	14	21	temperature	skin lesion	other clinical signs
I (10^3 $TCID_{50}$)	1	0	0	0	38,5	-	-
	2	0	0	0	38,7	-	-
	3	0	0	0	38,5	-	-
II (10^4 $TCID_{50}$)	4	0	0	0	38,6	-	-
	5	0	0	0	38,5	-	-
	6	0	0	0,75	38,7	-	-
III (10^5 $TCID_{50}$)	7	0	0	0	38,8	-	-
	8	0	0	0,5	38,6	-	-
	9	0	0	0,75	38,5	-	-
IV (control)	10	0	0	0	39,7	+	+
	11	0	0	0	39,5	+	+
	12	0	0	0	39,8	+	+
Notes: (–) – absence of clinical signs (+) – presence of clinical signs.							

However, despite the absence of virus neutralizing antibodies in calves, all vaccinated animals did not respond to the control infection with the virulent strain «Cowpox-CAM». At the same time, in the vaccinated groups during the observation period, no deviations from the physiological norm were noted, regardless of the

immunizing dose of the vaccine. Whereas in the control group of animals on the 3rd day there was a lack of appetite, a slight increase in body temperature, as well as lesions (variolas) on the skin of animals developed at the site of the introduction of the virus.

Discussion

At present, the human population has practically no immunity to orthopoxvirus infections that cause smallpox viruses, monkey pox, cowpox, buffalo pox. Every year, more and more massive outbreaks of orthopoxvirus infections among humans and animals are registered on different continents [17]. Therefore, vaccination is the only way to protect people, animals and birds from this infectious disease. In this study, we used the BIEMG-51 strain of the vaccinia virus, which was successfully used for preventive immunization of camels against camel pox in 1996 in the Mangystau region [18]. In addition, this strain was successfully used until 1980 in the production of smallpox vaccine for healthcare during the Soviet Union. In this regard, in this study, the specified strain was chosen as a comprehensively tested and most safe strain for creating a vaccine, and studies were carried out to determine its immunizing dose for cattle, and its immunogenicity was determined. It is known that the immunogenicity of vaccines, as a rule, is directly dependent on the concentration of the antigen in the vaccination dose, that is, the higher the activity of the drug, the more significant immunogenicity it has, while causing intense immunity in animals in the short term and for a long period after vaccination. [19]. According to some authors [18], the duration of post-vaccination immunity against chicken pox in birds depends on the vaccination dose of the vaccine preparation. At the same time, the duration of immunity in birds that received small doses of the vaccine was relatively short. Such similar studies on the selection of the minimum immunizing field dose were carried out during the development of monovaccines against sheep pox, *peste des petits ruminants* (PPR), lumpy skin disease and associated vaccine against sheep pox and PPR [19-21]. When determining the immunizing dose of the associated vaccine, the authors prepared 4 samples of a vaccine preparation with different immunizing doses of 10, 100, 1000, 10000 $TCID_{50}$. According to the results of the conducted studies, it was found that animals vaccinated at a dose of 100 $TCID_{50}/cm^3$ of the PPR and sheep pox virus acquired protect to challenge with sheep pox, and at the same time the effectiveness of immunization was 67%. At a dose of 1000 – 10000 $TCID_{50}/cm^3$, the virus neutralizing antibodies to the sheep pox virus was in titer 1:8 – 1:32, to the PPR virus in titer 1:2 - 1:8 and the animals didn’t respond to the control infection, the effectiveness of immunization was 100%. While animals vaccinated at a dose of 10 $TCID_{50}/cm^3$ and intact animals became ill after control infection with epizootic sheep pox virus. A similar study conducted on calves that received different doses of the vaccine showed protect to challenge with the lumpy skin disease virulent virus of cattle without showing any clinical signs of the disease [19]. In

our study, no antibodies were produced in immunized animals regardless of the administered dose concentration of the vaccine preparation. But, despite this, during experimental infection, vaccinated animals did not show clinical signs characteristic of cowpox, remained healthy and alive during the entire observation period. A possible explanation for this conclusion may be a decrease in immune properties during attenuation of poxviruses due to a long passage in the biological system or a simultaneous increase in paraspecific effects in poxvirus infections [22]. It is important to note that in poxvirus infections, cellular factors play a more prominent role as protective factors of immunity. Humoral factors may be absent or present at a low level of antibodies, which cannot be detected using available tests (SNT, ELISA). Animal protection or the presence of immunity in such cases is confirmed by resistance to infection with a virulent virus. These data are based on such results of studies in which animals whose blood serum lacked specific antibodies, and they remained immune to the virulent virus during experimental infection [23]. The same data were obtained by other researchers who experimentally studied the noted phenomenon regardless of previous authors. Based on the results of their research, they attribute this phenomenon to cellular immunity observed in smallpox diseases [24]. However, this question remains open and requires additional research.

Conclusion

Thus, the results of the experiments showed that the experimental sample of the vaccine against cowpox was evaluated as safety against laboratory animals. Also, when determining the immunizing dose of the vaccine, it was found that animals vaccinated at doses of 10^3 $TCID_{50}$, 10^4 $TCID_{50}$, 10^5 $TCID_{50}$ were resistant to control infection, while the unvaccinated group reacted to challenge and fell ill with characteristic clinical signs of cowpox.

Based on the foregoing, the optimal immunizing dose of the vaccine for calves was 1000 $TCID_{50}$. However, taking into account unforeseen circumstances during storage and transportation, we propose a fivefold immunizing dose - 5000 $TCID_{50}$.

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