PRIMATOLOGY

The Use of Interferon for Emergency Prophylaxis of Marburg Hemorrhagic Fever in Monkeys

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The possibility of emergency prophylaxis of Marburg hemorrhagic fever with leukocytic and recombinant interferons was studied in experiments on *Cercopithecus aethiops*. None of the agents protected monkeys from the action of lethal doses of Marburg virus. Recombinant interferon-α_2 administered according to the emergency prophylaxis schedule prolonged the mean life-span of monkeys injected with Marburg virus in doses of 100 and 1000 LD_{50} by 1.9 and 6.1 days, respectively.

Key Words: *Marburg virus; interferon; reaferon; monkey; emergency prophylaxis*

Marburg hemorrhagic fever induced by viruses of the *Filoviridae* family is an acute viral disease characterized by complex clinical picture, shock syndrome, and high mortality in humans and primates [2,6]. Marburg fever is endemic only for some African regions, however, tourism and widening economic relations make this infection potentially dangerous for other countries. At present, there are no effective means of prevention and treatment of this disease.

There is convincing evidence for high efficiency of recombinant interferon-α_2 (IFN-α_2) in the treatment of wide spectrum of viral diseases [3,5]. This drug is most effectively used for emergency prophylaxis, although it is also used for therapy [7,9,10]. There are data that recombinant IFN-α_2 shortens viremia and prolongs the life-span in monkeys infected with Ebola virus, another member of the *Filoviridae* family [8].

Our aim was to evaluate the possibility to use natural and recombinant IFN-α_2 for emergency prophylaxis of Marburg hemorrhagic fever.

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MATERIALS AND METHODS

Experiments were carried out on outbred guinea pigs weighing 200-220 g and monkeys *Cercopithecus aethiops* weighing 3.8-5.1 kg (Vector Research Center of Virology and Biotechnology). The animals were maintained under vivarium conditions on a standard ration. Before the experiments, the absence of antibodies to Marburg virus (MV) was confirmed by immunofluorescence assay. Marburg virus (strain Popp) was obtained from Belorussian Institute of Epidemiology and Microbiology.

For emergency prophylaxis of Marburg fever, human recombinant IFN-α_2 (reaferon, dry preparation, Vector) and human leukocytic IFN (Biomed, Perm') were used. The monkeys were infected with 10% liver homogenate from guinea pigs injected with a lethal dose of MV. Virus titer in guinea pigs was 8.1 lg LD_{50}/ml [1].

The monkeys of the control and two test groups (n=6, n=7, and n=7, respectively) were subcutaneously infected with MV into the front paw in doses of 10, 100, 1000, and 10,000 LD_{50} per animal (two monkeys from each group per dose; MV in a dose of 10,000

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TABLE 1. Body Temperature (°C)/Virus Titer ($lgLD_{50}$/ml) in Blood of Monkeys Infected with VM

Drug	VM dose, LD_{50}/animal	Monkey No	Time after infection with MV, days							
			2	4	6	8	10	12	14	17
IFN	10	8	37.5	38.2/2.25	38.6/2.75	39.8/3.50	39.0/4.75***			
	16		36.8	37.1/1.00	37.2/2.25	38.2/3.50	40.1/4.00	39.9	39.6***	
	100	18	36.9	37.3/3.25	38.9/4.00**					
		17	36.6*							
	1000	24	37.7	38.2/3.75	39.7/4.75**					
	23	36.7**	—/1.25+							
	10 000	3	37.1	37.8/3.50	38.4/5.25	38.8/6.25*				
Reaferon	10	21	36.8*							
		22	36.6	37.1/1.50	38.2/3.50	39.5/3.75	38.8/4.75	39.4*		
	100	12	37.0	37.7/2.25	38.1/2.75	39.6/3.75	40.4/5.50	40.8*		
		13	37.2	38.1/2.75	38.5/3.25	39.8/4.50	40.4/5.75	40.6*		
	1000	19	37.1	38.1/2.00	38.0/3.25	38.4/4.00	40.0/5.50	40.5*		
		20	36.8	37.5/1.50	37.9/2.25	38.8/3.75	38.8/4.75	37.9	37.2	37.5/3.0*
	10 000	7	37.3	38.4/3.25	39.2/4.00	39.6/4.75**				
Placebo (viral control)	10	2	36.7	37.3/2.00	37.5/3.50	38.6/4.25	39.2/4.75	41.0*		
		9	36.8	37.9/2.00	37.9/3.75	39.8/4.75	39.0/5.00**			
	100	1	36.6	38.2/2.25	39.0/3.75	39.2/4.50	39.2/5.25**			
	6	36.7	37.2/2.50	37.4/4.25	36.2/4.75					
	1000	14	36.7	38.8/2.50	39.4/4.25	40.0/5.75*				
		15	36.8	38.6/3.00	39.1/4.75	39.6/6.00*				
IFN	—	10	37.5	37.4/<1	37.7/<1	36.8/<1	36.7/<1			36.8/<1
Reaferon	—	11	37.3	37.7/<1	37.3/<1	36.4/<1	36.3/<1			36.5/<1

Note. Monkey died *on the next day, **on day 2, and ***on day 3 after the last temperature measurement. +Blood was taken from dead monkey.

LD_{50} was injected to one monkey of each test group). Group I and II monkeys were injected intramuscularly with leukocytic IFN or reaferon, respectively, according to the emergency prophylaxis: IFN — 10,000 MU/animal 30 min after infection, then 5000 MU/animal 60 and 120 min, and 2, 3, 4, and 5 days after infection. Reaferon: 2000 MU/animal 30 min after infection, then 1,000,000 MU/animal at the same terms as in group I animals. Control monkeys (group III) were treated with placebo (5 mg/ml human serum albumin, a stabilizing component of reaferon) for 5 days after infection. Two monkeys not infected with MV received IFN or reaferon only for 5 days according to group I and II schedule, respectively. On day 4, 6, 8, 10, or 17 postinfection, the blood was drawn to measure viremia. MV titer in the blood of infected monkeys was measured on guinea pigs and expressed in lg LD_{50}/ml.

All experimental procedures were performed under ketamine narcosis.

The mean life-span (MLS) was determined as described previously [5].

RESULTS

Two monkeys from group I and one monkey from group II died on days 3-4 after injection of MV. Autopsy revealed no specificity of monkey death. Blood MV tests by the method of serial passages on sensitive animals revealed the presence of MV only in monkey No. 23 (Table 1).

On days 4-5 after viral infection the animals demonstrated anorexia, and some of them refused to eat. Starting from days 8-9 after infection, nervous disorders (aggressiveness, or apathy) were noted in some animals. It should be noted that classical symptoms of Marburg disease (hemorrhage and bloody diarrhea) were weakly expressed in these animals. During the entire period of experiment (20 days) the Group IV monkeys did not refuse to eat and had no signs of neural disorder throughout of the experiment.

Body temperature was measured throughout the experiment. In some monkeys infected with MV body temperature increased by 0.5-1°C on day 2 postinfection. This was most likely associated with interferon injections, because no fever was observed at this term in group III animals (not treated with interferon). In groups I, II, and III animals fever (more that 1.5°C) developed on days 4-6 postinfection and persisted to death. In two monkeys the death occurred againt the background of temperature decease to normal (monkey No. 6, group III) and to subfebrile level (monkey No. 20, group II). In group IV monkeys temperature increased on day 2 and returned to normal on day 8.

The blood titers of MV in monkeys increased throughout the experiment (Table 1), except monkey No. 20 (group II), in whom MV titers tended to decrease.

All monkeys in groups I and II died before the end of the experiment (Table 1). Postmortem examination revealed histopathologic features characteristic of this disease: enlarged clay-sandy liver with necrotic foci at the surface; enlarged dark-red spleen; ulcers and the necrotic foci in the large intestine. Melena was noted in some monkeys.

MLS in reaferon-treated monkeys increased by 1.02 days (11.85 *vs.* 10.83 day in the control value). MLS of monkeys injected with 100 and 1000 LD_{50} virus dose increased by 1.9 and 6.1 days, respectively. In monkeys treated with leukocytic IFN, MLS was lower than in the control group (8.85 day). This can be explained by the fact that reaferon contains highly purified recombinant human IFN-α_2 possessing antiviral activity, while leukocytic IFN is a mixture of interferons, lymphokines, cytokines, and other biologically active agents, products of stimulated leukocytes. Prolongation of MLS after emergency prophylaxis of Marburg fever is very important, because the time factor plays a dominant role for rapidly progressing hemorrhagic fevers, which may lead to the fatal outcome as early as day 5 postinfection.

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