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Comparative analysis of residual neurovirulence of vaccine and low attenuated rubella virus (*Matonaviridae: Rubivirus: Rubella virus*) strains in the experiments on the macaque rhesus (*Macaca mulatta*) monkeys

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Introduction. Rubella is currently an infection controlled by specific prophylaxis. Not only the right vaccine prophylaxis strategy and tactics, but also the use of effective and safe vaccine preparations is crucial for the elimination of this disease.
The **aim** of the investigation was to study the morphological and pathogenetic patterns of changes developing in the central nervous system (CNS) and internal organs of monkeys (*Haplorhini*) during intracerebral inoculation with 2 strains of rubella virus (*Matonaviridae: Rubivirus: Rubella virus*) (RV): highly attenuated Orlov-B, and low attenuated Orlov-14.
Material and methods. In the experiments, seronegative rhesus macaque monkeys (*Macaca mulatta*) weighing 3.3–5.1 kg (*n* = 7) were used. Neurovirulence of the strains was determined by a complex of clinical, pathomorphological, and virological methods.
Results and discussion. It was found that during attenuation, the Orlov-B strain lost the ability to replicate in CNS cells and induce moderate/expressed specific changes in them, as well as to overcome the blood-brain barrier and cause the damage of sensitive organs and tissues. This fact indicates a low level of residual neurovirulence of the vaccine strain.
Conclusion. The results obtained in this study regarding the clinical symptoms of CNS lesions and the nature of the pathological process in its tissues in experimental animals can be significant for the improvement of safety control of live rubella vaccines. These data indicate that the Orlov-B strain can be considered as a candidate strain for further study on the development of a rubella vaccine based on the domestic vaccine strain.

Key words: *residual neurovirulence, rubella virus (RV), attenuation, macaque rhesus (Macaca mulatta) monkeys*

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НАУЧНАЯ СТАТЬЯ
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Сравнительный анализ остаточной нейровирулентности вакцинного и низкоаттенуированного штаммов вируса краснухи (*Matonaviridae: Rubivirus: Rubella virus*) в эксперименте на обезьянах вида макак резус (*Macaca mulatta*)

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Введение. Краснуха в настоящее время представляет собой инфекцию, управляемую средствами специфической профилактики. Для ликвидации этого заболевания решающее значение имеют не только правильно выбранные стратегия и тактика вакцинопрофилактики, но и применение эффективных и безопасных вакцинных препаратов.
Цель исследования – изучение морфологических и патогенетических характеристик изменений, развивающихся в центральной нервной системе (ЦНС) и внутренних органах обезьян (*Haplorhini*), при интрацеребральной инокуляции 2 штаммами вируса краснухи (ВК) (*Matonaviridae: Rubivirus: Rubella virus*): высокоаттенуированным «Орлов-В» и низкоаттенуированным «Орлов-14».
Материал и методы. В экспериментах использовали серонегативных к ВК обезьян вида макак резус (*Macaca mulatta*) массой 3,3–5,1 кг (*n* = 7). Определение нейровирулентности штаммов выполняли посредством комплекса клинических, патоморфологических и вирусологических методов. Клиническое наблюдение за животными осуществляли ежедневно на протяжении 28 сут после инокуляции. Титрование вируса проводили в соответствии со стандартной методикой по цитопатическому действию (ЦПД) (показателю $ТЦД_{50}$/мл – тканевая цитопатическая доза) в культуре клеток ВНК-21. Титр ВК рассчитывали по методу Рида и Менча.
Результаты и обсуждение. Установлено, что в процессе аттенуации штамм «Орлов-В» утратил способность к репликации в клетках ЦНС и индуцированию в них умеренных/выраженных специфических изменений, а также к преодолению гематоэнцефалического барьера с поражением чувствительных органов и тканей. Указанный факт свидетельствует о низком уровне остаточной нейровирулентности вакцинного штамма.
Заключение. Полученные в рамках настоящего исследования результаты относительно клинических симптомов поражения ЦНС и характере патологического процесса в её тканях у экспериментальных животных моделей могут быть значимыми для совершенствования контроля безопасности живых краснушных вакцин. Эти данные позволяют рассматривать штамм «Орлов-В» в качестве кандидатного при дальнейшей работе по созданию краснушной вакцины на основе отечественного вакцинного штамма.

Ключевые слова: *остаточная нейровирулентность; вирус краснухи (ВК); аттенуация; обезьяны макак резус (Macaca mulatta)*

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Introduction

Rubella virus (*Matonaviridae: Rubivirus: Rubella* virus) (RV) is the causative agent of anthroponous infection that occurs in the form of mild infectious exanthematosis in children and severe intrauterine damage to the fetus in pregnant women, which leads to disruption of human ontogenesis, the development of congenital malformations and as a consequence to spontaneous abortion, stillbirth and the birth of children with congenital rubella syndrome (CRS) [1, 2].

Currently, specific prophylactic of rubella infection, and especially the prevention of CRS, is carried out using live vaccines [3, 4]. Due to the fact that live vaccines contain although weakened but live neurovirulent strains, increased requirements are imposed on vaccine strains in terms of their attenuation level [5, 6]. Thus, when testing the derivative of the first licensed HPV-77 strain, HPV-77DE5 [7] and an experimental series of vaccines prepared from the 16th passage the Orlov strain [8] on volunteers, the clinical reactions typical of rubella were revealed in a number of cases: rash, fever, and enlargement of the occipital lymph nodes. In this case, the reactogenicity of the «Orlov» strain was 30%. Therefore according to the current regulatory documents, the safety of attenuated vaccines, along with immunogenicity, is the most important criterion for the suitability of a strain as a live attenuated vaccine [9].

Thus, the study of the character and features of the rubella pathological process in the central nervous system and extra neural organs of monkeys (*Haplorhini*) intracerebrally infected with rubella virus strains with different levels of attenuation is very relevant.

The aim of this work was to study the pathogenesis features of the rubella pathological process developing in the central nervous system (CNS) and internal organs of monkeys intracerebrally inoculated with a highly attenuated strain Orlov-V and a low-attenuated one Orlov-14.

Material and methods

The highly attenuated vaccine strain Orlov-V (39 passages in a primary trypsinized culture of rabbit kidney cells (PPK)), and a low-attenuated strain Orlov-14 (14 passages in PPK) of rubella virus obtained from the collection of the Pasteur' St. Petersburg FSBI «Saint Petersburg Pasteur Research Institute of Epidemiology and Microbiology» («RI MP») were used.

The study was carried out on 7 clinically healthy *Macaca mulatta* weighing 3.3–5.1 kg, born and kept in the colony of the FSBRI «RI MP». The animals selected for experiment did not have the neutralizing antibodies to RV. The monkeys were randomized into 3 groups. The animals of the first group ($n = 3$) were injected intracerebrally with a preparation of highly attenuated strain Orlov-V with an infectious titer of 4.7 lg $TCID_{50}$/0.5 ml. Monkeys of the second group ($n = 3$) were injected differentially with a material containing a low-attenuated strain Orlov-14 with an infectious titer of 4.7 lgTCD_{50}/0.5 ml or 3.8 lg TCD_{50}/0.5 ml. An animal of the third group ($n = 1$) was injected with a solvent for a lyophilized commercial rubella vaccine (water for injection).

The virus-containing material was administered to monkeys under deep anesthesia, which was achieved by the injection of 0.1 ml of xyla (InterchemiVerken De Adelaar Eesti AS, Estonia) and 0.05 ml of zoletil (Valdepharm, France) per 1.0 kg of animal weight. The material was injected in a volume of 0.25 ml into the optic tubercle of each cerebral hemisphere. With the aim of the subsequent formation of a «biological suture» at the time of infection, the scalp was pulled back and fixed by hand. The needle 5.0 cm long and 0.6 mm in diameter was used for infection. The virus-containing material was injected into the trepanation hole made with a drill with a diameter of 1.5 mm, retreating 0.5 cm back from the coronary suture and 1.0 cm lateral to the sagittal suture to a depth of 2.5 cm.

Clinical observation of the monkeys was carried out on daily basis for 28 days, during which the presence or absence of general clinical symptoms (loss of appetite, fever, lethargy, anxiety) and signs of CNS damage (tremor of the extremities, impaired coordination, paresis, paralysis) were recorded.

The animals were sacrificed on the 12, 21 and 28 days. Before euthanasia, the monkeys were put into deep anesthesia by injecting 1.0 ml of zoletil (Valdepharm, France) and 4.0 ml of xyly (Interchemiverken De Adelaar Eesti AS, Estonia) into the inguinal vein of the animal. After

the animal entered deep sleep, 5.0 ml of listenone (Takeda Austria GmbH, Austria) was injected into the same vein, which led to complete cardiac arrest.

For histological and virological studies during autopsy, tissues of the CNS (brain and spinal cord), lymph nodes (submandibular and posterior cervical), internal organs (lung, liver, kidney, spleen), and cerebrospinal fluid (CSF) were taken.

Rubella virus titration was performed by cytopathic action (CPE) on BHK-21 cell culture. A 10% suspension in 0.9% NaCl was prepared using the material from various parts of the CNS and internal organs. To obtain the supernatant the suspension was centrifuged for 10 min at 1000 rpm. The resulting supernatant was used to prepare serial 10-fold dilutions ranging from 10^{-1} to 10^{-8}. Then, 100 µl of each of the dilution was added to 4 wells of a 96-well culture plate. Infected and control cell cultures were cultured in incubator with 5% CO_2 at 35 °C. The results were scored on the 12 day according to the CPE reaction. The virus titer was calculated by the method of Reed and Mench.

The material taken for histological examination was fixed in 10% neutral buffered formalin solution, dehydrated in alcohols according to the standard technique, embedded in paraffin blocks and cut into sections with a thickness of 4–5 µm. Sections were stained with hematoxylin, eosin, and cresyl violet according to the Nissl method. Morphological analysis was performed using a biological microscope for laboratory studies AXIOLAB.A1 (CarlZeiss Microscopy GmbH, Germany). Axiocam 105 color digital camera (CarlZeiss Microscopy GmbH, Germany) was used for microphotography.

The anterior and posterior central gyrus of the right and left hemispheres, the thalamus (visual hillocks), the midbrain, the varoliev bridge, the cerebellum, the medulla oblongata, the cervical and lumbar thickening of the spinal cord, as well as the submandibular, occipital and posterior cervical lymph nodes, lung, spleen and the liver were subjected to histological analysis. The degree of pathomorphological changes in the CNS of animals was determined using a four-point scale recommended by the Ministry of Health of the Russia for «Assessment of the specific safety of industrial strains and inoculum viruses of measles, mumps and rubella» OFS.1.7.2.0010.15.

Results

During the period of clinical observation none of the animals of the 1st and 3rd groups of monkeys infected with the Orlov-V strain showed the manifestation of general clinical and neurological symptoms typical for this disease: fever, appetite disorders, lethargy, anxiety, tremor of the limbs, and lack of coordination, paresis and paralysis. The clinical state of the experimental animals corresponded to the physiological norm. In monkey No. 42 884 (group II), inoculated with a low-attenuated strain of RV Orlov-14 in a titer of 4.7 $lgTCD_{50}$/0.5 ml on the 10th day of the experiment, an increase in body temperature up to 39.5 °C was observed (**Table 2**), as well as a lack of appetite and lethargy. On the 11th and 12th days of the experiment, the following deviations from the physiological norm were recorded: lethargy, weakness of the limbs, tremor of the left upper limb, lack of jumping and climbing the cage. In animals Nos. 43 389 and 43 419, also infected with a low-attenuated strain of the rubella virus Orlov-14, but with a lower dose, 3.8 lg TCD_{50}/0.5 ml, there was a slight increase in body temperature and a decrease in appetite, but no other disorders of the CNS were noted.

In monkey No. 43 389, increased in body temperature was recorded for 3 days, from 15 to 17 days of the experiment; in monkey No. 43419 for 2 days, from 14 to 15 days of the experiment. After that the general clinical state of the animals did not differ from the physiological norm.

According to virological studies, the infectious virus was not isolated from any samples of monkeys infected with the Orlov-V strain and euthanized on the 12, 21 and 28 days after infection (**Table 1**). However, in monkeys infected by the Orlov-14 strain with infectious titers of 4.7 lg TCD_{50}/0.5 ml and 3.8 $lgTCD_{50}$/0.5 ml and sacrificed on the 12 and 21 days, the virus was detected in various parts of the CNS, lymph nodes, and peripheral organs. It should be noted that in a monkey inoculated with the Orlov-14 strain with an infectious titer of 3.8 lg TCD_{50}/0.5 ml and withdrawn from the experiment on the 28 day, the infectious virus was not detected either in the CNS or in the peripheral organs.

Localization and degree of pathomorphological changes in the CNS of monkeys were determined on a four-point scale. According to the histological results of the central nervous system of monkeys inoculated with the vaccine strain Orlov-V (group I) with an infectious titer of 4.7 $lgTCD_{50}$/0.5 ml, there were no moderate and pronounced degenerative changes in the brain and spinal cord of the animals. In monkey No. 38 976 euthanized on day 12 the spread of the pathological process was revealed in the motor area of the cerebral cortex and in the thalamus. The average score for monkey CNS lesions was 0.3 (**Table 2**).

In monkey No. 43 764 euthanized on day 21 and in monkey No. 43 456 euthanized on day 28 after infection with the Orlov-V vaccine strain no pathological changes in neurons and glial cells were found in the brain and spinal cord. The brain membranes were thin, there was no hyperemia and structure corresponded to the parts. The nuclei of the nerve cells were light with distinct nucleoli. The Nisslevian substance was clearly contoured. Glia had no changes (**Fig. 1**). The average score for CNS lesions in these monkeys was 0.0.

According to the results of a histological study of the CNS of group II monkeys, depending on the observation period (12, 21 and 28 days), both single vasculitis and dystrophic changes in neurons were observed. Pronounced inflammatory changes characteristic for severe meningoencephalomyelitis was also disclosed. So, in monkey No. 42 884 euthanized on the 12 day after the inoculation with the low-attenuated strain Orlov-14 with a titer of 4.7 lg TCD_{50}/0.5 ml the pronounced lymphocytic infiltration, multiple or focal vasculitis at the injection

site and behind it along with meningoencephalomyelitis were found. Focal inflammatory changes were found in almost all parts of the brain, in the cervical and lumbar spinal cord. The average score for CNS lesions was 3.0.

CNS damage was also detected in monkey No. 43 389 inoculated with a low-attenuated strain Orlov-14 in a titer of 3.8 lgTCD$_{50}$/0.5 ml and sacrificed on the 21 day after infection. In this animal the characteristic lesions of focal encephalitis were found, namely, moderate lymphohistiocytic infiltration of the meninges with involvement of the vascular plexus of the brain and focal inflammatory changes in both the gray and white matter of the brain. The average score for CNS lesions was 1.8.

In monkey No. 43 419, euthanized on the 28th day after the injection of the low-attenuated strain Orlov-14 with a titer of 3.8 lg TCD$_{50}$/0.5 ml, a weak infiltrative-productive reaction was revealed. Isolated vasculitis, dystrophic changes in nerve cells and small infiltrates were found in both white and gray matter of the brain (**Fig. 2**). The mean score for monkey CNS lesions was 1.3.

In monkey No. 36 518 (group III), euthanized on 28 day after infection, no pathological changes in brain and spinal cord neurons and glial cells were found. The nuclei of the nerve cells are light with distinct nucleoli. The Nisslevian substance was clearly contoured, and glia was without features. The average score for CNS lesions in this monkey was 0.0.

Histological examination of the internal organs of experimental animals did not reveal pronounced pathological changes in all the examined tissues. All organs and tissues had a typical anatomical and histological structure. In all experimental animals the structure of the lungs, liver, kidneys, pancreas and spleen was characterized as typical without any abnormal characteristics. Hyperemia and extraneous cellular infiltration were absent. Lung tissue was defined as airy. The interalveolar septa were thin, the lumens of the alveoli and bronchi were free. In monkey No. 42 884, infected with a low-attenuated strain Orlov-14 and sacrificed on the 12 day, signs of activation of the occipital, posterior cervical and submandibular lymph nodes were found. In animals Nos. 43 389 and 43 419 infected with the low-attenuated strain Orlov-14 and sacrificed on the 21 and 28 days, respectively, signs of activation were found only in the submandibular lymph nodes.

Discussion

Comparative analysis of the data obtained in the study of residual neurovirulence of the highly attenuated strain Orlov-V and the low-attenuated one Orlov-14 in the intracerebral infection test of monkeys is of great theoretical and practical importance. Nonhuman primates (*Primates*) are the most appropriate laboratory model for the experimental study of attenuated rubella virus strains [12, 13]. Rubella virus is pathogenic, in particular for monkeys

Table 1. Virus titers in various parts of the central nervous system and peripheral organs of monkeys inoculated intracerebrally with a highly attenuated strain Orlov-V and a low attenuated strain Orlov-14 of rubella virus

Таблица 1. Титры вируса в различных отделах центральной нервной системы и внутренних органах обезьян, интацеребрально инокулированных высокоаттенуированным штаммом «Орлов-В» и низкоаттенуированным штаммом «Орлов-14» вируса краснухи

	Group I Группа I			Group II Группа II			Group III Группа III
Monkey ID Номер животного	38 976	43 764	43 456	42 884	43 389	43 419	36 518
Time of euthanasia, day Время эвтаназии, сут	12	21	28	12	21	28	28
Tested sample Исследуемый образец	Virus titer, lg TCD$_{50}$/ml Титр вируса, lg ТЦД$_{50}$/мл						
Brain Головной мозг	0,0	0,0	0,0	4,0	0,0	0,0	0,0
Cervical part of the spinal cord Шейный отдел спинного мозга	0,0	0,0	0,0	3,2	0,0	0,0	0,0
Lumbar part of the spinal cord Поясничный отдел спинного мозга	0,0	0,0	0,0	1,8	0,0	0,0	0,0
Cerebrospinal fluid Цереброспинальная жидкость	0,0	0,0	0,0	2,4	0,0	0,0	0,0
Submandibular lymph nodes Поднижнечелюстные лимфатические узлы	0,0	0,0	0,0	0,0	2,6	0,0	0,0
Posterior cervical lymph nodes Задние шейные лимфатические узлы	0,0	0,0	0,0	2,3	1,8	0,0	0,0
Lung Лёгкое	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Spleen Селезёнка	0,0	0,0	0,0	2,0	2,0	0,0	0,0
Liver Печень	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Table 2. Assessment of the intensity of pathomorphological changes in the parts of the central nervous system of experimental primates

Таблица 2. Оценка выраженности патоморфологических изменений в исследованных отделах центральной нервной системы экспериментальных приматов

			Symbols of the departments of the central nervous system of primates subject to histological examination Условные обозначения отделов центральной нервной системы, подлежавших гистологическому исследованию														
			Brain divisions Отделы головного мозга												Spinal cord divisions Отделы спинного мозга		
			L **Л**	**A** **А**	**D** **Д**	**G** **Г**	**T** **Т**	V V	HP HP	O З	SA xxxx	OP xxx	CP xx	CAP x	CSC ш.о.	HSC п.о.	
Group Группа	Monkey ID № животного	Time of euthanasia, day Время эвтаназии, сут	Intensity of pathomorphological changes, scores Выраженность патоморфологических изменений, баллы														Mean score Средний балл
	38 976	12	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0,3
I	43 764	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0
	43 456	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0
	42 884	12	3	3	3	4	3	3	2	3	3	3	3	3	3	3	3,0
II	43 389	21	2	2	2	2	2	2	2	1	2	2	2	2	1	1	1,8
	43 419	28	1	2	2	2	2	2	2	1	1	1	1	1	0	0	1,3
III	36 518	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0

Note. L, frontal cerebral cortex; A, anterior horns of the lateral ventricles and sections of the subcortical ganglia: the head of the caudate nucleus, the anterior leg of the inner capsule, lenticular nucleus; D, motor area of the cerebral cortex; G, central parts of the lateral ventricles, third ventricle, caudate nucleus, thalamus, inner capsule, lenticular nucleus; T, parietal cerebral cortex; V, central parts of the lateral ventricles and the third ventricle, the body of the caudate nucleus, the nuclei of the thalamus, the posterior leg of the inner capsule; HP, hippocampus, lower horns of the lateral ventricles; O, occipital cerebral cortex; SA, cerebral aqueduct (aqueduct Sylvii), substantia nigra (Nisslevian substance), nuclei of the midbrain; OP, oral part of the fourth ventricle, own nuclei of the pons (Varolii) and the sections of the pathways located in it; CP, the central part of the fourth ventricle, nuclei and sections of the pathways located at the bottom of the rhomboid fossa of the medulla oblongata; CAP, caudal part of the fourth ventricle, olive nuclei, reticular formation, cranial nerve nuclei, sections of the pathways; CSC, cervical spinal cord: the nuclei of the gray matter and the pathways of the white matter; HSC, lumbar spinal cord.

Примечание. Л – лобная зона коры большого (головного) мозга; А – передние рога боковых желудочков и отделы подкорковых ганглиев: головка хвостатого ядра, передняя ножка внутренней капсулы, чечевицеобразное ядро; Д – двигательная зона коры большого (головного) мозга; Г – центральные части боковых желудочков, III желудочек, хвостатое ядро, таламус, внутренняя капсула, чечевицеобразное ядро; Т – теменная зона коры большого (головного) мозга; V – центральные части боковых желудочков и III желудочек, тело хвостатого ядра, ядра таламуса, задняя ножка внутренней капсулы; HP – гиппокамп, нижние рога боковых желудочков; З – затылочная зона коры большого (головного) мозга; xxxx – водопровод мозга (сильвиев), чёрное (нисслевское) вещество, ядра среднего мозга; xxx – оральная часть IV желудочка, собственные ядра моста (варолиева) и расположенные в нём участки проводящих путей; xx – центральная часть IV желудочка, ядра и участки проводящих путей, расположенные в дне ромбовидной ямки продолговатого мозга; x – каудальная часть IV желудочка, ядра олив, ретикулярная формация, ядра черепных нервов, участки проводящих путей; ш.о. – шейный отдел спинного мозга: ядра серого вещества и проводящие пути белого вещества; п.о. – поясничный отдел спинного мозга.

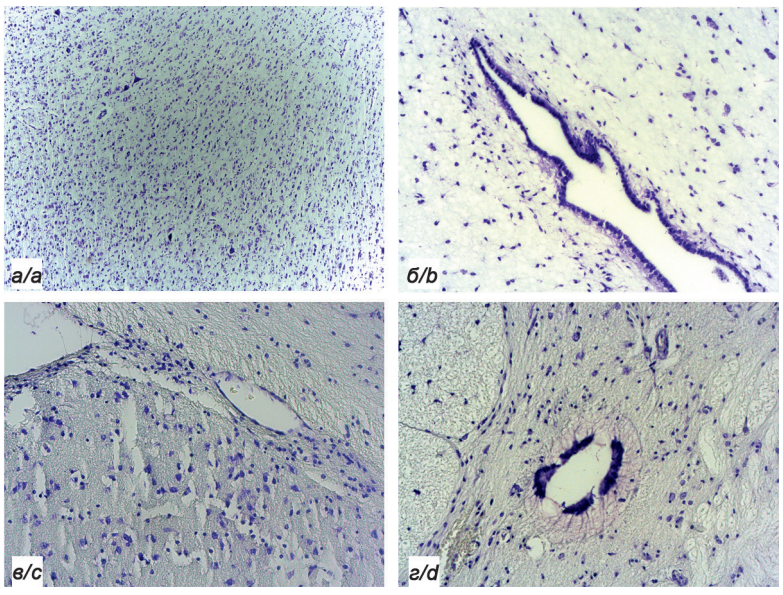

Fig. 1. Histological structure of the brain tissue of monkey No. 43 456, euthanized on day 28 after infection with the vaccine strain «Orlov-V»: *a)*, place of viral injection. There is typical histological structure of brain tissue. No pathological changes are found; *b)*, thalamus. No pathological changes are found; *c)*, precentral gyrus. Ependymal epithelium has a typical histological structure; *d)*, lumbar part of the spinal cord. The structure of the brain tissue is normal. Glial elements are without activation phenomena. Microphotographs, Nissl staining (cresyl violet dye), magnification: ×50 (*a*); ×200 (*b–d*).

Рис. 1. Гистологическая структура мозговой ткани обезьяны № 43 456, эвтаназированной на 28 сут после заражения вакцинным штаммом «Орлов-В». *а)* – место введения. Типичное гистологическое строение мозговой ткани. Патологических изменений не выявлено; *б)* – таламус. Патологические изменения отсутствуют; *в)* – предцентральная извилина. Эпендимный эпителий типичного гистологического строения; *г)* – поясничный отдел спинного мозга. Структура мозговой ткани без особенностей. Глиальные элементы без явлений активации. Микрофотографии, окраска по методу Ниссля (краситель крезиловый фиолетовый), увеличение ×50 (*а*), ×200 (*б–г*).

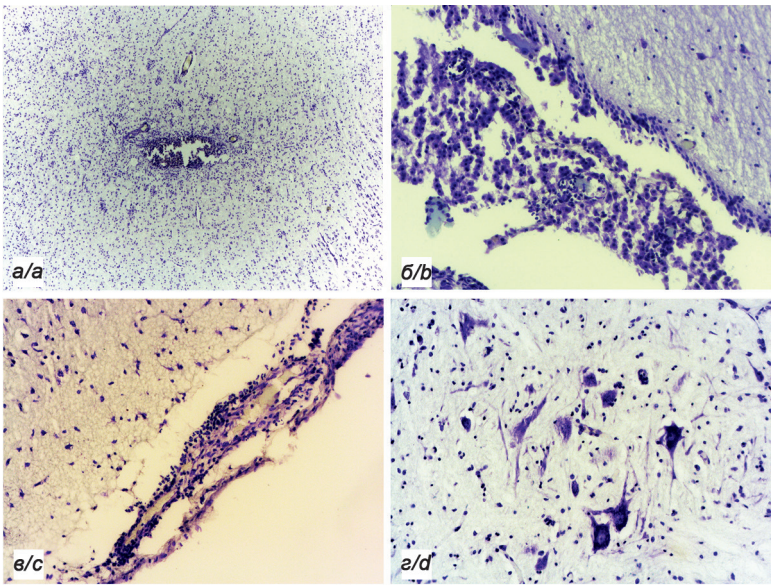

Fig. 2. Histological structure of the brain tissue of monkey No. 43 419, euthanized on day 28 after infection with a low attenuated strain «Orlov-14» in a titer of 3.8 lg TCD_{50}/0.5 ml. *a)*, place of viral injection. There are focal gliosis and moderate dystrophic changes in nerve cells in the inoculation zone; *b)*, thalamus shows the dystrophic changes in neurons and signs of vasculitis; *c)*, precentral gyrus. There are focal lymphohistiocytic infiltration of the pia mater and moderate dystrophic changes in neurons in the area of inflammation; *d)*, lumbar part of spinal cord. The histological structure of the tissue is the typical one. No pathological changes are found. Microphotographs, Nissl staining (cresyl violet dye), magnification: ×50 (*a*); ×200 (*b–d*).

Рис. 2. Гистологическая структура мозговой ткани обезьяны № 43 419, умерщвлённой на 28 сут после заражения низкоаттенуированным штаммом «Орлов-14» в титре 3,8 lg $ТЦД_{50}$/0,5 мл. *а)* – место введения. Очаговый глиоз, умеренно выраженные дистрофические изменения нейронов в зоне инокуляции; *б)* – таламус. Дистрофические изменения нервных клеток, явления васкулита; *в)* – предцентральная извилина. Очаговая лимфогистиоцитарная инфильтрация мягкой мозговой оболочки. Умеренные дистрофические изменения нейронов в зоне воспаления; *г)* – поясничный отдел спинного мозга. Типичное гистологическое строение ткани. Патологические изменения отсутствуют. Микрофотографии, окраска по методу Ниссля (краситель крезиловый фиолетовый), увеличение ×50 (*а*), ×200 (*б – г*).

of *Macaca mulatta* species, which are sensitive to intranasal, intramuscular and intravenous infection [14]. According to the published data the pathogenesis of the disease caused by the rubella causative agent is similar to that in humans in terms of such parameters as the duration of the incubation period and viral shedding, the presence of viremia, the dynamics of the formation of virus-specific antibodies [15].

In our experiment the reactogenic strain Orlov-14 was used as a positive control, which was administered in the same (4.7 lg TCD_{50}/0.5 ml) and lower (3.8 lg TCD_{50}/0.5 ml) doses than strain Orlov-V.

During the study the data on the clinical observation of experimental animals were obtained. In animals infected with the low-attenuated strain Orlov-14 depending on the dose of the infectious virus the appearance of clinical and neurological symptoms typical of rubella was observed. In animals of group I no clinically significant symptoms indicative of damage to the central nervous system (tremor, paresis and paralysis) were found. There were also no changes in the behavioral characteristics of animals. They retained their standard movements in the cages (jumping, climbing, grabbing objects). Thus, the results of clinical observation of animals underline the main criteria for the suitability for live attenuated vaccines strains – the absence of reactogenicity of the Orlov-V strain [16].

Taking into account that the histological study of the CNS of monkeys in the test of intracerebral infection is currently the only recommended method for assessing the specific safety of attenuated vaccine strains, a pathomorphological study of the CNS of experimental animals was carried out. Comparative analysis of the results of histological examination of various parts of the CNS of monkeys generally shows the loss of ability of the vaccine strain Orlov-V to propagate in the cells of the central nervous system causing the development of moderate and pronounced degenerative changes in the brain and spinal cord of animals.

The data obtained during the morphological and histological examination of the internal organs of experimental animals (the absence of virus-specific lesions in the studied organs and tissues) points to a loss of the ability to penetrate the blood-brain barrier during the Orlov-V vaccine strain attenuation which is an important indicator of the safety of the strain.

Results of the virological analysis of various parts of the CNS and peripheral organs of *Macaca mulatta* also indicate the loss of the vaccine strain Orlov-V its ability to infect CNS cells and cross the blood-brain barrier and confirms the data of histological studies of the CNS and internal organs of experimental animals, which indicates a low level of residual neurovirulence of the vaccine strain Orlov-V.

Conclusion

This work evaluates the degree of neurovirulence of the highly attenuated strain Orlov-V and the low-attenuated strain Orlov-14 in the intracerebral challenge test of virus infection of monkeys. The results obtained for clinical symptoms of CNS damage and the nature of the pathological process in the cells of the central nervous system of experimental animals will be significant for improving the safety control of live rubella vaccines, and allow us to consider the strain Orlov-V as a candidate for further research on the creation of a live rubella vaccine based on the domestic vaccine strain.

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