Construction and Titration of a Recombinant Canine Adenovirus Expressing the Rabies G Protein

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Abstract

Rabies is a fatal disease known since ancient times, and the only weapon against them is the vaccination. Starting with the 19th century has developed rabies vaccines which registered a real success, but the current trend is to search for safer and more economical formulas. In this paper we designed a structural model of a rabies vaccine using the molecular cloning techniques represented by a recombinant adenovirus (CAV-2) where the E3 region was replaced with an insert represented by G rabies protein. Has started from a plasmid containing the canine adenovirus type 2 virus (CAV-2) and another one which contain rabies virus glycoprotein, and both plasmids were subjected to enzymatic digestion by restriction enzyme such as *Swa*I, *Mbo*I, *Asc*I and *Not*I, thus obtaining the pRecCAV2CMV-G vector. This vector was transfected in DKcre cells, multiplied and purified. The results after titration in DKcre cells revealed us a 10^8 TCID_{50%}/ml virus concentration and $10^{10.4}$ infectious particles using real time PCR.

Keywords: *canine adenovirus, molecular cloning, rabies, recombinant, vaccine*

INTRODUCTION

Rabies is a fatal disease of global importance resulting in encephalitis affecting both humans and animals (Warrel, 2004; Rupprecht *et al.*, 2008; Tordo *et al.* 2005) produced by a lyssavirus transmitted by the bite of an infected animal or by aerosols in several species of bats especially in North and South America (Xiang *et al.*, 1994). From Pasteur to the present rabies vaccine production technologies have evolved tremendously with the benefits of biotechnology development (Rosset, 1985). For rabies immunization of domestic animals inactivated vaccines are used. These vaccines have different degrees of purity and after vaccination may occur neurological accidents which are caused by the presence of whole viral particle, or incomplete inactivation or adjuvants used. To overcome these drawbacks subunit vaccines (Yang *et al.*, 2008; Yuan *et al.*, 2008; Yusibov *et al.*, 1997, Zhou *et al.*, 2010) why not use whole viral particles, vaccines that use only

the viral genome and recombinant vaccines have been researched and tested (Szelechowski *et al.*, 2012). All these new types of vaccines have been shown to represent an alternative to conventional vaccines. In addition, because of the current economic crisis that shows no sign they would end soon, vaccine manufacturers are looking for solutions on the production process, and to solve old problems that can occur after vaccination. To eliminate these drawbacks a promising solution is the use of vaccinal vectors obtained using DNA recombinant technology.

In this paper we developed a new vector derived from CAV-2 expressing the G protein of rabies virus which could bring improvements in animal rabies vaccination strategy.

MATERIALS AND METHODS

Dog kidney cells (DK-cre), were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal

calf serum at 37°C in 5% CO₂. Construction of CAV-G R⁺ and CAV-GFP used laboratory plasmids (polyG and poly GFP, pNavette 311-319 pA CMV), *Sma*I, *Mbo*I, *Asc*I and *Not*I restriction enzyme (BioLabs Laboratories) and CAV-2 strain Manhattan (ATCC VR-800). A model was realised using SerialCloner 2 program. For titration we used real time PCR (Roche-Applied), PCR (Eppendorf) and the biophotometer Eppendorf.

RESULTS AND DISCUSSIONS

Rabies virus glycoprotein which represent gene of interest in present experiment, it was supplied into a plasmid named generically poly A from an authorised laboratory.

The plasmids poly A and pNavette 311-319pA CMV was digested and separated using *Sma*I and *Mbo* I restriction enzyme. The plasmid thus obtained was designated as pNavette 311-319 G CMV and it has a length of 6111 bp.

The next level of this research was homologous recombination using DHC cloning subpopulation (Invitrogen) of pNavette 311-319 G/GFP CMV which was digested by *Asc*I and *Sma*I thus obtaining pCAV311-319nuII (33473pb) plasmid.

That plasmid was digested by *Not*I and result pCAV311-319G/GFP CMV (33473bp). This one was digested by *Asc*I and result the vector pRecCAV2 CMV G/GFP (35531bp) who was after that transfected, multiplied and purified.

After titration on DKcre cells and using the Reed-Muench formula was obtained 10^{8,6}TCID₅₀/ml vector titer. Using real time PCR was established a concentration of 10^{10,4} infectious particles in 2 µl of vaccine solution. The biophotometer measurement at DO₂₆₀ nm revealed a 1,1x10¹² concentration of CAV-G virus.

Conventional vaccines have proven to be very successful in preventing rabies. However, there is a concerted effort to clarify safer and more effective vaccines that would overcome the disadvantages of current preparations. Since the pioneering activity of the early 1990s, immunisation with plasmid DNA has proven to be a valid alternative to conventional vaccines, both for human and veterinarian use. In this study, our team has developed a new rabies vaccine candidate derived from CAV-2 vector and using recombinant DNA technology.

Viral vectors offer several advantages for vaccine delivery compared to conventional vaccines. First of all, because of the possibility of delivery multivalent vaccines using one type of vector can result in one manufacturing process, rather than more, and possibly even one vaccination process. The vector only expresses the antigens of the pathogen that are required to elicit a protective immune response and, therefore, reduces or eliminates chance of being exposed to all pathogenic disease as a live vaccine killed or modified (Schultz, 1999). At the level of research for the development of new

Fig.1. Enzymatic digestion of polyA and pNavette 311-3119 pACMV 5406bp using the *Sma*I and *Mbo*I restriction enzyme (a) and construction of a pNavette 311-319 G/GFP 6111bp (b)

Fig.2. Separation of pNavette 311-319 G/GFP CMV using Ascl and SwaI restriction enzyme

Fig.3. After digestion with NotI result pCAV311-319G/GFP CMV 33473 bp (a) the final vector pCAV311-319G/GFP CMV 35531 bp was born after enzymatic action of AsclI enzyme (b)

vaccines have been used as vectors many types of viruses. Poxviruses are large viruses containing a DNA genome who can accommodate large amounts (over 25 kb) of extra DNA (Merchinsky, 1992) thus several transgenes can be expressed simultaneously providing a multivalent vaccine approach (Perkus *et al.*, 1985, Boyle *et al.*, 2004), and the recombinant vaccinia virus to be used in the field was the Copenhagen strain expressing the rabies virus surface glycoprotein (Blancou *et al.*, 1986) in 1982. From then until now many strains have been engineered to express a variety of antigens from a number of pathogens in various animal species (Brun *et al.*, 2008). Several baculoviruses (Abe *et al.*, 2003) and herpesviruses have been engineered to serve as vaccine vectors (Xuan *et al.*, 1998). RNA viruses classified in the family Paramyxoviridae, Bunyaviridae, Rhabdoviridae, Coronaviridae, Retroviridae, Alphaviridae or Flaviviridae have served as vaccine vector for some pathogens.

Adenoviruses (Ad) have been isolated from humans and many animals, including bovine, ovine, porcine, canine and avian species. Human

Ad are by far the best characterised, and the most tested was the vectors derived from serotype 5 of Ad (Ad5). Vectors derived from several animal adenoviruses have been evaluated as vaccines delivered by parenteral routes with promising results. In this experiment was developed a vector derived from canine adenovirus type 2. Using CAV-2 vectors presents certain advantages such as infecting a large number of cells both in vivo and ex vivo, does not integrate into the host cell genome, can transfer large foreign DNA sequences (30kb) and has particular affinity for the nervous system. Rabies vaccines based on CAV-2 having as insert rabies glycoprotein were constructed and tested in mice (Li *et al.*, 2006), cat (Hu *et al.*, 2007), dog (Hu *et al.*, 2006), pig (Lyu *et al.*, 2008) and sheep (Redwan *et al.*, 2009) with good results.

Rabies virus glycoprotein were used in different vaccine construction regardless of type of vector used, mainly because has increased immunogenic potential, but were also used formulations with rabies virus nucleoprotein as insert (Prehaud *et al.* 1999).

In our research were obtained a stable product and after titration was obtained the same value for the standard strain of CAV-2 and Adh-5.

CONCLUSION

In the present research work, we developed a new vaccine candidate that uses canine adenovirus type 2 (CAV-2) vector expressing the rabies virus glycoprotein which may represent an alternative to current immunological preparations available on the market.

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REFERENCES

1. Abe T, Takahashi H, Hamazaki H, Miyano-Kurosaki N, Matsuura Y, Takaku H (2003). Baculovirus induces an innate immune response and confers protection from lethal influenza virus infection in mice. *J Immunol* 171:1131-9.
2. Blancou J, Kieny MP, Lathe R, Lecocq JP, Pastoret PP, Soulebot JP, et al. (1986). Oral vaccination of the fox against rabies using a live recombinant vaccinia virus. *Nature* 322(July (6077)):373-5.
3. Bouet-Cararo C, Contreras V, Fournier A, Jallet C, Guibert JM, Dubois E, Thierry R, Bréard E, Tordo N, Richardson J, Schwartz-Cornil I, Zientara S, Klonjowski B (2011). Canine adenoviruses elicit both humoral and cell-mediated immune responses against rabies following immunisation of sheep. *Vaccine* 29(6):1304-10.
4. Boyle DB, Anderson MA, Amos R, Voysey R, Coupar BE (2004). Construction of recombinant fowlpox viruses carrying multiple vaccine antigens and immunomodulatory molecules. *Biotechniques* 37(July (1)):104-6, 8-11.
5. Brun A, Albina E, Barret T, Chapman DAG, Dixon LK, Keil GM, Klonjowski B, Czub M, Le Potier MF, Libeau G, Ortego J, Richardson J, Takamatsu HH (2008). Antigen delivery systems for veterinary vaccine development-Viral-vector based delivery systems. *Vaccine* 26:6508-6528.
6. Hu R, Zhang S, Fooks AR, Yuan H, Liu Y, Li H, et al. (2006). Prevention of rabies virus infection in dogs by a recombinant canine adenovirus type-2 encoding the rabies virus glycoprotein. *Microbes Infect* 8(April (4)):1090-7.
7. Hu RL, Liu Y, Zhang SF, Zhang F, Fooks AR. (2007). Experimental immunization of cats with a recombinant rabies-canine adenovirus vaccine elicits a longlasting neutralizing antibody response against rabies. *Vaccine* 25(July (29)):5301-7.
8. Li J, Faber M, Papaneri A, Faber ML, McGettigan JP, Schnell MJ, et al. (2006). A single immunization with a recombinant canine adenovirus expressing the rabies virus G protein confers protective immunity against rabies in mice. *Virology* 356(December (1-2)):147-54.
9. Liu Y, Zhang S, Ma G, Zhang F, Hu R (2008). Efficacy and safety of a live canine adenovirus-vectored rabies virus vaccine in swine. *Vaccine* 26:5368-5372.
10. Merchlinsky M, Moss B (1992). Introduction of foreign DNA into the vaccinia virus genome by in vitro ligation: recombination-independent selectable cloning vectors. *Virology* 190 (September (1)):522-6.
11. Perkus ME, Piccini A, Lipinskas BR, Paoletti E (1985). Recombinant vaccinia virus: immunization against multiple pathogens. *Science* 229(September (4717)):981-4.
12. Prehaud C, Takehara K, Flamand A, Bishop DHL (1989). Immunogenic and protective properties of virus glycoprotein expressed by baculovirus vectors. *Virology* 173:390-399.
13. Redwan RM, Fahmy A, El Hanafy A, El Baky NA, Sallam SMA (2009). Ovine anti- rabies antibody production and evaluation. *Comparative Immunology, Microbiology and Infectious Diseases* 32:9-19.
14. Roland D Schultz (1999). Veterinary Vaccines and Diagnostics. *Advances in Veterinary Medicine* 41:1-815.
15. Rosset R (1985). Pasteur et la rage. Imprimerie Commerciale 175, Paris.
16. Rupprecht CE, Barrett J, Briggs D, Cliquet F, Fooks AR, Lumlertdacha B, et al. (2008). Can rabies be eradicated? *Dev Biol (Basel)* 131:95-121.
17. Szelechowski M, Bergeron C, Gonzalez-Dunia D, Klonjowski B (2013). Production and purification of non replicative canine adenovirus type 2 derived vectors. *J Vis Exp* 2013 Dec 3(82):50833.
18. Tordo N, Ceccaldi PE, Gaudin Y, Wandeler AI, Collier L, Mahy B, (2005). Topley & Wilson's microbiology and microbial infections 51 10th ed. London: Hodder Arnold. 1104-6. Rhabdoviruses: rabies.
19. Warrell MJ, Warrell DA (2004). Rabies and other lyssavirus diseases. *Lancet* 363(9413):959-69.
20. Xiang ZQ, Spitalnik S, Tran M, Wunner WH, Cheng J, Ertl HCJ (1994). Vaccination with a plasmid vector carrying the rabies virus glycoprotein gene induce protective immunity against rabies virus. *Virology* 199:132-140.
21. Xuan X, Tuchiya K, Sato I, Nishikawa Y, Onoderaz Y, Takashima Y, et al. (1998). Biological and immunological properties of rabies virus glycoprotein expressed by canine herpesvirus vector. *Vaccine* 16:969-76.
22. Yang S, Xia X, Qiao J, Liu Q, Chang S, Xie Z, Ju H, Zou X, Gao Y (2008). Complete protection of cats against feline panleukopenia virus challenge by a recombinant canine adenovirus type 2 expressing VP2 from FFPV. *Vaccine* 26:1482-1487.
23. Yuan Z, Zhang X, Zhang S, Liu Y, Gao S, Zhang F, Xu H, Wang X, Hu R (2008). Development of recombinant canine adenovirus type-2 expressing the Gn glycoprotein of Seoul virus. *Biologicals* 36:162-167.
24. Yusibov V, Modelska V, Stepiewski K, Agadjanyan M, Weiner D, Hooper DC, Koprowski H (1997). Antigens produced in plants by infections with chimeric plant-viruses immunize against rabies virus and HIV-1. *Immunology* 94:5784-5788.
25. Zhou JX, Xue JD, Yu T, Zhang JB, Liu Y, Jiang N, Li YL, Hu RL (2010). Immune responses in pigs induced by recombinant canine adenovirus 2 expressing the glycoprotein 5 of porcine reproductive and respiratory syndrome virus. *Vet Res Commun* 34:371-380.