ANTIBODY-MEDIATED VIRUS CLEARANCE FROM NEURONS OF RATS INFECTED WITH HEMAGGLUTINATING ENCEPHALOMYELITIS VIRUS

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1. INTRODUCTION

Swine hemagglutinating hencephalomyelitis virus (HEV) causes vomiting and wasting disease or encephalitis in piglets. In our experimental studies of rats[1] and mice,[2] HEV-67N spread trans-synaptically from peripheral nerve to the central nervous system (CNS) and infected neurons but not any glial cells. These neurotropic properties of HEV are similar to those of rabies virus, indicating that HEV might be a good experimental model for the investigation of control of neurotropic viral infections including rabies. To examine the effectiveness of antiserum treatment, rats were inoculated with HEV following antiserum treatment and analyzed virologically.

2. MATERIALS AND METHODS

Plaque-purified HEV-67N strain was propagated and assayed for infectivity in SK-K cells as described previously.[3] Specific pathogen free of 6-week-old Wistar male rats were inoculated into the right hindleg by subcutaneous (s.c.) route ($1x10^6$ pfu). Five rats were used in each group. Antiserum was prepared to inoculate into infected rats 3 times at weekly intervals intraperitoneally (i.p.). On day 7 after the last inoculation, blood was collected from rats. For antiserum treatment, rats were administrated i.p. with 1 ml of rat antiserum (HI titer; > 1:1000).

The same experiment was made to confirm preventing fatal infection from rats by treating with antiserum heated at 56ºC for 30 minutes. In addition, antiserum treatment was delivered by intravenous inoculation to compare the effectiveness of different routes.

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3. RESULTS

To prevent the virus spread into the CNS of rats infected with HEV by intracerebral (i.c.) or intraspinal (i.s.) inoculation, antiserum was administered before/after HEV inoculation. Among rats pretreated with antiserum 24 hr before i.s. inoculation, only 3 of 5 rats survived. Even with pretreatment 24 hr before i.c. inoculation, all rats infected by i.c. route died of encephalitis.

To measure virus growth in the spinal cord and brain of rats infected after s.c. inoculation, 3 rats per day were examined to detect virus in CNS. As shown in Figure 1, virus was first detected in spinal cord on day 2, and in brain on day 3. On day 4, the brain titers became higher than those of spinal cord, reaching 10^6 to 10^7 pfu/0.2 g.

As shown in Table 1, antiserum treatment at 0, 24, 48, 72 hr postinoculation (p.i.) prevented fatal infection in all rats after s.c. inoculation. At 96 hr p.i., all rats showed flapping ears as clinical signs. After antiserum treatment at 96 hr p.i., 3 of 5 rats survived.

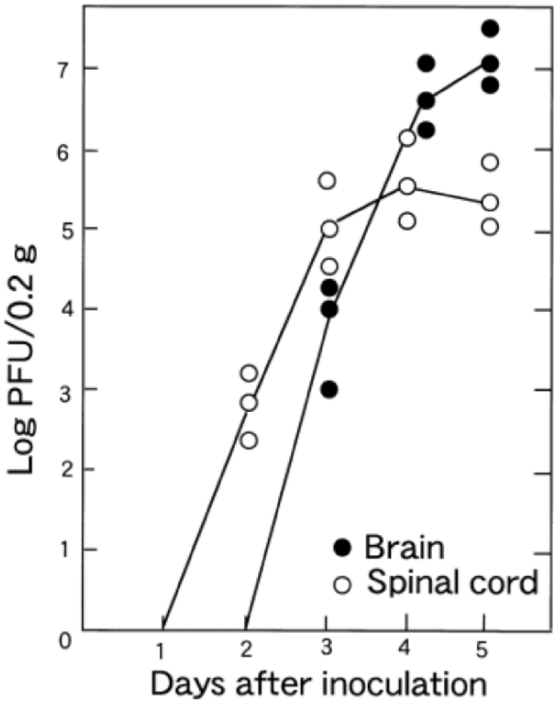

Figure 1. Virus growth in the spinal cord and brain.

Table 1. Antiserum treatment of rats after HEV inoculation into hind leg.

Antiserum treatment	CNS/tested	Dead/tested
0 hr	0/5	0/5
24	0/5	0/5
48	0/5	0/5
72	2/5	0/5
96	5/5	2/5
120	5/5	5/5
Nontreated	5/5	5/5

At 120 hr p.i., all rats developed CNS signs and died even with antiserum treatment. All nontreated rats died showing CNS signs within 7 days. The virus was detected in the brain of dead rats but not of the survivors. Approximately the same results were obtained in rats treated with heated antiserum as described above.

After antiserum treatment by both i.p., and i.v. routes, infected rats did not show any clinical signs and survived. The virus was not detected in brains of all of these animals on day 4 and 7 after treatment. Nontreated rats developed CNS signs and died within 7 days.

4. DISCUSSION

Antiserum treatment failed to prevent fatal infection from rats inoculated by i.c. and i.s. routes. However, rats inoculated s.c. were rescued by antiserum given at 0, 24, 48, and 72 hr p.i. By treatment at 96 hr p.i., 3 of 5 rats with CNS signs were protected. As shown in Figure 1, the virus already replicated in neurons of the brain at 72 to 96 hr p.i. These findings suggest that antibody mediated clearance of HEV was established in neurons of the brain of rats infected. Our previous studies demonstrated that HEV spreads trans-synaptically from peripheral nerve to CNS of rats.[4] Antibody might inhibit the spread of HEV in synaptic pathways via axons. Although this mechanism is unclear at present, antibody treatment might be useful tool for preventing neurotropic virus infection in the CNS of animals and humans.

5. REFERENCES

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