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The Effect of an Attenuated Rabies Virus SRV_9 on Suckling Mouse Growth After Intracerebral Inoculation

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Abstract: Rabies virus is a kind of virus having strict neurotropic property. Intracerebral inoculation method was commonly used to RV-related research. SRV_9 is an attenuated RV vaccine strain. In the present study, we inoculated intracerebrally on suckling mouse with SRV_9 strain. The results showed that SRV_9 could cause a mortality rate of 100% on suckling mice below 13 day old. The mortality rates of 14-17 day old mice were 68, 33, 33 and 17%, respectively without incidence above 18 day old. Meanwhile, the results showed that the growth of survival suckling mice was inhibited remarkably after intracerebral inoculation SRV_9. The 16 day old survival suckling mice were chosen to perform body weight test and the result showed that the weight growth was very slow with 14% less than normal growth rate. However, 2 weeks latter the weight of the mice began to recover gradually but slowly. It was suggested that the intracerebral RV reproduction could take a significant effect on the growth and development of the mice and with RV being eliminated by antibody, the weight of the mice which were inoculated with SRV_9 could gradually recover.

Key words: Rabies Virus (RV), SRV_9 strain, suckling mouse, growth, intracerebrally inoculation, vaccination

INTRODUCTION

Despite the fact that rabies is one of the oldest known human infections, the pathogenic mechanism by which Rabies Virus (RV) infection leads to the development of neurological diseases and death is not well understood (Dietzschold *et al*., 2005; Dhingra *et al*., 2007). RV causes a non-lytic infection of neurons leading to a fatal myeloencephalitis in mammals including humans (Baloul and Lafon, 2003).

Many results support the hypothesis that fatal rabies may result from neuronal dysfunction rather than from structural damage (Munzel and Koschel, 1981; Tsiang, 1982; Dhingra *et al*., 2007; Weihe *et al*., 2008). When rabies virus is passaged in animals, chick embryos or cultured cells, the virulence of some strains may be gradually attenuated for example SAD B19, ERA, SAG, SRV_9 and so on.

The attenuated RV vaccine strains (Titoli *et al*., 1982; Lawson *et al*., 1987; Masson *et al*., 1996; Vos *et al*., 1999) are not lethal to adult animals however, the young ones may actually die. The survivals will be dysfunction. Experimental studies shown that the immune response and possible immune suppression are largely influenced by strain, dose and route of inoculation (Nandi and Kumar, 2011).

SRV_9 is an attenuated RV vaccine strain. In the present study, we inoculated intracerebrally on suckling mouse with SRV_9 strain. The results showed that SRV_9 could cause a mortality rate of 100% on suckling mice below 13 day old but without incidence above 18 day old.

Meanwhile, the 16 day old survival suckling mice were chosen to perform body weight test and the result showed that the weight growth was very slow with 14% less than normal growth rate. However, 2 weeks latter the weight of the mice began to recover gradually but slowly.

It was suggested that the intracerebral RV reproduction could take a significant effect on the growth and development of the mice.

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MATERIALS AND METHODS

This research project was conducted from March 2009-June 2010.

Virus: SRV_9 is a plaque cloned vaccine strain that has been kept in the laboratory for over 20 years. It can cause suckling mice death by intracerebrally inoculating with a dose of 300 LD_{50}/0.03 mL. The brain mixtures were homogenized, aliquoted and frozen at -80°C before using. The LD_{50} of SRV_9 was tested in the suckling and 3 day mice, the virus aliquots followed the protocols described elsewhere (Meslin *et al*., 1996).

Animal and grouping: All Kunming strain mice used in the experiment were purchased from the Changchun H and N Animal Breeding Center for Medicine, Changchun, China. They were fed *ad libitum* and kept away from the healthy mice after being challenged with SRV_9. Grouping refers to 2.3, 2.6 and 2.7.

The death age determination of suckling mice infected with SRV_9: About 1-22 day old suckling mice divided into 22 groups with 12 per group were intracerebrally inoculated with 30 μL of 10^4 LD50 mL of the SRV_9. The mice were checked twice daily and the mortality rate was recorded.

The direct Fluorescent Antibody Test (FAT): The dead and survived mice brain was tested by FAT which followed the protocols described elsewhere (Meslin *et al*., 1996).

The neutralizing antibodies detection by Fluorescent Antibody Virus Neutralization test (FAVN): The presences of rabies virus specific antibodies were detected by an OIE prescribed method described elsewhere (Cliquet *et al*., 1998). The neutralizing antibody titers were calculated by Spearman-Karber formula.

The effect of RV SRV_9 on 16 day old sucking mouse growth after intracerebral inoculation: The 16 day old suckling mice were divided into three groups: group I included 50 suckling mice with the dose of 300 LD_{50}/30 μL/pieces SRV_9 by intracerebrally inoculating brain suspension, 20 survivals mice were tested (A group); group II included 20 suckling mice with the dose of 30 μL/pieces by intracerebrally inoculating normal brain suspension (B group); group III was without any treatment (C group). Then, all the mice were weighed each 2 day, respectively. The results were recorded and drawn the growth curves by SPSS 17.0.

The effect of intracerebrally inoculating with SRV_9 to 26 day old on weight: The 26 day old mice were divided into three groups with 20 mice in each group. The doses were as follows: 300LD_{50}/30 μL/pieces SRV_9 by intracerebrally inoculating with brain suspension group (D group); 2.30 μL/pieces by intracerebrally inoculating normal brain suspension group (E group); 3. The control group was not treated (F group). Then, all the mice were weighed each 2 day. The results were recorded and drawn the growth curves by SPSS 17.0.

RESULTS AND DISCUSSION

The death rate determination of suckling mice infected with SRV_9: A number of 12 mice per group were intracerebrally inoculated with 30 μL of 10^4LD50 mL of the SRV_9. The mortality rate was recorded (Table 1).

FAT: All the dead sucking mice brain were tested by FAT and the results were all positive. Rabies viruses were detected in the brain of the survival suckling mice 2 weeks after intracerebral inoculation with SRV_9 (Fig. 1).

FAVN: About 2 weeks after intracerebral inoculation with RV SRV_9 strain, all the survival mice sera were tested by FAVN and the titer centered at 23.38±30.77 IU. It confirmed that all of them were infected by the rabies virus and then the RVs were eliminated by antibody about 2 weeks latter.

The effect on weight of survival 16 day old suckling mice after intracerebral inoculation SRV_9: Growth of the 16 day old survival suckling mice was significantly inhibited (Fig. 2). The mean weight of SRV_9 group increased 1.42 g, untreatment group and normal brain tissue control increased 10.12, 9.55 g, respectively. The weight growths of the survivals within 2 weeks were very slow with less than normal growth rate of 14%.

The effect on weight of 26 day old mice after intracerebral inoculation SRV_9: The effect of intracerebrally inoculating RV SRV_9 strain on 26 day old mice growth was without obvious variance (Fig. 3).

RV SRV_9 as the oral vaccine in China for many years is an attenuated vaccine strain from the SAD B_{19} strain by plaque cloning and mouse inoculation test screening. This study showed that the growth of survival mice was significantly inhibited after being intracerebrallyin-oculated with RV SRV_9 strain (Fig. 2). However, the

Table 1: The death rate of 1-21 day old suckling mice infected with SRV_9

Age (days)	1-13	14	15	16	17	18-22
Mortality (%)	100%	68%	33%	33%	17%	0

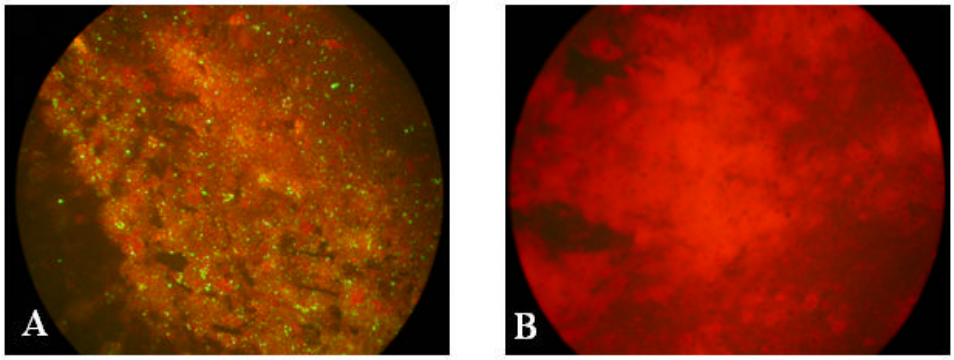

Fig. 1: The results of FAT. All the dead sucking mice brain were positive tested by FAT (A); negative control (B)

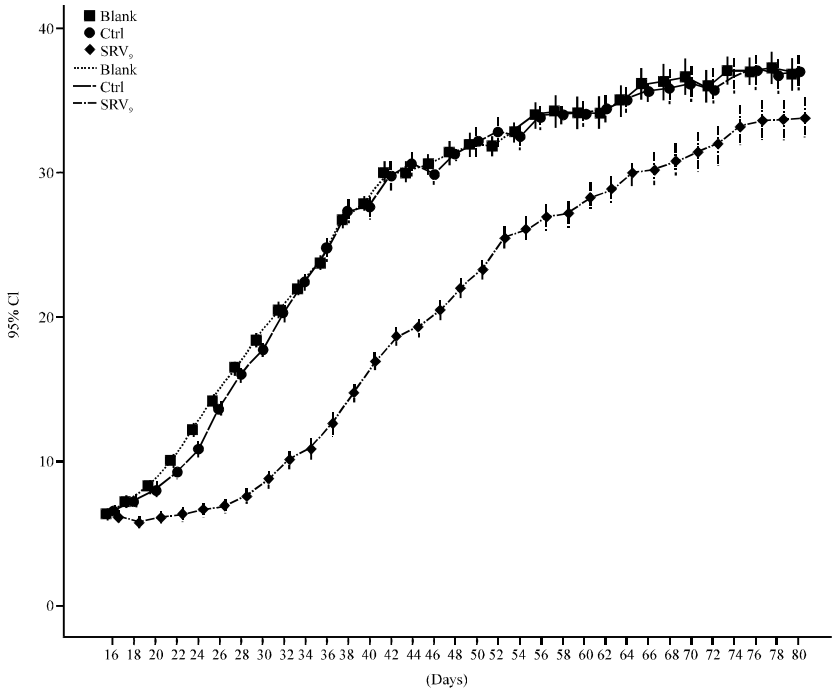

Fig. 2: The growth curves of 16 days old sucking mice were drawn by SPSS 17.0; SRV_9: 300 LD50/30 uL/pieces SRV_9 by intracerebrally inoculating with brain suspension group; Ctrl: 30 uL/pieces by intracerebrally inoculating normal brain suspension group and Blank: the control group was without any treatment

effect was not so obvious in adult mice (Fig. 3). The real reason for this phenomenon is unclear. Presumably, the RV could invade the brain tissue when sucking mice were in crucial phase of growth during the proliferation of the virus which affected or even terminated a variety of genes expression including genes related to growth.

Compared with virus reproduction effect, the pathological damage and physical damage are not the main factors in the affect to the growth of mice because the control groups are normal. As for SRV_9 injection group, the reason for slower weight gain maybe is that with the virus existed and reproduced the antibody to SRV_9 increased and neutralized the virus till the virus was cleaned up. The genes that controlled the growth had been expressed and even completely expressed during the growth when the mice developed to the adult so the affect of the virus reproduction to the growth was not significant.

The effects of virus reproduction in the brain are certainly multi-faceted. However, the inhibition to the growth is apparent phenomenon or most relatively obvious. This phenomenon is not the specific characters of attenuated RV but also refers to other neurotropic viruses such as the polio virus, encephalitis virus.

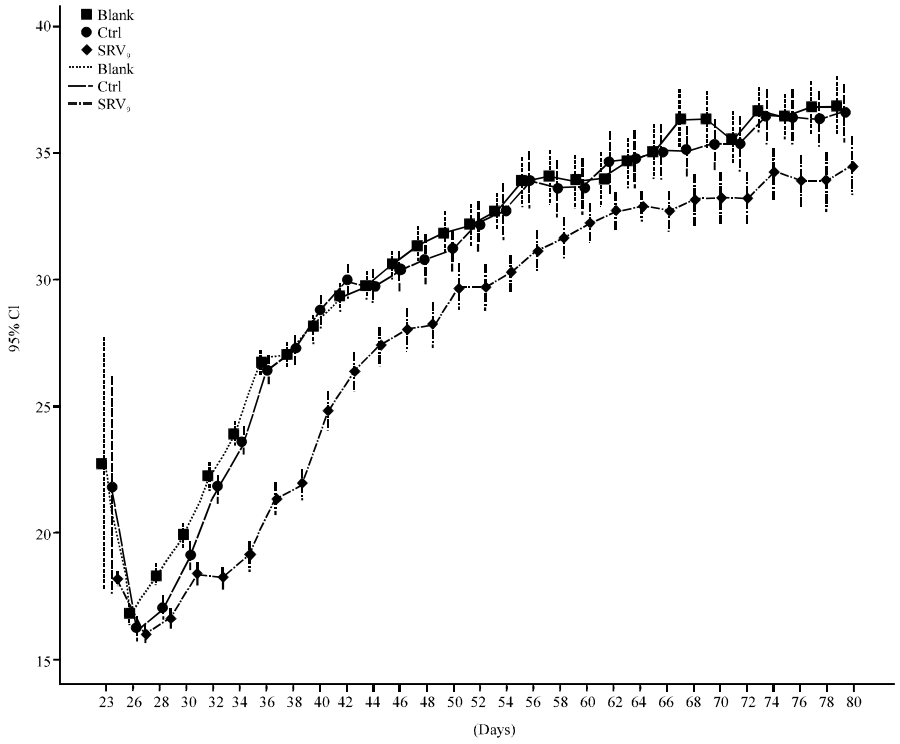

Fig. 3: The growth curves of 26 day old mice were drawn by SPSS 17.0; SRV_9: 300 LD_{50}/30 uL/pieces SRV_9 by intracerebrally inoculating with brain suspension group; Ctrl: 30 uL/pieces by intracerebrally inoculating normal brain suspension group; Blank: the control group was without any treatment

It was reported that cachexia, a severe loss of body weight often complicates the late stages of retroviral infections such as Human Immunodeficiency Virus (HIV), visna/maedi virus and Feline Leukemia Virus (FeLV), (Kotler *et al*., 1985; Hartke *et al*., 1995; Diao *et al*., 2001; Baricevic *et al*., 2004; Tremolada *et al*., 2008). Certainly, there is much theoretical and conflicting experimental evidence that retroviruses alter the cytokine milieu and in doing so alter metabolic rates and energy intake (Beutler and Cerami, 1989; Grunfeld *et al*., 1992; Grunfeld and Feingold, 1992).

It suggested that it should be pay more attention to the effect to young animals when using attenuated vaccine or living virus vector vaccine, especially neurotropic virus vaccine. In addition, the mouse model can performed related research to examine growth inhibition using this method.

CONCLUSION

It is concluded that normal brain suspension inoculation control group did not show any different. Meanwhile, the 26 day old mice treated with the same method could not observe abnormal of the weight.

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