Article @ Virology

Establishment of Rabies Virus Abdominal Challenge Model with Golden Hamster

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ABSTRACT

Objective: To establish a stable and reliable model of rabies virus infection by golden hamsters, which can be used to evaluate the protective effects of vaccine prevention, immunoglobulin and antiviral drugs after viral infection. Methods: Golden hamsters were injected intraperitoneally with different concentrations of rabies CVS strain virus to record animal death. Explore the best amount of virus attack and establish a model of the abdominal attack hamster. On this basis, the protective effects of CpG adjuvant rabies vaccine and anti-rabies immunoglobulin on rabies virus challenge were evaluated. Results: When the amount of virus attack remained above 4.1 $lgLD_{50}$/0.5ml, the mortality of the golden hamster in the control group was 100% within 14 days. Injection of adjuvant vaccine and immunoglobulin after viral infection can significantly reduce the mortality of golden hamsters. Conclusion: The golden hamster abdominal attack rabies virus model was successfully established, and it has simple operation and stable results, which will contribute to the development and screening of antiviral drugs and new adjuvant vaccines.

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Rabies is a fatal disease caused by rabies virus infection, which is 100% dead. Rabies virus is sensitive to all mammals[1]. The infection model of rabies virus is usually a viral attack using brain cavity and intramuscular injection[2]. For the abdominal cavity, it is often used as a vaccine immunization site for both mice and golden hamsters. A viral challenge infection model for golden hamsters by the abdominal route has not been reported.

Materials and Methods

1. Materials

1.1 Virus, animals and Vaccines

Rabies virus CVS standard strain, provided by the arbovirus vaccine room of National Institute for Food and Drug Control.

Female Syrian golden hamster and mice, 6-8 weeks old, were been provided by Beijing Weitong Lihua Experimental Animal Technology Co., Ltd.

Diploid rabies vaccine, PM strain rabies virus culture inactivated and purified vaccine stock solution, no human albumin, batch number:20160701 Storage temperature 4°C.

Human rabies immunoglobulin, 120 IU /mL, 2mL/bottle, Guangdong Shuanglin, batch number 20170403.

1.2 Solutions

PBS(pH7.2) buffer, accurately weigh 8.5g NaCl, Dissolve with ultra pure water, take 85mL of liquid A, 15mL liquid A, then The liquid A is accurately weighed 9.465g of Na_2HPO_4, dissolved in ultrapure water, and made up to 1000mL; The liquid B is accurately weighed KH_2PO_4 9.07g, dissolved in ultra pure water, constant volume to 1000mL.

Virus dilution: autoclaved ultra pure water containing 2% new born calf serum;

CpG adjuvant (1mg/mL): batch number is 20151001, provided by Anhui Zhifei Longkema Bio-Pharmaceutical Co., Ltd.

1.3 Instrument

pH meter(Mettler-toledo Co., Ltd.) ; 4°C -20°C Refrigerator (Haier Group Co., Ltd.); -80 ° C Ultra-low temperature ref--rigerator (SANYO Co., Ltd); Pure water meter(MILLIPORE); the 96 well plates was Greiner CELLSTAR® .

2. Method

2.1 Feasibility exploration of CVS virus challenge in abdominal cavity

10 female SPF-level golden hamsters of 6-8 weeks old were selected, and rabies virus CVS strain was been prepared by mice brain and the virus titer is $1x10^5$ $lgLD_{50}$/ml. The incidence of death of golden hamsters was observed daily by intraperitoneal injection CVS virus by 0.5 mL/mouse.

2.2 Determination of the CVS virus dose of the abdominal challenge by golden hamster

CVS was diluted to 4.5 $lgLD_{50}$/0.5ml, 4.0 $lgLD_{50}$/0.5ml, 3.75 $lgLD_{50}$/0.5ml and 3.5 $lgLD_{50}$/0.5ml in an ice bath; golden

dilute to 1000mL, autoclave, store at 4 °C. hamsters were intraperitoneally injected with 0.5 mL/dose. There are 6 hamsters per group.

The actual virus titer was detected by mice brain challenge. The challenge virus was been 10 times serial dilution, and each mouse was challenged with 0.03 mL. There are 10 mice per group.

3. Preliminary application of the golden hamster abdominal cavity challenge model.

3.1 Virus attack

CVS was diluted to 4.0 $lgLD_{50}$/0.5ml and placed in ice bath; golden hamsters were intraperitoneally injected with 0.5 mL/dose, There are 10 hamsters per group.

The actual virus titer was detected by mice brain challenge. The challenge virus was been 10 times serial dilution, and each mouse was challenged with 0.03 mL.

3.2 Immunization and treatment

After the golden hamster was been attacked with the CVS virus for 6 hours, the vaccine was immunized by muscle injection to 0.1 ml/mouse, and the immu--nization procedure was 4 needles: 0d, 3d, 7d and 14d.

After the golden hamster was been attacked with the CVS virus for 6 hours, the HRIG (human rabies immunoglobulin) was been injected by another leg gastrocnemius of golden hamster. The injection dose was 30μl, 15μl and 7.5 μl respectively. That is the usage standard was 20 IU, 10 IU and 5 IU/KgBW).

Results

1. Feasibility and determination of the CVS virus dose of CVS virus attack through the abdominal cavity

10 female SPF-level golden hamsters that intraperitoneal injection CVS virus by 0.5 mL/mouse were all rabid death. It's feasibility of CVS virus attack through the abdominal cavity.In order to study about the rabid death after the virus attack,the incidence of hamster death was been observed and recorded day by day. the results showed that the death usually occurred after 7 days, and all died after 14 days, as shown in Figure 1.

Twenty fourth SPF 6-8 week old female golden hamsters were used, and The 24 experimental animals were randomly divided into four groups. We challenged them with different CVS titers.The experimental results showed that when the viral attack amount was 3.75 $lgLD_{50}$/0.5ml or more, the mortality rate of the golden hamster was over 80%, and the experimental system was established, which can be used for further research (see Table 1 for details).

2. Preliminary application of the golden hamster abdominal cavity challenge model.

Take 6-8 weeks old SPF-class golden hamsters, 70 females. each group of 10, divided into 7 groups for experiments.

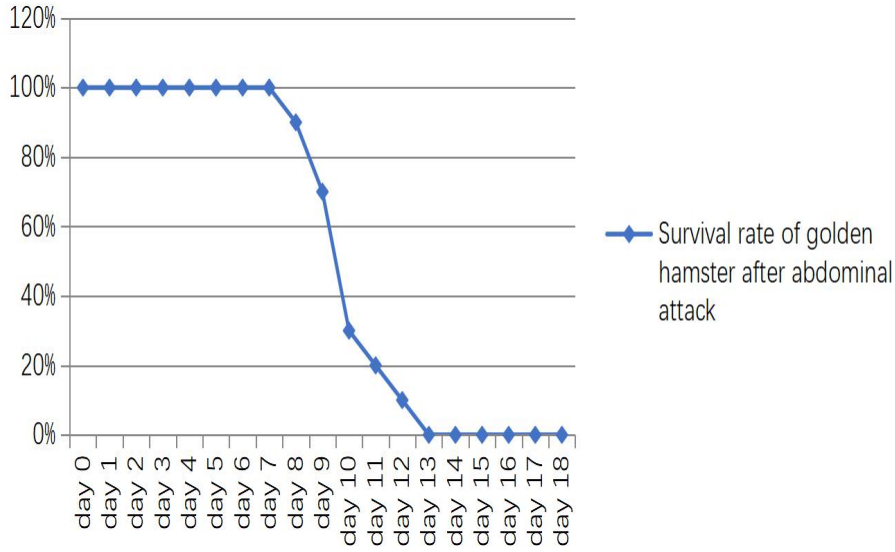

Figure1: Survival rate of golden hamster after abdominal attack

Table 1: Results of viral challenge dose in the abdominal cavity of golden hamster

Titer ($lgLD_{50}$/0.5ml)	Actual viral attack* ($lgLD_{50}$/0.5ml/each)	Number of animal deaths	Animal mortality
4.5	4.3	6/6	100%
4.0	4.1	6/6	100%
3.75	3.5	5/6	83.3%
3.5	3.0	4/6	66.7%

*: 11-13g SPF kunming mice were used for virus titration, 40 mice were used and randomly divided into four groups.

The actual viral attack titer was 4.1 lg LD_{50}/0.5ml, and that is the same as expected.Six hours after CVS challenge, adjuvant vaccine immunization and human rabies immunoglobulin intervention were performed.The results showed that the PBS control group had a mortality rate of 100%, indicating that the experimental system was established.

The animal protection rate of the simple vaccine group was 10%, and the protection rate of the adjuvant vaccine group was 20%, indicating that the addition of the vaccine antigen and the adjuvant can enhance the antiviral ability of the mice after the virus was exposed to the abdominal cavity.

Simultaneous injection of 5 IU/kg of rabies immunoglobulin (right gastro--cnemius) and adjuvant vaccine (left gastrocnemius), the survival rate of the animal was 70%; simultaneous injection of 10 IU/kg of rabies immunoglobulin and vaccine antigen, The survival rate after the day was 100%, and 20 IU/kg of rabies immunoglobulin and adjuvant vaccine were injected, and the survival rate of the animals was 100%. The survival rate of the rabies immunoglobulin group injected with 20 IU/kg alone was 100%. See Table 2 for details.

Table 2: Results of immunological experiments after CpG adjuvant human rabies vaccine for abdominal cavity exposure

Group	Virus titer ($lgLD_{50}$/0.5ml)	Antigen amount (ml/each)	CpG (μg)	IgG (IU/kg)	Mortality	Protection rate (%)
1		0（PBS）	/	/	10/10	0
2		0.25	/	/	9/10	10
3		0.25	75	/	8/10	20
4	4.1	0.25	75	5	3/10	70
5		0.25	75	10	0/10	100
6		0.25	75	20	0/10	100
7		/	——	20	0/10	100

Discussion

Rabies virus belongs to the genus Rabies, *Rhabdovirus*, and single-stranded RNA. Five structural proteins are encoded: nuclear protein, phosphoprotein, matrix protein, glycoprotein, and an RNA-directed RNA polymerase (L protein). Natural infection of rabies virus is usually caused by a bite or scratch of a host animal which carrying the virus. Peripheral nerve tissue is infected through the junction of the neuromuscular. After the virions are encapsulated, they are retrogradely transported under the regulation of dynein, from post-synaptic to presynaptic neurons, until eventually infecting the central nervous system [3] .

The literature shows that after inoculation of yellow fever virus in the peritoneum of golden hamsters, hamsters developed high titer viremia for 5-6 days, and showed inhibitory antibodies 4 or 5 days after infection, which was yellow fever virus[4]. The clinical and pathological changes in infected golden hamsters are very similar to those described in macaques and human cases. Therefore, golden hamsters have the potential to be used as animal models for studying neurodegenerative viruses. The mammalian autonomic nervous system balances the regulation of various organ tissues in the peritoneal cavity through two branches of sympathetic and parasym--pathetic nerves. The abundant intra--peritoneal ganglia in mammals provide reliable conditions for virus replication and proliferation. It has been shown that viral antigens were detected in the abdominal ganglia 12 hours after vaccination with the horse herpes virus in the peritoneal cavity of hamsters. At 36, 48, 72 h after inoculation, the dorsal spinal ganglia, lumbar central spinal cord nerves, and muscles Viral antigens were also detected in the interstitial plexus, and viral antigens were also detected at the end of the brain 96 hours after inoculation[5]. It is speculated that the horse herpes virus infects the abdominal ganglia or intermuscular plexus after initial reproduction in abdominal macrophages, and then moves to the brain through the peripheral nerves and spinal cord.

Tang et al. used golden hamsters as animal models to observe the post--exposure immune effects of different antigenic doses of rabies vaccine[6]. It was found that golden hamsters can be effectively evaluated for different quality vaccines as an animal model of rabies exposure. Our results showed that the golden hamster was been challenged intraperitoneally with 4.0 $lgLD_{50}$/0.5ml of rabies virus . After 14 days, the mortality rate of the animal was 100%, and the repeatability was finel. When the amount of virus attack remained above 4.0 $lgLD_{50}$/0.5ml, the mortality rate of the golden hamster in the control group was 100%, and the experimental system was established, which can be used for further research.

Using the abdominal challenge model, the golden hamster was infected with CVS strain rabies and the hamster was

injected with rabies immunoglobulin IgG in the right leg gastrocnemius muscle 6 hours later, and the vaccine or adjuvant vaccine was injected on the left side. The vaccine and immunoglobulin are injected separately on the left and right sides to avoid the binding reaction between the vaccine antigen and the rabies immunoglobulin, which affects the immune and protective effects. The CpG adjuvant vaccine is injected into the left gastrocnemius muscle, away from the immunoglobulin injection site to avoid the binding reaction, and the immune stimulation of the CpG adjuvant vaccine can be better and more durable.

When the model was used to evaluate the protective effect of adjuvant vaccine and immunoglobulin on hamsters, different concentrations of immunoglobulin gastro--cnemius injection (5, 10, 20 IU/ml) could protect the golden hamster (40%, 80%, 100%). It indicated that with the decrease of the concentration of injected immuno--globulin, the protection rate decreased, and the experimental groups injected with 20 IU/ml IgG had the best protection effect, up to 100%. Simultaneous immunization with a vaccine or adjuvant vaccine based on injection of immunoglobulin (5 IU/kg) increased the survival rate of golden hamsters (60%, 70%). When the concentra--tion of immunoglobulin used is increased to 10 IU/kg and 20 IU/kg, the golden hamster can produce 100% protection at the same time as the immunization vaccine or the adjuvant vaccine.

The conventional rabies virus mouse challenge model uses cranial virus attack [7], which has a rapid infection, generally 4-5 days of onset, and the virus directly infects nerve tissue, which is difficult to intervene in the subsequent process, thereby limiting its application. In this experiment, golden hamsters were used as experimental animals, and it took a period of time to attack from the abdominal cavity to the cranial nerves after viral infection. Therefore, the incidence is relatively lagging, which is conducive to drug intervention in the later stage, and the abdominal cavity is also a traditional immune route. Experiments such as mixing drugs and viruses in the visceral cavity. Therefore, the establishment of the model of abdominal challenge has strong innovation and practical application, and can be used in the pathogenesis of rabies virus, evaluation of vaccine immunologi--cal effects, and screening of antiviral drugs.

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