Review
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Analysis of aerobiological studies with orthopoxviruses by U.S. Department of Defense

Gennadiy G. Onishchenko[1,2], Igor A. Kirillov[3], Sergey V. Borisevich[2,4]✉, Tatiana E. Sizikova[4], Victor T. Krotkov[4]

[1]I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia;
[2]Russian Academy of Sciences, Moscow, Russia;
[3]Management of the Chief of the Nuclear, Biological and Chemical Protection Troops of the Armed Forces of the Russian Federation, Moscow, Russia;
[4]48 Central Scientific Research Institute, Sergiev Posad-6, Russia

Abstract

Discontinuation of vaccination after the completion of Smallpox global eradication program led to a sharp decrease in the level of collective immunity not only to smallpox but also to other orthopoxvirus infections. Over the past 10–15 years, the world has seen an increase in the frequency of diseases caused by smallpox viruses of cows, buffaloes, camels. The outbreak of mpox (a disease caused by the monkey pox virus) occurred in 2022–2023. Analysis of the literature data on the organization of the orthopoxvirus genome suggest that smallpox could have occurred in the past as a result of evolutionary changes in the zoonotic progenitor virus. In this regard, there is a threat of a new particularly dangerous anthropozoonosis, the pathogen of which can occur both naturally and artificially.

The **aim** of the review is to analyze open science published data on aerobiological research with OPVs conducted by the U.S. Department of Defense from 1994-2013, which was a period of restricted research and storage of smallpox virus samples. The authors did not find any publications of the results of aerobiological research with orthopoxviruses conducted by the US Department of Defense after 2013 in open scientific sources.

The review presents a data analysis in Russian and English-speaking scientist publication as well as those posted on the Internet.

The presented results of aerobiological studies with orthopoxviruses indicate the interest of the US military department in carrying out experimental work of dual use, including monitoring of the properties of orthopoxviruses and a possible change in their pathogenicity for humans, selection of optimal laboratory models for studying the properties of orthopoxviruses, and the possibility of modeling the properties of the smallpox virus when using other orthopoxviruses (cowpox virus, rabbit pox virus, monkey pox virus), modeling of the main characteristics of the disease caused by the smallpox virus in humans and evaluation of the effectiveness of existing and newly developed vaccines against smallpox, comparative study of effectiveness of antiviral drugs for regular or post-exposure prophylaxis of naturally occurring smallpox and monkey smallpox.

Keywords: *orthopoxviruses, smallpox virus, rabbitpox virus, monkeypox virus, cowpox virus, laboratory model, modeling of virus properties, medical protection products*

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Анализ аэробиологических исследований с ортопоксвирусами, проводимых Министерством обороны США

Онищенко Г.Г.[1,2], Кириллов И.А.[3], Борисевич С.В.[2,4]✉, Сизикова Т.Е.[4], Кротков В.Т.[4]

[1]Первый Московский государственный медицинский университет им. И.М. Сеченова (Сеченовский университет), Москва, Россия;
[2]Российская академия наук, Москва, Россия;
[3]Управление начальника войск радиационной, химической и биологической защиты Вооруженных Сил Российской Федерации, Москва, Россия;
[4]48 Центральный научно-исследовательский институт, Сергиев Посад-6, Россия

Аннотация

Прекращение вакцинации после завершения «Программы глобальной ликвидации натуральной оспы» привело к резкому снижению уровня коллективного иммунитета не только к натуральной оспе, но и к другим ортопоксвирусным (ОПВ) инфекциям. За последние 10–15 лет в мире произошло увеличение частоты заболеваний, вызванных вирусами оспы коров, оспы буйволов, оспы верблюдов. В 2022–2023 гг. произошла вспышка mpox (заболевание, вызываемое вирусом оспы обезьян). Анализ данных литературы об организации генома ОПВ позволяет предположить, что возбудитель натуральной оспы мог в прошлом возникать в результате эволюционных изменений зоонозного вируса-прародителя. В связи с этим существует угроза возникновения нового особо опасного антропозооноза, возбудитель которого может возникнуть как естественным, так и искусственным путём.

Целью обзора является анализ опубликованных в открытых научных источниках данных об аэробиологических исследованиях с ОПВ, проводимых Министерством обороны США в 1994–2013 гг. — в период ограничения научных исследований и хранения образцов вирусов оспы. Публикации результатов аэробиологических исследований с ортопоксвирусами, проводимых Минобороны США после 2013 г., в открытых научных источниках авторами не найдены.

Результаты аэробиологических исследований с ОПВ свидетельствуют о заинтересованности военного ведомства США в проведении экспериментальных работ двойного назначения, включают мониторинг за свойствами ОПВ и возможное изменение их патогенности для человека, выбор оптимальных лабораторных моделей для изучения свойств ОПВ и возможности моделирования свойств вируса натуральной оспы при использовании других ОПВ (вирусы оспы коров, оспы кроликов, оспы обезьян), моделирование основных характеристик заболевания, вызываемого вирусом натуральной оспы, у человека и оценка эффективности имеющихся и вновь разрабатываемых вакцин против натуральной оспы, сравнительное изучение эффективности противовирусных лекарственных средств для профилактики или экстренной профилактики натуральной оспы и оспы обезьян.

Ключевые слова: *ортопоксвирусы, вирус натуральной оспы, вирус оспы кроликов, вирус оспы обезьян, вирус оспы коров, лабораторная модель, моделирование свойств вируса, средства медицинской защиты*

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

The discontinuation of vaccination after the completion of the Global Smallpox Eradication Program has led to a dangerous situation, as a significant proportion of the world's population has become susceptible to both smallpox and other human pathogenic orthopoxviruses (OPVs) due to the loss of population immunity [1, 2]. The latter can lead to a global epidemic emergency [2, 3].

A clear example of this is the development of monkeypox outbreak in 2022-2023 (since 28.11.2023 the disease has been renamed by the Taxonomic Committee and is now known as “mpox”) [4], the increase in the frequency of diseases caused by cowpox, buffalo pox and camelpox viruses in the world over the last 10-15 years [5].

In nature, representatives of various zooanthroponotic OPVs circulate practically on all continents and periodically cause diseases among animals and humans. For example, isolated cases of poxvirus infections have been reported in Brazil and other parts of South America [6, 7]. Isolates from humans and livestock have been found to have a high degree of affinity to the vaccine virus [8, 9]. When investigating the possible role of primates as carriers of vaccine-like viruses, a high percentage of seropositive results was found [10].

Analysis of literature data on the organization of the OPV genome suggests that the smallpox pathogen may have arisen in the past as a result of evolutionary changes in the zoonotic progenitor virus. In this regard, there is a threat of a new particularly dangerous anthropozoonosis [11–13].

The **aim** of the review is to analyze open scientific published data on aerobiological research with OPVs conducted by the U.S. Department of Defense during the years 1994–2013. During this period, the World Health Organization imposed restrictions on research and storage of smallpox virus samples for all institutions worldwide, except for two international repositories: the Center for Disease Control and Prevention (USA) and the State Scientific Center for Virology and Biotechnology "Vector" of Rospotrebnadzor (Russia)[1].

The authors did not find any publications of the results of aerobiological studies with orthopoxviruses conducted by the US Department of Defense after 2013 in open scientific sources.

To study numerous aspects of the infection, specialists of research institutions of the US Department of Defense actively use various laboratory animals and pathogenic OPVs for them. These are white mice, lower primates (mainly Javan macaques, rhesus macaques) and rabbits. Ectromelia, cowpox and smallpox vaccine viruses were used to infect mice, rabbitpox and smallpox vaccine viruses were used to infect rabbits, and smallpox vaccines were used to infect monkeys [14–16]. According to American researchers, the optimal model should combine the possibility of using a low infectious dose to infect animals and the transmission of the virus from a sick animal to a healthy one. The peculiarities of the spread of smallpox are modeled to the greatest extent using experimental work with smallpox of rabbits and monkeys.

The importance of rabbit pox virus as a model agent for studying OPV infections was demonstrated in the early 1960s, when it was shown that hyperimmune sera provided protection to aerogenically infected rabbits when administered immediately after infection at a dose of 175 PFU (plaque forming units) per individual or even 3 days after infection. In this experiment, a dry biological formulation with an average particle size of about 1 μm was used [17].

Because aerosol particles larger than 10 μm are trapped in the upper respiratory tract, in almost all aerogenic infection experiments conducted by US Army Medical Research Institute of Infectious Diseases (USAMRIID) staff, the median diameter of generated particles penetrating the lower respiratory tract is 1 μm [18]. A number of parameters characterizing the course of rabbit smallpox in aerogenically infected animals makes it possible to model human smallpox disease (**Table 1**).

Thus, at low infectious doses (< 200 PFU), the incubation period was 4–6 days. The first clinical sign of the disease was fever, followed by anorexia, weakness, rapid weight loss, depression, lethargy, drop in body temperature to subnormal values and death on the 8–14th day after infection.

At high infectious doses (more than 200 PFU), rabbit pox virus caused a rapidly progressive lethal infection resembling the hemorrhagic form of smallpox. The incubation period of the disease in this case was 2–3 days. The disease ended with death on the 6th day.

According to the data of the USAMRIID Aerobiology Research Center, the LD_{50} value for aerogenic infection of rabbits with rabbit pox virus is 15 PFU [19]. This result coincides with the data obtained by H.S. Bedson et al. in 1963 using a dry preparation of rabbit pox virus [20].

When rabbits are aerogenically infected with a fine aerosol and the process of disease spread from one animal to another is studied, the above parameters are modeled for smallpox. Consequently, rabbit pox virus can be used to test protective agents against smallpox [19]. Rabbit smallpox virus can be used to model such characteristics of smallpox virus as the ability to cause aerogenic infection under conditions of low infectious dose and the ability to transmit infection from patients to healthy individuals [19]. M. Nicas et al. evaluated a mathematical model that determines the in-

[1] World Health Organization. Report of the meeting of the Ad hoc Committee on orthopoxvirus infections (Geneva, 09.09.1994). URL: https://iris.who.int/bitstream/handle/10665/59062/WHO_CDS_BVI_94.3.pdf?sequence=1

Table 1. Similarities and differences between smallpox and rabbit pox (by aerosol route of infection) [19]

Parameter	Nosological form		
	нSmallpox (common type)	rabbitpox (infectious dose < 200 PFU)	rabbitpox (infectious dose > 200 PFU)
Transmission method	Aerosol		
Incubation period, days	7–17	4–6	2–3
Prodromal phase, days	2–4		0–2
Clinical signs of the disease	Fever, pharyngitis, skin lesions	Fever, pharyngitis, skin lesions, erosions in the nasopharynx	Fever, pharyngitis, skin lesions, erosions in the nasopharynx
Characterization of skin lesions	Macules — papules — vesicles — pustules — crusts — pospinas	Macules — papules — vesicles — pustules	Macules — papules — vesicles
Complications	Pneumonia, blindness, encephalitis	Pneumonia, multiple necroses	
Lethality of the disease, %	≈ 30	≈ 100	100
Time of death, day from the beginning of the disease	22–28	8–14	5–7

Table 2. Results of evaluation of the effectiveness of specific and nonspecific means of protection against OPV (using rabbit pox virus, Utrecht strain, as a model agent in case of aerogenic infection) [22]

PFU infectious dose, Me, D	Preparation	Administration process that provides:	
		total protection	partial protection
175 (146–175)	Purified hyperimmune serum	10 ml of 1 : 100 dilution when administered 1 day after infection or 10 ml of whole drug when administered on the 3rd day after infection	10 ml of 1 : 10 dilution when administered on the 3rd day after infection
> 1000	Thiosemicarbazone	None	100–200 mg/kg of animal weight daily for 4 days
2860 (1140–5000)	ST-246	40 mg/kg of animal weight for 14 days at the first injection immediately after infection	40 mg/kg animal weight for 14 days at first injection, 24, 48 or 72 h after infection
296 (96–468)	Цидофовир Cidofovir	10 mg/kg animal weight for 3 days at first injection, either immediately or 24 h after infection	1 mg/kg animal weight for 3 days at the first injection either immediately or 24 h after infection

Note. Me — median infectious dose; D — range of variation of infectious dose during the experiment.

fectious dose of smallpox virus for conditions of aerogenic infection [21]. The authors concluded that one complete virion is sufficient to infect a person.

C.J. Roy et al. conducted a comparative study of the efficacy of nonspecific defenses against smallpox [22]. The antiviral drugs thiosemicarbazone, cidofovir and ST-246 were tested using rabbit smallpox virus as a model agent. For comparison, experiments were carried out with administration of a specific protective agent, namely a purified hyper-immune rabbit serum, to animals. Data on the efficacy of these antiviral preparations at aerogenic infection of animals with rabbit pox virus (**Table 2**) indicate that full protection of animals was revealed when using cidofovir at a dose of 10 mg/kg of animal weight for 3 days at the first injection either immediately or 24 h after infection, and ST-246 at a dose of 40 mg/kg of animal weight for 14 days (at the first injection immediately after infection). Thiosemicarbazone provided only partial protection.

A. Nalca et al. [19] and N.L. Garsa et al. [23] tested the efficacy of a third-generation smallpox vaccine (MVA-BN) in aerogenic infection of rabbits with rabbitpox virus. During a single immunization with a low dose of the vaccine, some rabbits showed some signs of disease, but all animals survived (**Table 3**). In case of double immunization with an interval of 14 days or a single immunization with a high dose of vaccine there were no signs of disease in animals.

Based on these studies, specialists from the USAMRIID Pathology, Toxinology and Aerobiology

Table 3. Results of the evaluation of the efficacy of the third-generation smallpox vaccine (MVA-BN) in aerogenic infection of rabbits with rabbit pox virus [19]

Animal group	Percentage of animals with signs of disease, %	Percentage of surviving animals, %
Once immunized with a low dose of vaccine followed by infection	30	100
Twice immunized with a low dose of vaccine followed by infection	0	100
Once immunized with a high dose of vaccine	0	100
Control group (infected animals without immunization)	100	0
Control group (once immunized with a high dose of vaccine without infection)	0	100

Note. An infectious dose of rabbit pox virus 200 CFU/animal was used in challenge experiments. The low vaccine dose was 1×10^3 PFU/specimen, and the high vaccine dose was 1×10^5 PFU/specimen.

Departments consider rabbitpox virus to be a promising agent mimic for smallpox virus [24, 25].

In 1999, monkeypox virus was included by the Ad Hoc Group of States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction in the List of Biological Agents – Human Pathogens, which was recognized as significant in the context of developing a list of biologically destructive agents for specific measures to strengthen the "Convention..." [26]. It should be noted that according to the public opinion formed before the middle of 1998, smallpox of monkeys was considered as a zoonotic infection, which is not significant for human pathology.

When analyzing the outlined studies (including aerobiological studies) conducted with the monkeypox virus at the leading specialized foreign military-medical center, the US Army Medical Research Institute of Infectious Diseases, two significant directions can be distinguished: modeling of the main characteristics of the disease caused by the smallpox virus in humans and evaluation of the effectiveness of existing and newly developed smallpox vaccines.

According to N. Hahon, a member of the U.S. Army Chemical Corps, monkeypox virus allows modeling some basic characteristics of the disease caused by human smallpox virus. Thus, according to the literature, 4 species of lower primates (*Macaca cynomolgus*, *M. irus*, *M. rhesus*, and *Saimiri*) are susceptible to aerogenic infection with smallpox virus [14].

Experimental infection in Javan macaques by aerogenic infection with monkeypox virus was studied [27]. Monkeypox virus, strain Zaire-79, isolated in 1979 during a fatal human disease, was used in this work. The inoculum for aerosol formation was the supernatant of infected Vero cells. The average mass diameter of the aerosol particles was 1.2 μm, and the calculated infectious dose ranged from 1.0×10^4 to 1.4×10^5 PFU. Javan macaques of both sexes weighing 1.6–4.7 kg were used in the experiment. The infectious dose was determined for each monkey during the entire exposure period (10 min). Aerosol sampling was performed in DMEM medium with defoamer. The virus concentration in the aerosol samples was determined by subsequent titration of the obtained samples using the negative colony method on a monolayer of Vero cells.

All infected monkeys died from the 10th to 17th day after infection (the average life time before the animals died was 11.2 days). Lethal outcome was associated with the development of bronchopneumonia. There was no correlation between the time of death and infectious dose.

Subsequently, an additional study of experimental infection was carried out during aerogenic infection of Javan macaques with monkeypox virus [28]. Infection was carried out using an automated system of biological aerosol exposure, which allows for precise administration of the infectious dose to each animal depending on its individual respiratory characteristics. The Zaire strain V79 was used in the aerogenic infection experiments. According to the data presented in **Table 4**, the outcome of the disease is apparently determined by the individual characteristics of the infected animals; in any case, no correlation between the administered dose and the proportion of dead animals was observed. At the same time, according to foreign military specialists, the clinical features of the disease in Javan macaques resemble the course of smallpox in humans [29].

Subsequently, the possibility of using reverse transcription polymerase chain reaction (RT-PCR) for quantitative determination of monkeypox virus in bioassays obtained from aerogenically infected Javan macaques was evaluated [30]. The sensitivity of the method was found to be 200 PFU/cm^3. The infectious dose ranged from 2.5×10^4 to 9.3×10^5 PFU. The mean median aerosol particle size was 1.07 μm and varied for each individual experiment between 1.06 and 1.09

Table 4. Results of the study of experimental infection indices during aerogenic infection of Javan macaques with monkeypox virus [28]

Indicator	Infectious dose, PFU			
	$4,3 \times 10^4$	$1,4 \times 10^5$	$4,4 \times 10^5$	$1,1 \times 10^4$
Fever				
onset, day after infection	4.7	3.8	2.8	4.3
duration, h	215.3	244.7	266.7	278.1
Temperature. °C				
maximum increase from normal	2.5	3.3	3.4	3.5
average increase from normal	1.9	1.9	2.1	2.3
Life expectancy before death, days	10.0	9.0	9.6	8.5
Percentage of dead animals	2/3	4/6	5/6	2/3

Table 5. Estimated virus concentration in the blood of monkeys aerogenically infected with monkeypox virus, PFU/cm³ [31]

Infectious dose, PFU/cm³	Time after infection, days								
	0	2	4	6	8	10	14	18	21
4.2×10^4	< 200	< 200	5.1×10^3	7.0×10^4	1.5×10^5	4.0×10^5	3.1×10^5	1.2×10^3	< 200
2.5×10^4	< 200	< 200	< 200	7.4×10^4	3.0×10^5	2.6×10^5	4.1×10^4	< 200	< 200
1.2×10^5	< 200	< 200	7.8×10^3	1.8×10^5	2.7×10^5	**	–	–	–
2.8×10^5	< 200	< 200	< 200	9.1×10^4	3.6×10^5	**	–	–	–
3.9×10^5	< 200	< 200	9.3×10^3	4.8×10^5	*	–	–	–	–
9.3×10^5	< 200	< 200	2.5×10^4	6.9×10^5	4.5×10^6	4.8×10^6	***	–	–

Note. Here and in Table 6: < 200 — concentration of the pathogen in blood is lower than the limit of detection of RT-PCR assay (200 PFU/cm³). *The animal died on the 7th day; **the animal died on the 9th day; ***the animal died on the 12th day after aerogenic infection.

Table 6. Estimated virus concentration in nasopharyngeal washings of monkeys aerogenically infected with monkeypox virus, PFU/cm³ [31]

Infectious dose, PFU/cm³	Time after infection, days								
	0	2	4	6	8	10	14	18	21
4.2×10^4	< 200	< 200	< 200	2.5×10^3	1.1×10^5	2.5×10^5	3.1×10^5	6.3×10^3	< 200
2.5×10^4	< 200	< 200	< 200	< 200	1.4×10^5	4.1×10^5	4.1×10^4	3.5×10^3	< 200
1.2×10^5	< 200	< 200	< 200	< 200	3.0×10^4	**	–	–	–
2.8×10^5	< 200	< 200	< 200	8.4×10^2	2.1×10^5	**	–	–	–
3.9×10^5	< 200	< 200	3.2×10^2	9.0×10^5	*	–	–	–	–
9.3×10^5	< 200	< 200	5.3×10^3	5.8×10^4	1.9×10^6	4.6×10^6	***	–	–

μm. The LD_{50} for monkeys for this mode of infection was approximately 7.8×10^4 PFU, and the time to death was 7–10 days after infection. Viremia and virus concentration in nasopharyngeal washings from infected animals were determined by extrapolation of the results of quantitative RT-PCR.

As follows from the data presented in **Table 5** and **Table 6**, monkeypox virus is detected in blood and nasopharyngeal washings on the 4th–18th day after aerogenic infection. The onset of detection of the pathogen correlates with the infecting dose.

Taking into account that the course of monkeypox in Javan macaques can simulate human smallpox disease, it can be concluded that the probability of virus transmission from a sick person to a healthy person reaches a maximum on the 8th–10th day (the virus con-

Table 7. Results of evaluating the protective efficacy of 2nd and 3rd generation smallpox vaccines (against monkeypox virus during aerogenic infection of Javan macaques) [32]

Group	Immunization process	Characterization of the course of the disease				
		clinical signs	time of papule appearance, days	average number of papules	duration of papule disappearance, days	survival rate, %
1	Administration of buffered saline 28 days before infection (control)	+++	6	51	Didn't disappear	0*
2	Injection of Acam2000 once at a dose of (2.5–12.5) × 10^5 PFU 28 days before infection by skin scarification	+	9	3	5	100
3	Subcutaneous injection of Imvamune once at a dose of 2.0 × 10^8 $TCPD_{50}$ 28 days before infection	++	9	10	5	67**
4	Subcutaneous injection of Imvamune twice at a dose of 2.0 × 10^8 $TCPD_{50}$ 28 days before infection	+	6	7	5	100

Note. $TCPD_{50}$ — 50% tissue cytopathic dose. + — mild; ++ — moderate; +++ — expressed signs of disease.
*Animals died on the 7th–11th day; **Animals died on the 7th and 9th day after aerogenic infection.

centration in nasopharyngeal washings has the highest values and approximately corresponds to the virus concentration in blood).

Specialists of the U.S. Department of Defense together with the U.S. Center for Disease Control and Prevention evaluated the protective efficacy of second-generation (Acam 2000) and third-generation (Imvamune) vaccines. In experiments on aerogenic infection of Javan macaques, monkeypox virus, strain Zaire 79, was used, with an infectious dose of (2.1–3.1) × 10^5 CFU per animal. The results presented in **Table 7** indicate that, although the level of virus-neutralizing antibodies was not significantly different between animals of groups 2 and 4, the signs of disease were slightly more pronounced in group 4. It is concluded that the use of aerogenic infection of Javan macaques provides an assessment of the efficacy of various vaccines intended for human immunization under conditions where clinical trials are not feasible [32]. It was found that the dynamics of antibody production in vaccinated Javan macaques is similar to that in vaccinated humans [33, 34].

Despite the fact that the cowpox virus is not considered a potential biologically destructive agent, leading foreign military medical centers, including the

Table 8. Results of evaluation of susceptibility of BALB/c white mice to aerogenic infection with cowpox virus [35]

Average weight of animals, g	Infectious dose, PFU	Disease symptoms	Average survival time to death, days	Percentage of dead animals, %
12	5 × 10^6	Reduced body weight, ruffled coat, significant decrease in functional activity	12	100
	5 × 10^4	Decrease in body weight, slight decrease in functional activity	–	0
	5 × 10^2	None	–	0
17	5 × 10^6	Decrease in body weight and functional activity	12	65

Table 9. Results of a study of the susceptibility of white mice to aerosolized infection with ectromelia, vaccinia and cowpox viruses [16]

Virus	Strain	White mouse line	Infection method	Infectious dose, PFU	Disease symptoms
Ectromelia	Hampstead	Autobred animals	Intranasal	1 × 10^6	Inflammation of the bronchi, alveoli, pleura
			Aerosol	1 × 10^6	Inflammation of the bronchi, alveoli, pleura
Vaccines	WR	BALB/c	Intranasal	1 × 10^6	Bronchopneumonia with manifestations of necrosis
Cowpox	Brighton	BALB/c	Aerosol	5 × 10^6	Bronchopneumonia, rhinitis, sinusitis, meningitis, exanthema

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U.S. Army Medical Research Institute of Infectious Diseases, conduct research with this pathogen. Analysis of the data published in the open press indicates that cowpox virus is also used in studies to evaluate the efficacy of existing and newly developed nonspecific prophylaxis against smallpox.

The results of the assessment of the susceptibility of BALB/c mice to aerogenic infection with cowpox virus (**Table 8**) indicate that aerogenic infection of BALB/c mice weighing 12 g with cowpox virus, Brighton strain, at a dose of 5×10^6 PFU causes 100% mortality of the animals.

Data on the susceptibility of inbred white mice to intranasal and aerosol infection with various OPVs (**Table 9**) indicate that all tested viruses caused respiratory tract damage. In case of aerosol infection with cowpox virus, symptoms of meningitis and exanthema were also recorded.

The study of morphologic changes in tissues of white BALB/c mice during intranasal or aerosol infection with cowpox virus, Brighton strain (**Table 10**) indicates that this pathogen is a promising model agent for screening tests of nonspecific prophylaxis against smallpox. This is due to the fact that the disease caused by this strain in the aerosol method of infection of white mice is characterized by a variety of symptoms, as well as the fact that this pathogen is pathogenic for humans, which simplifies the possibility of extrapolating the obtained data regarding the antiviral efficacy of the investigated therapeutic and prophylactic agents.

Thus, the antiviral effect of cidofovir (1-[(S)-3 hydroxy-2]-(phosphonomethoxy)-propyl cytosine) was

Table 10. Results of morphologic changes in tissues of white BALB/c mice during intranasal or aerogenic infection with cowpox virus, Brighton strain [24]

Tissues and organs	Morphologic changes	Infection method	Presence of cowpox virus antigen in organs
Lungs, bronchi, bronchioles, alveoli	Inflammation, eczema, necrosis, hemorrhages, inclusion bodies	Aerosol	+
		Intranasal	+
Bronchiolar vessels	Inflammation, necrosis, degeneration, inclusion bodies	Aerosol	+
Pleura	Inflammantion	Aerosol	–
Trachea	Inflammation, eczema, necrosis, corpuscles	Aerosol	+
Nasal tract	Inflammation, eczema, necrosis, hemorrhages, inclusion bodies	Aerosol	+
		Intranasal	+
Glands	Inflammation, eczema, necrosis, hemorrhages, inclusion bodies	Aerosol	+
		Intranasal	+
Connective tissues	Inflammation, hemorrhages	Aerosol	+
		Intranasal	+
Mammary gland ducts	Inflammation, necrosis, inclusion bodies	Aerosol	–
			+
Nasopharyngeal ducts	Inflammation, eczema, necrosis, hemorrhages, inclusion bodies	Intranasal	+
Eustathian pipe	Inflammation, inclusion bodies	Intranasal	+
Middle ear	Inflammation, necrosis, hemorrhages, inclusion bodies	Aerosol	+
		Intranasal	–
Muscles	Inflammation, necrosis, inclusion bodies, tissue regeneration	Aerosol	+
Bone marrow	Myelogenous hyperplasia	Aerosol	–
		Aerosol	+
Tail, skin	Inflammation, necrosis, inclusion bodies, epidermal proliferation	Aerosol	–
		Intranasal	+

Note. + — detection of labeled viral antigen by immunohistological method; – — No detection of labeled viral antigen.

studied on the model of white BALB/c mice aerogenically infected with cowpox virus, Brighton strain [35]. This strain causes bronchopneumonia in BALB/c mice when aerogenically infected with a fine aerosol (particle size 1 μm) with subsequent death. Subcutaneous administration of cidofovir at a dose of 100 mg/kg (once) provided 90–100% protection of aerogenically infected animals when administered not later than 4 days after infection. When cidofovir was administered on the day of infection, the virus titer in the lungs decreased 10–100 times, the severity of viral pneumonia decreased and pulmonary hemorrhages were prevented.

Administration of cidofovir did not cause an increase in the concentration of urea, creatine, aspartate aminotransferase and alanine aminotransferase in the blood sera of infected and intact animals. It was found that the disease did not develop with daily subcutaneous administration of cidofovir at doses of 20.5 and even 1 mg/kg. The time of the first administration of the drug is important. The dose of 5 m/kg protected almost 100% of mice when the drug was administered on the day of infection. However, if the beginning of drug administration was delayed for at least 1 day, daily administration of higher doses was required to protect the animals. Aerosol application of cidofovir was significantly more effective [36, 37]. The results of determining body weight, virus concentration in the lungs, pathologic changes in the lungs, and survival of infected animals established that a dose of cidofovir in the range of 0.5–5.0 mg/kg was always more effective than a dose of 25 mg/kg, and sometimes even more effective than a dose of 100 mg/kg when administered subcutaneously. Consequently, the antiviral efficacy of cidofovir is largely due to the drug retention in the respiratory tract of animals. Subsequently, the dependence of the antiviral efficacy of cidofovir on the scheme of its administration into the body of white mice aerogenically infected with cowpox virus was determined [37] (**Table 11**). Based on the results obtained, the authors of the study concluded that cidofovir when administered aerosolized can be effective in the prophylaxis or emergency prophylaxis of smallpox or monkeypox.

Analysis of the presented data indicates that specialists of the US Department of Defense use cowpox virus as a model agent for screening tests and methods of application of nonspecific medical defenses against smallpox. When summarizing the results of the presented studies, it is possible to conclude about the dual-use character of the conducted research. Thus, it can be stated that the USAMRIID staff has substantiated the

Table 11. Results of antiviral efficacy of cidofovir when administered by aerosol or subcutaneous injection to BALB/c white mice aerogenically infected with cowpox virus, Brighton strain, at a dose of 5 × 10^6 PFU [37]

Method of drug administration	Dose, mg/kg	Period of drug administration, day	Ratio of surviving to infected animals	Percentage of surviving animals, %	*p*
Aerosol	0.5–5.0	–2	8/10	80	< 0.05
		–1	9/10	90	< 0.05
		0	10/10	100	< 0.05
		+1	10/10	100	< 0.05
		+2	9/10	90	< 0.05
	0.06–0.50	–2	0/10	0	N. d.
		–1	7/10	70	< 0.05
		0	10/10	100	< 0.05
		+1	9/10	90	< 0.05
		+2	7/10	70	< 0.05
Subcutaneously	100	–2	7/10	70	< 0.05
		–1	7/10	70	< 0.05
		0	10/10	100	< 0.05
		+1	10/10	100	< 0.05
		+2	10/10	100	< 0.05
Placebo		0	0/10	0	–

Note. –2 — administration of cidofovir 2 days before infection; 0 — administration of cidofovir on the day of infection; +2 — administration of cidofovir 2 days after infection. *p* — reliability level of differences in relation to the experiment variant with placebo administration. N. d. — differences are not reliable.

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choice of rabbit pox and monkey pox viruses as agent mimics of the smallpox virus.

At the same time, the data obtained in the early 2000s were compared by US military specialists with the results obtained in the early 1960s using a dry agent mimic based on rabbitpox virus [20]. In their opinion, the rabbit pox virus can simulate such characteristics of the smallpox virus as the level of reproduction in various systems, including cell cultures in suspension cultivation, and resistance when transferred to aerosol. The LD_{50} value for rabbits during aerogenic infection is quite low (in contrast to other laboratory animals during aerogenic infection with other OPVs) [19].

When conducting aerobiological studies, special attention was paid to the fractional-disperse composition of the agent simulant. Such specification is obviously unnecessary for the declared by the authors goals of the conducted studies. As an infectious preparation for these studies, in most of the research projects of USAMRIID staff, the direct culture of monkey pox and rabbit pox virus strains was used. However, according to a number of indirect signs (composition of sampling fluids, presence of different concentrations of defoamer, different concentrations of fetal calf serum), it can be concluded that in reality, during a number of aerobiological tests, virus-containing materials obtained by growing the pathogen in suspension cell culture were used as an infectious preparation.

Conclusion

The presented results of aerobiological studies with OPVs indicate the interest of the U.S. Military Department in conducting dual-purpose experimental work, including monitoring for the properties of OPVs and possible changes in their pathogenicity for humans, selection of optimal laboratory models for studying the properties of OPVs as well as the possibility of modeling the properties of smallpox virus using other OPVs (cowpox, rabbitpox, monkeypox viruses), modeling the main properties of the disease caused by smallpox virus in humans and evaluation of the efficacy of available and newly developed smallpox vaccines, and the comparative study of the efficacy of antiviral drugs for regular or post-exposure prophylaxis of naturally occurring smallpox and monkey smallpox.

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Information about the authors

Gennadiy G. Onishchenko — D. Sci. (Med.) Professor, RAS Full Member, Head, Department of human ecology and environmental hygiene, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia,
https://orcid.org/0000-0003-0135-7258

Igor A. Kirillov — Cand. Sci. (Military), Chief, Nuclear, Biological and Chemical Protection Troops of the Ministry of Defence of the Russian Federation, Moscow, Russia, https://orcid.org/0000-0001-5001-3326

Sergey V. Borisevich✉ — D. Sci. (Biol.), Professor, RAS Full Member, Head, 48 Central Scientific Research Institute of the Ministry of Defense of the Russian Federation, Sergiev Posad-6, Russia,
48cnii@mil.ru, https://orcid.org/0000-0002-6742-3919

Tatiana E. Sizikova — Cand. Sci. (Biol.), senior researcher, 48 Central Scientific Research Institute of the Ministry of Defense of the Russian Federation, Sergiev Posad-6, Russia,
https://orcid.org/0000-0002-1817-0126

Victor T. Krotkov — Cand. Sci. (Med.), senior researcher, 48 Central Scientific Research Institute of the Ministry of Defense of the Russian Federation, Sergiev Posad-6, Russia,
https://orcid.org/0000-0002-7674-2321

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Информация об авторах

Онищенко Геннадий Григорьевич — д.м.н., профессор, академик РАН, зав. каф. экологии человека и гигиены окружающей среды ПМГМУ им. И.М. Сеченова (Сеченовский университет), Москва, Россия, https://orcid.org/0000-0003-0135-7258

Кириллов Игорь Анатольевич, канд. воен. наук, начальник войск радиационной, химической и биологической защиты Вооруженных Сил Российской Федерации, Москва, Россия,
https://orcid.org/0000-0001-5001-3326

Борисевич Сергей Владимирович✉ — д.б.н., профессор, академик РАН, начальник 48 ЦНИИ Минобороны России, Сергиев Посад-6, Россия, 48cnii@mil.ru,
https://orcid.org/0000-0002-6742-3919

Сизикова Татьяна Евгеньевна — к.б.н., с.н.с. научно-исследовательского отдела 48 ЦНИИ Минобороны России, Сергиев Посад-6, Россия, https://orcid.org/0000-0002-1817-0126

Кротков Виктор Тимофеевич — к.м.н., с.н.с. научно-исследовательского отдела 48 ЦНИИ Минобороны России, Сергиев Посад-6, Россия, https://orcid.org/0000-0002-7674-2321

Участие авторов: *Онищенко Г.Г., Кириллов И.А.* — разработка концепции статьи, обобщение полученных данных; *Борисевич С.В.* — сбор и анализ данных, обобщение полученных данных, редактирование текста статьи, утверждение окончательного варианта статьи; *Сизикова Т.Е.* — сбор и анализ данных, формирование текста статьи; *Кротков В.Т.* — оценка полученных данных. Все авторы подтверждают соответствие своего авторства критериям Международного комитета редакторов медицинских журналов, прочли и одобрили финальную версию до публикации.

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