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BIOLOGICAL PROFILE OF A VIRUS BELONGING TO CALIFORNIA ENCEPHALITIS COMPLEX ISOLATED IN KARELIAN AUTONOMOUS SOVIET SOCIALIST REPUBLIC

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Summary. — We investigated in white mice and in Syrian hamsters the pathogenesis of infection with a virus belonging to the California encephalitis complex isolated in the Karelian Autonomous Soviet Socialist Republic. The pathogenic properties of the virus were investigated also in green monkeys. The localization and character of lesions in the organs of given animals appeared to be similar. The virus appeared to possess neurotropic activity, it propagated in the animal body by haematogenous route. The virus was found to be heat-resistant and pH-sensitive.

Key words: *virus of California encephalitis complex*; *pathogenesis*; *biological profile*

Introduction

Our many years studies provided data on the circulation in the Karelian A.S.S.R. of arboviruses ecologically connected with blood-sucking vectors (Lvov *et al.*, 1984*c*; Skvortsova *et al.*, 1984). Some of these viruses (Ťahyňa, Karelian fever) caused — as determined in both isolated cases — local outbreaks of disease in humans (Lvov *et al.*, 1984*a*,*b*). Based on virological studies in *Aedes communis* mosquitoes collected in Muezer region of the Karelian A.S.S.R. a virus belonging to *Bunyaviridae* family (strain LEIV-9251) had been isolated and found to be a representative of the viruses of California encephalitis complex. The present paper describes the studies on biological profile of the isolated virus and on its role in the infectious pathology of animals.

Materials and Methods

Virus. Strain LEIV-9251 Kar was isolated in 1983 from *Aedes communis* mosquitoes collected in Muezer region of Karelian A.S.S.R. by intracerebral (i.c.) inoculation of suckling white mice. A neuroadapted variant of strain LEIV-9251 Kar was used. After 3 serial i.c. passages in 2-week-old white mice the infectious titre of the virus amounted to 7.0 log LD_{50}/0.03 ml.

Animals. Experiments were carried out in 3 types of animals: in 2-week-old albino mice weighing 6—7 g; in 3 to 4-week-old Syrian hamsters weighing 60 g, and in 2 to 2.5-year-old green monkeys. Altogether 4000 albino mice, 100 hamsters, and 4 monkeys were used.

Virus suspension (10 %) of the adapted variant was inoculated into mice and hamsters by i.c. (0.03 ml and 0.05 ml, respectively) and by subcutaneous (s.c.) routes (0.1 ml and 0.5 ml, respectively). The blood and organs of infected animals (brain, lungs, liver spleen, kidneys) were tested for the presence of the virus after 24, 48, 72, 96, 144 hr and within 21 days (or until the death of the animal). LD_{50} was measured by i.c. infection of white mice. Pieces of the same organs were simultaneously fixed in 10 % neutral formaline and embedded for histolcg.c examination. The paraffin sections were stained by haematoxylin-eosin and according to Nissl.

Green monkeys were inoculated with 1.5 ml and 1.0 ml of the suspension by s.c. and intravenous (i.v.) route, respectively. Before infection and 1 month post-infection (p.i.) (observation time) the blood of monkeys was tested for virus presence. The sera were serologically tested in complement fixation (CF) test and in neutralization test (NT). After the observation period virological and histological examinations of the organs of monkeys were also made. All manipulations were carried out under hexenal anesthesia.

Serological tests. CF was performed according to a standard procedure using sucrose-acetone antigen prepared from strain LEIV-9251 Kar. In the NT strain LEIV-9251 Kar was used.

For thermostability studies the suspension was exposed to following temperatures: −10 °C, 4 °C, 22 °C, 37 °C, and 56 °C. pH-sensitivity was determined by comparison of virus infectivity at 4 °C in media of pH 7.2, 6.0, and 9.0, respectively. The changes in virus activity were determined according to virus titre as measured by i.c. inoculation of white mice before and after the test. The virus titre was calculated according to Reed and Muench and expressed in log LD_{50}/0.03 ml.

Results

On day 3 after i.c. inoculation white mice showed the first signs of disease: adynamia and pareses of hind limbs. All animals died on day 4 or 5 exhibiting signs of hind limb paralysis. From 24 hr p.i. the virus was detected in internal organs, in titres increasing by 72 hr (Table 1). The highest virus titre was detected in the cerebral tissue. Histological examinations showed after 24 hr circulatory disorders in the CNS and focal inflammatory cellular infiltration in the pia mater. After 72 hr a well developed encephalitis was observed. Parenchymatous degeneration in the liver was observed as early as 48 hr p.i. The mice inoculated by s.c. route died on days 6 or 7 p.i. Alike to i.c. inoculation, the animals showed paralysis of the hind limbs before death. From 24 hr until death the virus titre in the blood and brain ranged from 1.0 to 3.25 log LD_{50}/0.03 ml, in other organs from 0.75 to 3.0 log LD_{50}/0.03 ml (Table 1).

Pathomorphological changes in internal organs of animal sacrificed after s.c. infection were similar to that after i.c. infection. After 24 hr, single foci of productive vasculitis, perivascular infiltrates and capillaritis were found in the brain. Moderate focal degeneration in the liver was observed. The same changes were also seen after 48 hr. By hour 72 pathomorphological changes in the brain indicated encephalitis: multiple haemorrhages, fibrinous swelling of vessel walls, marked productive vascular reactions, multiple lymphoid foci and single foci of neuroglial proliferation and neuron degeneration. On day 6 p.i. the same lesions were found in the brain of dead mice. Macrofocal interstitial pneumonia developed. Circulatory disorders were observed also in other organs.

At intracerebral infection of hamsters we observed adynamia and paresis of hind limbs. On day 7 the lethality of the disease was 70 %. Virus was detected in the blood and in internal organs of hamsters as early as 24 hr

Table 1. Time course of virus accumulation in the organs of albino mice and hamsters inoculated with the strain LEIV-9251 Kar

Animal species	Inoculation route	Observation time (hr)	Virus titre (log LD_{50}/0.03 ml)						Outcome
			blood	brain	lungs	liver	spleen	kidneys	
Albino mice	Intracerebral	24	1.50	1.75	1.25	1.50	1.50	1.0	Death on days 4 or 5
		48	2.50	2.50	2.25	2.25	2.50	2.25	
		72	3.0	5.50	3.0	2.25	3.25	3.50	
		96	2.75	7.50	3.0	2.0	3.0	2.0	
	Subcutaneous	24	1.0	1.25	0.75	1.25	0.75	1.25	Death on days 6 or 7
		48	1.25	1.50	0.75	1.25	1.0	0.75	
		72	2.25	2.25	2.25	2.50	2.25	2.50	
		96	2.25	2.25	2.0	1.75	2.25	1.75	
		144	2.0	3.25	3.0	1.25	2.0	1.50	
Hamsters	Intracerebral	24	2.0	2.0	1.0	1.50	1.25	1.0	Death on day 7
		48	1.50	2.25	2.25	1.75	1.50	2.0	
		72	2.0	3.0	3.0	2.0	1.75	2.0	
		96	3.0	4.0	3.25	3.0	2.25	2.75	
		168	2.50	4.0	3.0	2.50	2.0	2.50	
	Subcutaneous	24	1.0	1.0	1.0	0.75	1.0	1.25	Survival
		48	2.0	2.25	2.25	2.0	2.25	2.25	
		72	1.25	2.25	2.25	1.75	1.75	2.0	
		96	2.25	2.50	3.25	3.25	3.50	3.50	
		144	2.50	2.50	3.50	3.25	3.50	3.50	
		21 days	1.25	1.50	1.25	1.25	1.75	0.75	

p.i. Alike to albino mice, the virus titre increased in the course of infection from 1.0 to 4.0 log LD_{50}/0.03 ml (Table 1). The virus titre was higher in the brain and in lungs. Autopsy showed plethora and enlargement of the liver and massive haemorrhages in the lungs and brain. Histological examination of the brain carried out after 48 to 72 hr showed single foci of vasculitis, perivascular infiltrates and oedema. At 96 hr p.i. expressed encephalitis and liver degeneration were observed. In addition to encephalitis, microfocal pneumonia was also found. On day 6 after s.c. inoculation of hamsters clinically pronounced hind limb paresis was observed but all animals survived. One day p.i. the virus was detected in low titres in the blood and in organs of the sacrificed animals (Table 1). At 48 hr the infectious titre reached 2.0—3.50 log LD_{50}/0.03 ml. Autopsy carried out 6 days p.i. showed spleen enlargement, haemorrhages in the lungs and necrotic microfoci in the liver. Histologic examination by 48 to 72 hr p.i. showed neuronal degeneration in the brain as well as vasculitis with single foci of lymphoid infiltration. In the liver a lymphohistiocytic infiltration was found. Pronounced encephalitis was observed from 6 to 21 days p.i. (observation time).

Table 2. Virus distribution in the organs of monkeys inoculated with the strain LEIV-9251 Kar

Inoculation route	Virus titre (log LD_{50}/0.03 ml)						Antibody titres in	
	blood	brain	lungs	liver	spleen	kidneys	CF	NT
Subcutaneous	1.0	1.0	1.0	0	1.25	0.75	0	2.50
Intravenous	0.75	0	1.0	0.75	0	0	0	1.50

Note. Blood sera were negative before infection. The animals were sacrificed 30 days p.i.
0 — virus not detected

Monkeys infected with strain LEIV-9251 Kar by s.c. and/ or i.v. routes survived. No clinical signs of the disease were observed throughout the whole observation time. Autopsy performed after this observation period showed no noticeable pathologic changes in internal organs. Low titres of virus (0.75—1.0 log LD_{50}/0.03 ml) were registered in blood, lungs, and liver upon i.v. inoculation and in all organs up on s.c. inoculation (Table 2). Histological examination revealed moderate encephalitis in the brain of s.c. inoculated monkeys: moderate productive capillaritis, perivascular and pericapillary oedema, neuronal degeneration; parenchymatous degeneration was seen in the liver and epithelial degeneration in kidneys.

Intravenous infection of monkeys was followed with single haemorrhages, moderate productive capillaritis, and neuronal degeneration in the brain. In the liver focal lymphohistiocytic infiltrates developed and in kidneys moderate degeneration of tubular epithelium and singular microfocal haemorrhages occurred. In the serum of monkeys collected after the observation time specific virus-neutralizing antibodies were found (Table 2). Thus, strain LEIV-9251 Kar caused irrespective of the inoculation route encephalitis-like lesions in CNS and, in addition, liver lesions. These changes occurred in the course of clinically inapparent infection.

Heat-resistance tests showed that the infectious virus titre remained unchanged over 1 month at exposure to —10 °C. The virus appeared to be preserved for 335 days at exposure to —20 °C. At 4 °C (original titre 5.25 log LD_{50}/0.03 ml) the virus retained its activity over the first 7 days; after 21 days the infectious titre decreased by 2.75 log LD_{50}/0.03 ml. The virus was completely unaffected within a 24 hr exposure to 24 °C. It was partially inactivated after 7 days (infectious titre decreased by 2.25 log LD_{50}/0.03 ml); after 21 days it has lost its infectivity. During incubation at 37 °C the infectious titre decreased by 2.0 log LD_{50}/0.03 ml within 24 hr and by 4.75 log LD_{50}/0.03 ml within 7 days. Heating at 56 °C for 30 min led to complete virus inactivation. Lyophilization of the virus-containing brain suspension without filler failed to reduce infectivity. After keeping the virus for 10 months at 4 °C the infectious titre was reduced from 0.0 to 3.25 log LD_{50}/0.03 ml.

Incubation of the brain suspension for 7 days at 4 °C at varying pH caused following reduction of the virus titre at pH 9.0—2.0 log, at pH 7.2—5.25 log, at pH 6.0—1.25 log; after 21 days incubation the titres were at pH 9.0—0.5 log, at pH 7.2—2.50 log, at pH 6.0—0.

Discussion

Results of virological and pathomorphological studies presented in this paper indicate a significant pathogenicity of the strain LEIV-9251 Kar for laboratory animals. In white mice administration by i.c. or peripheral routes was followed with paralysis of hind limbs and death of animals between days 4 to 7 p.i. In all organs increased virus titres indicated generalized infection. The highest virus titre was found in the brain. By time of death it was lower in internal organs, a characteristic of arboviral infections (Klisenko *et al.*, 1982).

Localization and character of lesions were similar in white mice inoculated by i.c. and s.c. routes: pronounced encephalitis, parenchymatous degeneration of liver and circulatory disorders. Syrian hamsters died on day 7 after i.c. infection. After s.c. infection, however, they did not die, thus exhibiting lower susceptibility. Histological examination of the organs of animals infected by different routes revealed similar localization and character of lesions: encephalitis and liver degeneration. The hamsters appeared to have encephalitic lesions in the brain even 21 days p.i. as confirmed by the presence of the virus in brain tissue. No clinical signs of infection were found in green monkeys by 30 days p.i. (observation time) after either i.v. or s.c. virus inoculations. The virus was detected in the blood and internal organs; in the brain, liver, and kidneys characteristic inflammatory and degenerative changes were observed.

Thus, peripheral administration of strain LEIV-9251 Kar caused in monkeys and Syrian hamsters an infection associated with prolonged circulation of the virus and development of characteristic morphological changes in the CNS and other organs, as well as the appearance of virus-neutralizing antibodies in the blood. The results indicate that according to its pathogenic properties the strain LEIV-9251 Kar was a pantropic virus which spreads in the body by haematogenous route. These data also suggest that rodents may carry chronic (latent) infection under natural conditions and, therefore, may be of epidemiologic importance.

Strain LEIV-9251 Kar is highly heat-resistant. It was completely inactivated after as long as 21 days at room temperature (24 °C) and after 30 min at 56 °C. The virus was found to be well preserved at neutral pH.

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