Development of an Oral Rabies Vaccine with improved safety and efficacy to control Canine Rabies in ownerless and stray dogs

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Abstract

Most of the rabies deaths in humans are due to dog bites in particular stray- or community-owned dogs. An effective and economical tool to combat this threat would be oral immunization of these dogs by a bait delivery system in addition to parental vaccination of reachable pets. Live oral Rabies vaccines have already been used for decades in Europe and USA to control wildlife rabies. However, the attenuated Rabies vaccine strains used worldwide are considered not safe enough under the Indian conditions. Our recent development leads to improve the safety of the original SAD strain. First a SAD mutant has been derived wherein the Arginine residue of Glycoprotein G at position 333 is stably replaced with Aspartic acid (to obtain strain ORA-D). ORA-D is non-pathogenic for adult mice given intracerebrally (IC), but possesses residual pathogenicity for baby mice if given IC. Then a further small deletion of 7 amino acids was introduced into the P-protein. This removes the LC8 binding site, thereby preventing axonal transport of the virion core along a neural tract. These steps lead to the development of a stable ORA-DP vaccine virus, which is now even safe in 1-2 days-old suckling mice after intramuscular inoculation. To enhance the immunogenicity, the ORA-DPC strain has been developed by inserting an additional glycoprotein G derived from the CVS-strain (which included the replacement of Arg to Asp at 333). The insertion even further improves the safety profile of the strain. Its enhanced immunogenicity induces higher neutralizing antibody titers even in immuno-suppressed dogs. ORA-DPC is the best candidate Rabies vaccine strain available for oral immunization of dogs.

Recently held WHO expert consultation meet on Rabies has identified mass immunization as the single most effective tool for dog rabies control whereas dog culling alone is ineffective one. In addition, it has also been recommended that use as a complementary measure of oral vaccines in dogs in addition to i/m and s/c routes. In such situation, ORA-DPC has a greater role to play.

Introduction

Rabies remains one of the most dreadful infectious diseases affecting human and animals despite significant scientific advances in prevention and control. Rabies presents as a distinct problem in different parts of the world. In the more industrialized nations, the risk to human beings has minimized significantly, mainly due to mandatory vaccination programs of dogs and other pet animals. Although wildlife rabies still exists in the developed countries, most impressive progress has been made in control and elimination of wildlife rabies using oral immunization of wild carnivores. In contrast, rabies remains a major threat to public health and persists to cause numerous human deaths in the less industrialized nations.

Rabies is still epizootic in most countries of Africa, Asia and South America and in these countries dogs are responsible for most human deaths from the disease. Dog rabies control relies principally on the mass immunization of dogs in order to achieve population immunity levels sufficient to inhibit rabies transmission. The presence of large numbers of “ownerless” dogs that are not accessible for parental immunization contributes greatly to failure of vaccination programs in these parts of the world. Therefore, introducing new control strategies in addition to the existing parental vaccination programs is a necessity.

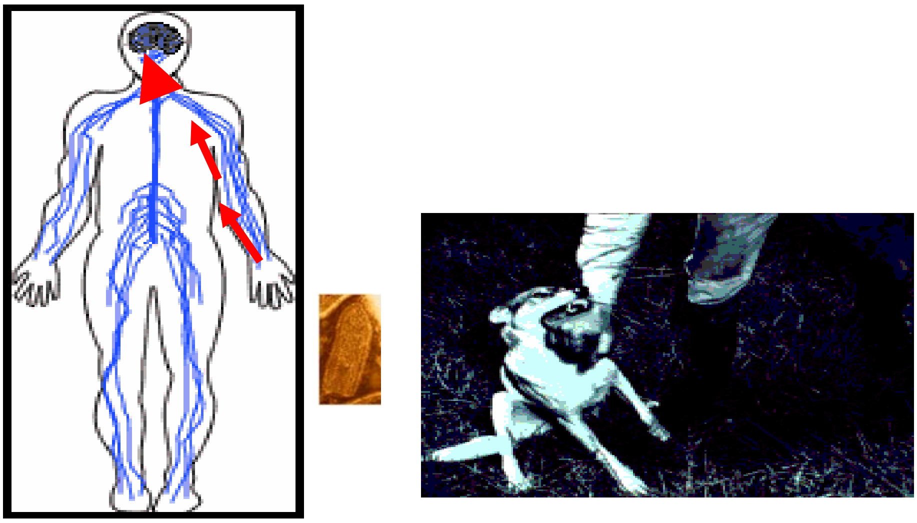


Fig.1

In India, Rabies is common in all parts of Indian Subcontinent except Andaman and Nicobar Islands, Lakshwadeep etc. Each year approximately 7 million people undergo post-exposure treatment after a dog-bite. India has a dog population of around 25 to 30 million animals. Most of them are stray dogs. The majority of this stray dog population is found in rural areas and they play a major role in the spread of the disease (Jayakumar, 1998). More than 99% of human deaths in the world occur in Tropical and Developing Countries. In India alone 30,000 to 50,000 people die of rabies every year (WHO). But a recent APCRI report estimates a lower number and that it would involve around 20,500 people, which is still a frequency of 1 human rabies death per every 30 minutes (APCRI 2004).

In order to increase vaccination coverage in dogs, several countries are presently volunteering to make use of oral vaccination of dogs as a valuable complement to parental dog immunization (WHO, 1998). The vaccine viruses involved include live modified viruses and recombinant vaccines expressing rabies virus glycoprotein (G protein).

For live vaccines that are prepared for oral vaccination of wild (or domestic) animals, innocuity and efficacy in target animals, and safety in non-target species must be demonstrated.

In this paper the vaccine virus strains for oral immunization of dogs against rabies are referred to as ORA-D for strain SAD-D29 (Mebatsion, 2001) and ORA-DP / ORA-DPC for the further derivatives obtained from the ORA-D, as is explained in the text below.

Materials and Methods

Cells and virus: BSR-T7/5 cells and BHK-21 cells are used for propagating the virus. Candidate Rabies virus vaccine strains ORA-D, ORA-DP and ORA-DPC (all are sequentially derivatives of SAD strain) are used for testing in the animals. Vaccine virus titers harvested were in the range of $10^{7.3}$ to $10^{8.3}$ FFU per ml.

Test animals: 6-week old female BALB/c mice for efficacy studies, whereas 4-6 week old Nude (nu/nu) and SCID (CB17) mice (immuno-compromised) for pathogenicity studies have been used. Beagle dogs 4 months of age were used for safety and efficacy studies.

Treatment and immunization of animals: 0.1 ml of vaccine virus is injected intramuscularly (i/m) to BALB/c mice and 2 weeks later, a similar dose has been given by the same route. Two weeks after booster vaccination, all animals including control ones are challenged with 0.03 ml of 100 LD_{50} of CVS strain. 50 µl of vaccine virus is injected into immuno-compromised mice by i/m route. Beagle dogs were treated with dexadresson (0.5 mg/kg body weight) by subcutaneous injection, 3 times per week for a 3-months period. Vaccine virus (10^8 FFU) was given orally the first week and a booster vaccination at 8 weeks after starting the corticosteroid treatment. The untreated control group of dogs was vaccinated similarly.

Observation of clinical signs: BALB/c mice were observed for clinical signs of Rabies for 4 weeks. Immuno-compromised mice were observed daily for rabies clinical signs for a period of 3 months. Both untreated and treated (dexadresson) groups of dogs were observed for general health conditions and behavioral change.

Virus isolation: Virus isolation has been attempted from the brain samples of dead and moribund mice or from saliva samples of vaccinated dogs.

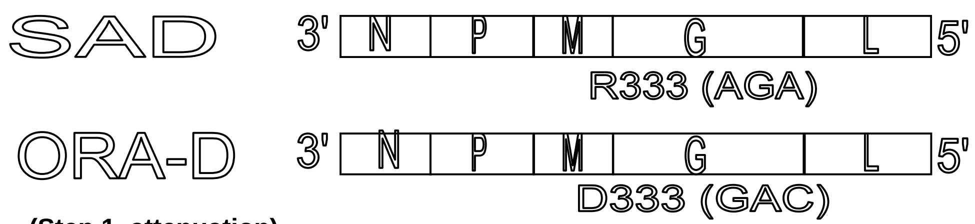
Serology: Sera samples from dogs were collected for antibody analysis using the Rapid Fluorescent Focus Inhibition test (RFFIT) as described elsewhere.

Results and Discussions

<u>ORA-DPC construct:</u>

Genetically stable engineered rabies virus

Fig.2

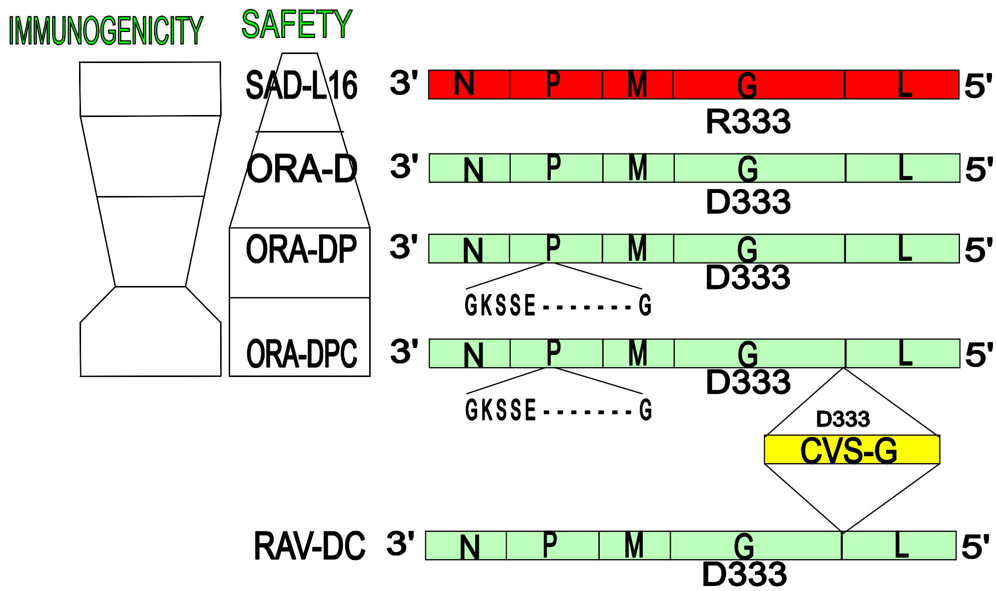


(Step 1, attenuation)

The triple mutant ORA-D is <u>genetically stable in suckling mice passages</u> and in <u>more than 25 cell culture passages</u> in the absence of any Mab.

Increasing immunogenicity without affecting safety

FIG. 3



I. Safety studies in immuno-compromised animals

1. <u>In Mice</u>:

To test the pathogenicity of the live oral rabies strains ORA-D and ORA-DP they were inoculated into 1-week and into 2-days old baby mice at a dose of 10^2 FFU with the parent strain SAD as a control. Whereas ORA-D is safe in 1-week old mice, only ORA-DP has no pathogenicity left for 2-days old mice. In Fig. 4 the increasing safety in mice is shown in relation to the steps taken for the attenuation.

Further testing of the pathogenicity was done in immuno-incompetent mice, i.e. Nude and SCID mice. Here ORA-D, -DP and -DPC, respectively, were inoculated by intramuscular route into Nude or SCID mice at doses of 10^5 FFU or 10^2 FFU (Fig. 5). All Nude mice infected remained healthy throughout the observation period of three months. In contrast, one out of six (~17%) SCID mice from each dose group treated with 10^5 and 10^2 FFU of ORA-D showed symptoms and died of rabies. No signs of rabies or mortalities have been observed in the SCID mice inoculated with ORA-DP, demonstrating extended attenuation of the ORA-DP mutant even in immuno-compromised mice. Also the further improved virus strain **ORA-DPC**, possessing two glycoproteins and in addition to the deletion of 7 amino acids in the LC8 binding site of the P-protein was studied in immuno-compromised mice. It was inoculated by intramuscular route into Nude or SCID mice but at a higher dose of 10^6 FFU. All the infected mice remained healthy throughout the observation period of three months (Fig. 5), demonstrating the safety of ORA-DPC in immuno-compromised mice. Thus, the safety of the ORA-DP and ORA-DPC modified vaccine viruses has been proven in the very susceptible, immuno-compromised mice, as well as in 2-days old suckling mice.

Fig. 4

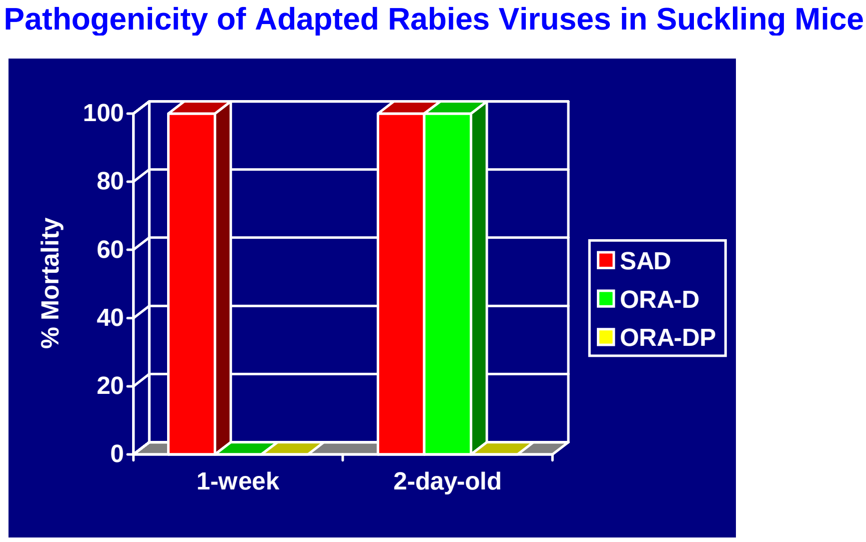


Fig. 5

Pathogenicity of the adapted viruses in Nude and SCID mice

Mortality (%)

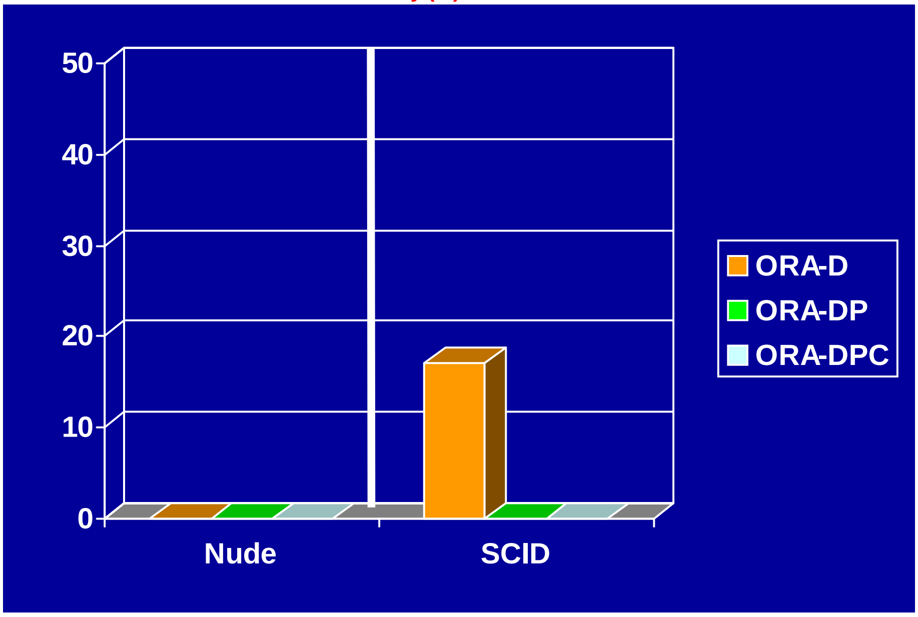


Fig. 6

Safety and Efficacy of ORA-DP/-DPC in dogs. Antibody response (IU/ml)

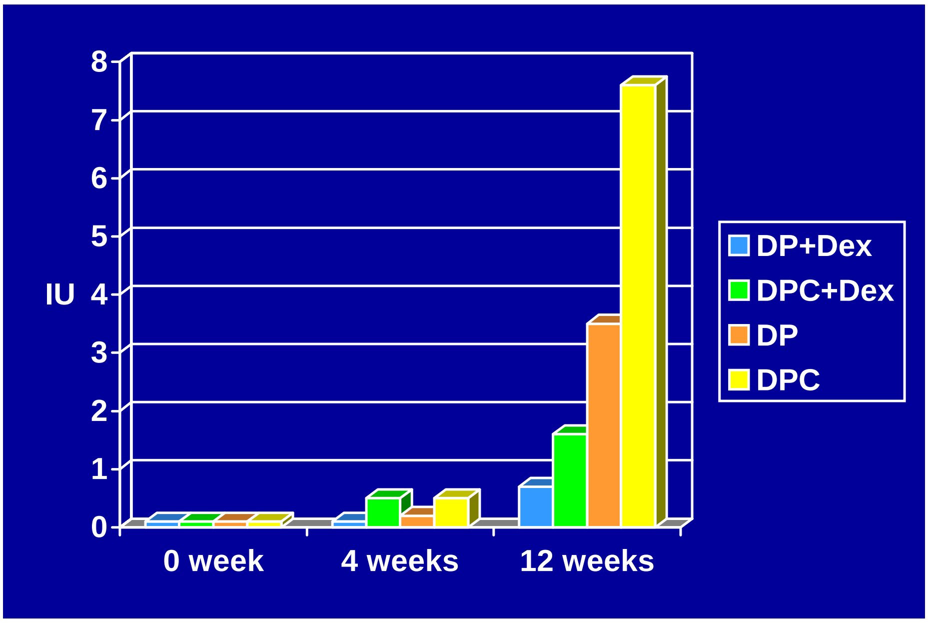
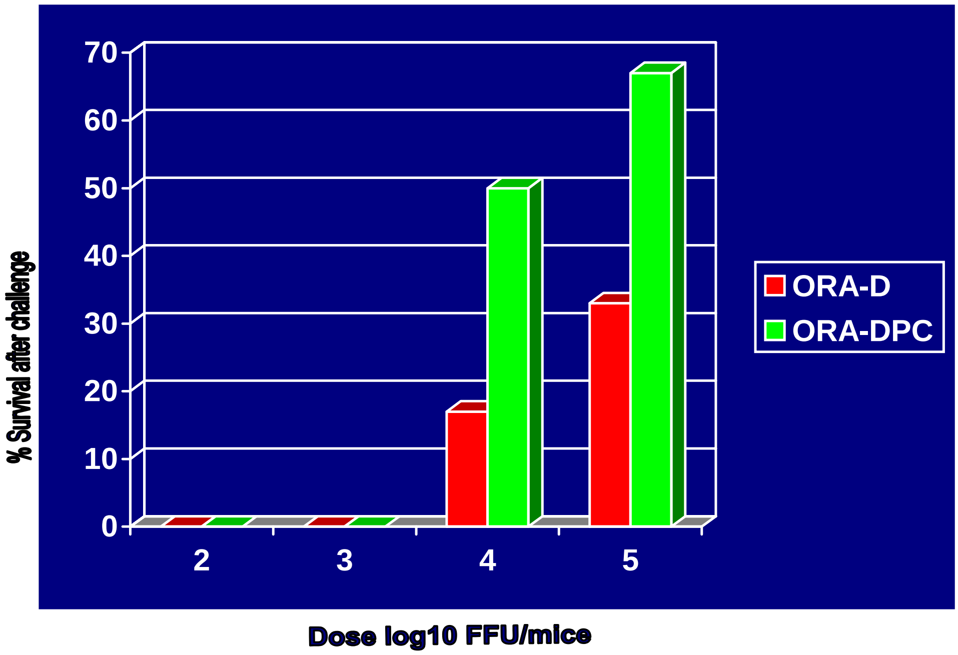


Fig. 7

Efficacy of Rabies Viruses in Mice



2. <u>In Dogs:</u>

The safety of two live oral rabies virus candidate vaccine mutants ORA-DP and ORA-DPC viruses was determined in immuno-compromised dogs after oral immunization. The development of antibodies in the presence and absence of dexadresson treatment is shown in Fig. 6. No rabies related signs or other adverse reactions were detected throughout the observation period of three months. No vaccine virus could be re-isolated from the saliva of the dogs 1 day p.i. Thus, safety of the ORA-DP and ORA-DPC modified vaccine viruses has been shown in the most susceptible species, i.e. immuno-compromised dogs.

II. Efficacy studies

<u>1. In Mice:</u>

Results of challenge experiments (NIH challenge model) demonstrated that survivorship after challenge strongly increased in a dose-dependant manner. Survivorship of mice immunized with ORA-DPC at doses of 10^5 FFU was 2- to 4-fold higher than that of the mice immunized with ORA-D. Fig. 7 demonstrates that over-expression of rabies virus G strongly enhances the protective ability of a live rabies virus vaccine against challenge.

<u>2. In Dogs:</u>

Interestingly, the immune response in ORA-DPC treated group was around two-fold higher than that of ORA-DP group at 4 and 12 weeks after the prime dose.

The results demonstrate that ORA-DPC appears more efficient than ORA-DP in inducing anti-rabies antibodies (Fig. 6). The immuno-suppresive effect of dexadresson on the antibody response is most notably at 12 weeks showing as expected much lower titers in the dexadresson treated group. Nevertheless the improved response resulting from the additionally expressed G-protein is present in both groups at each time point (4 and 12 weeks).

In conclusion the rabies vaccine virus strain ORA-DPC is highly efficacious for oral immunization of dogs.

Conclusion

The oral vaccine strains developed through *'reverse genetics'* technology are genetically stable and very safe, even in immuno-compromised animals. In addition, they are efficacious in raising protective antibody titers in the vaccinated dogs after oral immunization. The most promising strain being ORA-DPC, hence providing a effective tool for oral vaccination of stray and community-owned dogs, and with a suitable bait delivery system, it can be the best option to control canine rabies by mass immunization as suggested by WHO in addition to parental immunization.

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