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ANTIGENIC COMPARISON OF YULI AND VNUKOVO-32 VIRUS STRAINS IN MONKEYS: SPECIFIC PROTECTION BY COMMERCIAL ANTIRABICES PREPARATIONS

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Summary. - A combined vaccination schedule using commercial antirabies immunnoglobulin G and experimental vaccine from strains Vnukovo-32 or Yuli beginning 2 hr before intracerebral (i.c.) challenge with a high dose of Yuli virus conferred no protection to *Cercopithecus aethiops* monkeys. In monkeys inoculated into lip with a middle dose of Yuli virus, administration of large amounts of antirabic IgG (up to 5000 national units, NU/kg) had a clearcut effect. The disease in Yuli virus-infected monkeys showed typical signs of acute encephalitis with lethal outcome, although one animal which developed typical encephalitis recovered as evidenced by increased virus-neutralizing antibodies in its serum. Inflammatory and degenerative lesions developed in the CNS of animals with signs of acute encephalomyelitis; their intensity was less prominent in those monkeys which underwent the combined treatment. In the cytoplasm of brain neurons of monkeys infected with Yuli virus relatively small Babes-Negri bodies with more or less apparent internal structure were detected.

Key words: rabies-related virus Yuli; lyssavirus; Rabivac Vnukovo-32; antirabies immunoglobulin; prophylaxis; vervet monkeys

Introduction

Five lyssa-related viruses of chiropteric origin were isolated in the U. S. S. R. (Selimov *et al.*, 1987). Here we describe the results of prophylactic treatment with Rabivac Vnukovo-32 and commercial antirabies immunoglobulin (Selimov, 1985) after Yuli virus infection.

Materials and Methods

In the first experiment 6 Cercopithecus aethiops monkeys (group 1 and 2, Table 1) were given 2500 national units (NU) per kg of antirabic immunoglobulin (IgG) raised in horse by hyperimmuniza-

tion with the fixed rabies virus Moscow strain grown in sheep brain. By 2 hr later, these animals as well as three untreated monkeys (group 4) were inoculated into thalamus with 0.1 ml of 10 % brain suspension of Yuli virus ($10^{5.1}$ LD_{50}/0.03 ml) under deep hexenal anaesthesia. From 2 hr post-infection (p.i.) the animals were immunized with the experimental inactivated concentrated Vnukovo-32 vaccine or with the experimental Yuli virus vaccine (5 % mouse brain suspension inactivated at 37° C for 2.5 hr and for 24 hr 4° C with beta-propiolactone in dilution 1:4000 (relative immunogenicity 4 and 0.3, respectively).

Three monkeys (group 1) were immunized with the Vnukovo-32 vaccine and other 3 monkeys (group 2) with the Yuli vaccine in a dose of 1 ml virus by intramuscular route for 9-12 days until the first signs of disease developed. Three animals from group 3 were given 0.2 ml of Yuli virus suspension (20 % mouse brain homogenate) mixed with antirabies IgG diluted 1:50 (preincubated for 1.5 hr at 37°C) into thalamus.

In the second trial (12 monkeys) 4 animals from group 5 were given antirabies IgG (5000 NU/kg) by intramuscular (i.m.) route into anterior and posterior extremities. By 2 hr later these monkeys as well as 4 controls (group 7) were infected with 1.25 ml of 10 % Yuli virus suspension into the lip under deep hexenal anaesthesia. Then the immunization schedule was started overnight with the Vnukovo-32 vaccine strain as described above. The animals from group 6 were given by intralabial route 2.5 ml of virus suspension consisting of equal volumes of 20 % mouse brain homogenate of Yuli virus and undiluted antirabies IgG (preincubated at 37°C for 1.5 hr).

Different parts of the CNS of succumbed, sick or survived monkeys were examined i.c. mouse inoculation and by direct immunofluorescence (IF) test (Selimov *et al.*, 1964). For histology, different areas of CNS and of sciatic nerve were fixed in 10 % formalin and embedded into pataffin; the sections were stained with haematoxylin and eosin (HE) and cresyl violet according to Nissl (Merkulov, 1969). For the detection of Babes-Negri bodies the contact slides were fixed with absolute alcohol-ether, stained according to Muromtsev (1926) and viewed in a light microscope at magn. x 630.

Results

In the first trial, control monkeys infected with Yuli virus by i.c. route developed encephalitis within a short incubation period of 9–12 days (Table 1). A similar incubation period was observed in the animals groups 1 and 2 in spite of the fact that they were given 2500 NU/kg antirabies IgG 2 hr prior to infection and they were immunized either with the Vnukovo-32 vaccine or with the Yuli vaccine. In the third group of animals infected with Yuli virus previously incubated with antirabies IgG *in vitro* all monkeys developed disease, but one animal (No. 8) had a long incubation period of 37 days. It can be assumed that Yuli virus was not fully neutralized at the given IgG dilution (concentrated IgG would have been toxic at i.c. inoculation).

Complete protection was observed in the second trial, when an extremely high amount of antirabies IgG had been administered 2 hr prior infection (5000 NU/kg) and the animals were inoculated by labial route with a moderate virus dose. The antirabies IgG in dilution 1:2 completely neutralized the Yuli virus. In the control group 3 monkeys developed disease, their incubation period being 13, 15, and 17 days, respectively. One monkey (No. 20) survived, another (No. 19) survived after experiencing clinical disease with signs such as hypersensitivity, aggressiveness, impaired mobility associated with overt paresis of neck muscles and the muscles of right hind extremity. Within 15 days the

Table 1. Effects of commercial antirabic IgG administration and vaccination with Vnukovo-32 in monkeys challenged with Yuli virus

Experiment	Group	Monkey No.	Antirabic IgG	Vaccine	Sick out of total infected	Incubation time(days)	Course of illness (days)	Examination IIF	Examination Virus isolation
1	1	1, 2, 3	2500 NU/kg	Vnukovo-32	3/3	10, 13, 19	3, 3, 3	+++	+++
	2	4, 5, 6	2500 NU/kg	Yuli	3/3	10, 15, 9	3, 2, 3	+++	+++
	3	7, 8, 9	1:100** + virus		3/3	14, 37, 14	6, 2, 3	+++	+++
	4	10, 11, 12			3/3	12, 9, 9	1; 5; 2,5; 2,7	+++	+++
2	5	13, 14, 15, 16	5000NU/kg	Vnukovo-32	0/4			0	0
	6	21, 22, 23, 24	1:2**+ virus		0/4			0	0
	7	17, 18, 19*, 20			3/4	13, 15*, 17	5, 7, 15*	++	++

Note: * monkey recovered
** final dilution of IgG in the mixture with Yuli virus

Table 2. Neutralizing antibody titres to viruses Yuli and Vnukovo-32 in the sera of monkeys 18 and 19

Monkey No.	Antibody titres to Yuli					Antibody titres to Vnukovo-32					
	0*	22**	37	83	117	0*	22**	37	42	83	117
18	0				64	0	6	6	6	0	5
19	0	1185	3123	1396		0	724	625	ND		33,8

* before immunization
** days post-immunization
Note: 25 LD_{50} of Yuli virus or 79 LD_{50} of Vnukovo-32 virus, respectively;
Virus titres are dilution reciprocals
ND = not done

symptoms receded and full recovery was recorded on day 30. Mice were inoculated by i.c. route with the saliva of the latter monkey collected on day 2 since the onset of clinical disease but no virus was isolated. The monkey in question was sacrificed 120 days p.i.; neither virus was isolated from nor rabies antigen was detected in the brain tissue of this animal; histologic examination of CNS showed no inflammatory lesions. However, this animal developed an antibody response, titre against Yuli virus being 1185, i.e. higher than detected to Vnukovo-32 virus (1:724) (Table 2). Clinical features of rabies were as usual.

As mentioned above, monkeys inoculated by i.c. route developed disease after a shorter incubation period (9–12 days), the disease itself being short (3 days) leading to death under complete paralysis of extremities. Prolonged incubation of 14–37 days was observed in Yuli virus-infected monkeys, in which the virus was partially neutralized with the IgG during preincubation *in vitro*. For example, in monkey No. 7 the clinical signs lasted for 7 days; this animal developed anisocoria, asymetry of eyelids, facial paralysis, disturbance of the muscle tonus and of reflexes of extremities later on followed by tetraparesis, and during agony by full paralysis, mydriasis, and absence of any photoreaction. Monkeys inoculated into the lip with a lower dose of Yuli virus (3 animals developed disease out of 4 inocualted ones) showed no peculiar symptoms; their incubation period lasted 13–17 days and duration of the disease was 5–7 days.

Histological lesions in the brain of group 4 monkeys (scored grade ++ and +++) seen infected by i.c. route with Yuli virus were the most extensive (Figs. 1a-e). Meninges were thickened, infiltrated with lymphocytes and mononuclear cells. Perivascular cuffings were numerous in the grey and white matter, but most prominent in the vicinity of meninges and below the ependyma. The vessels were widened and filled with erythrocytes. Broad perivascular infiltrates containing activated microglial cells and wandering mononuclear cells were scattered in all parts of the brain. Neurons showed chromatolysis, oedema, nuclear asymetry, and occasional lysis. These changes were widespread in the cortex, in hippocampus, cerebellum, and most prominent in the brain stem, namely midbrain and medullar nuclei. Inflammatory lesions were seen in the anterior horns of spinal cord, in the spinal ganglia and sciatic nerve. The histological lesions were similar but less extensive (scored grade + and ++) in the CNS of monkeys inoculated i.c. with the mixture of Yuli virus preincubated with antirabies IgG. In the CNS of group 1 and 2 monkeys treated according to the immunization schedules shown in Table 1 less prominent perivascular cuffings were detected accompanied with glial modular infiltrates and dystrophic changes of neurons (scored grade +). In 2 control monkeys (group 7) which were given Yuli virus into lip the lesions were scored grade + or ++. In the CNS of the latter two animals (Nos. 18 and 19) which were sacrificed 4 months p.i. and showed no clinical signs of disease no histological lesions were found.

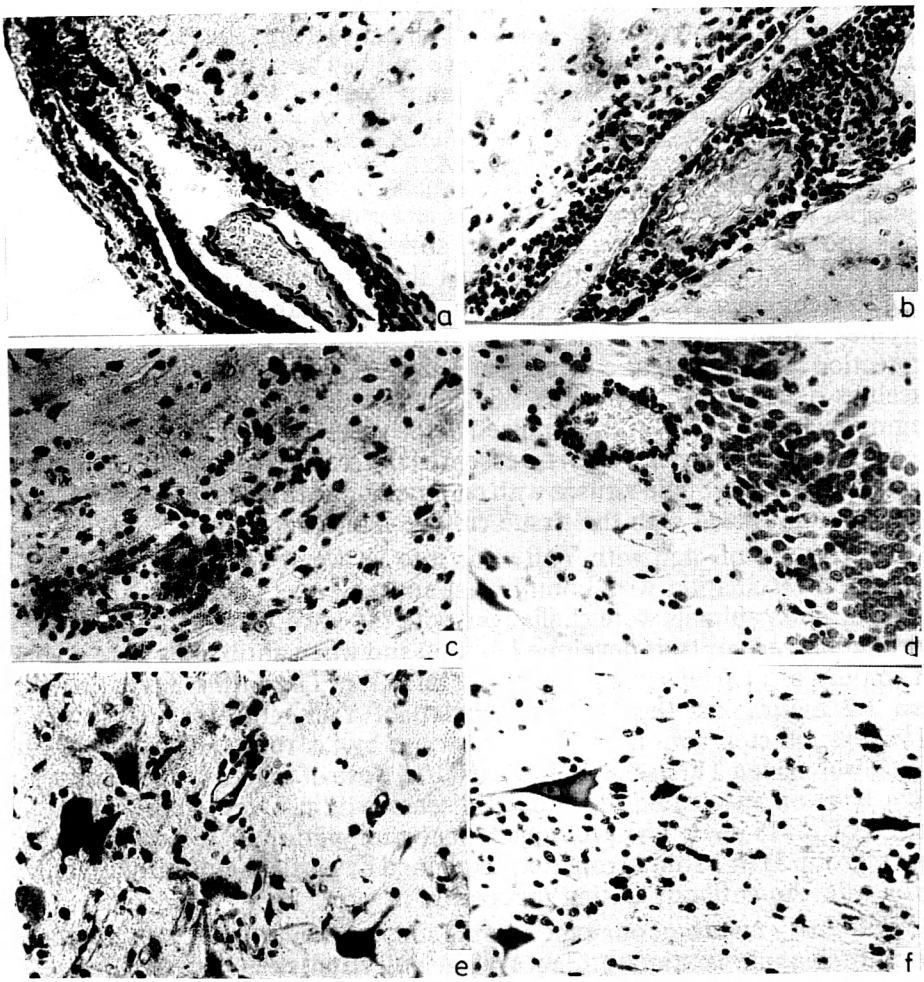

Fig. 1

Histologic changes in the CNS of monkeys inoculated with the rabies-related Yuli virus a + b - meninges are thickened, intensively infiltrated with lymphocytes and mononuclear cells; c, d - perivascular cuffing in cerebral cortex (c) and hippocampus (d), e - mononuclear infiltration and dystrophic changes of neurons in the grey matter of medulla oblongata; f - nodular mononuclear infiltration, chomatolysis and necrosis of neurons in the anterior horn of spinal cord. Stained with cresylviolet (according to Nissl), orig. magn. x 200 (reduced).

Attachment slides from different areas of the brain revealed cytoplasmic inclusions with more or less clearcut internal structure 1–4 μm in size. Histological examination of the CNS showed rabies encephalomyelis with diffuse infiltrates, degenerative changes of CNS and Babes-Negri bodies. The inflammatory lesions found in positive control monkeys were less abundant in those animals which succumbed clinical disease but had been treated with antirabies IgG and experimental vaccines from strains Yuli or Vnukovo-32.

Discussion

As known from literature, analysis of antigenic relationships between Stade virus and the classical fixed rabies virus showed controversal results. According to Schneider *et al.* (1986) mice immunized with commercial antirabies vaccines became resistant to Stade virus, while in other experiments the protection was less clear-cut; sera of subjects immunized with commercial vaccines neutralized Stade virus 5.8–104 times less than CVS virus and the commercial antirabies IgG neutralized Stade virus 1.3–19 times less than CVS. According to Lafon *et al.* (1986) no essential differences were found in neutralization of CVS and Stade viruses with commercial antirabies IgG or with sera of persons immunized with the tissue culture rabies vaccine.

In monkeys infected with Yuli virus into lip we showed the effectives of combined vaccination with commercial antirabies IgG and the Vnukovo-32 vaccine, when animals were challenged with the lower dose of Yuli (3 out of 4 nonimmunized controls developed illness) and when antibodies were given in an amount of 5000 NU/kg by 2 hr before infection. The antibodies in final dilution 1:2 neutralized the 10 % suspension of Yuli virus (exp. 2, group 6). Monkeys infected with high Yuli virus dose by i.c. route (10^4 LD_{50}/0.03 ml) which were given 2 hr earlier the 2500 NU/kg dose of antirabies IgG and which were later on vaccinated, developed disease within a very short incubation period of 10–15 days. The prolonged incubation perion of 37 days (lst experiment, group 3) in 1 animal might be explained by partial neutralization of Yuli virus with the antibody during incubation *in vitro*.

Preliminary results of our experiments support the notion that our commercial antirabies preparations protect against Yuli virus when it is administered by peripheral route. We suppose some qualitative and quantitative antigenic and immunogenic differences between the rabies-related Yuli and the vaccine viruses (Vnukovo-32 or Moscow strains). In our country 3 cases of succesful application of commercial antirabies preparations were reported after the bat bite. Therefore, prophylactic and therapeutic immunization of subjects bitten by bats may be continued using commercial antirabies IgG and the Vnukovo-32 vaccine. Also Lafon *et al.* (1986) recommended such treatment after bat bite in the territory of Europe.

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