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Immune Response after Rabies Oral Immunization in Mice

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ABSTRACT

The objective of this study was to formulate an anti-rabies oral vaccine from the cell culture vaccine PV TECPAR to elicit the production of antibodies against the rabies in mice. A vaccine was developed using $10^{7.5}$ DL_{50}/0.03 ml viral antigens homogenised in lanovaseline to facilitate oral administration. Mice were vaccinated two times for seroconversion. Sera of the vaccinated mice showed a higher level of antibody production than the control group. These results could be used to direct the development of an anti-rabies oral vaccine.

Key words: Rabies, Oral vaccine, Mice, ELISA

INTRODUCTION

Infected wildlife constitutes a significant reservoir of rabies virus for humans and domestic animals alike. Hematophagous bat (*Desmodus rotundus*) has been recognized worldwide as the predominant vector for rabies virus in farm animals, but terrestrial wild animals, such as wolves, coyotes, raccoons, mongooses, weasels, skunks, foxes and monkeys are also implicated in rabies epidemiology (Lima et al. 2005).

A rabies vaccine that could be orally administrated would facilitate the inoculation of large numbers of wildlife, limiting the viral dissemination among the rural and wildlife areas, as has been previously successfully demonstrated in North America and Europe (Blancou et al. 1986; Bingham et al. 1997). An oral rabies vaccine for mice was developed nearly 30 years ago (Atanasiou et al. 1982). However, since then, studies have focused on the concerns regarding the possible side effects of existing oral vaccines (Artois et al. 1992), rather than the development of new vaccines.

Only recently a study, which used a cell-culture-inactivated rabies vaccine mixed with different bait media, has been published (Salome and Gowda 2010). The objective of this study was to formulate an anti-rabies oral vaccine from the cell culture vaccine PV TECPAR in a paste form for oral administration.

MATERIALS AND METHODS

Male and female CALB 21 mice (weight, 20–25 g; TECPAR, Curitiba, Brazil) were used in this study (n = 66). Mice were maintained in white standard cages in a controlled environment at 21–22°C (±2°C) with 50-55% humidity. The animals received a standard diet in the form of pellets

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(Chorili et al. 2007) and had *ad libitum* access to food and water. They were randomly divided into groups of approximately 10 per cage. The cages were divided into the following groups: A (n = 7), B (n = 9), C1 (n = 10), C2 (n = 10), D (n = 10), E1 (n = 10), and E2 (n = 10). Intracardiac blood was collected using 3 mL syringes as previously described by Hoff (2000). All animal procedures were authorised by the Ethical Committee from the Catholic University of Paraná (PUCPR).
Sera were analysed by ELISA and spectrophotometry (model µQuant- Bio-Tek Instruments, INC) at wavelength = 490 nm. Viral suspensions of *Lyssavirus* Pasteur virus (PV) (TECPAR) were cultured in baby hamster kidney (BHK) cells. The suspension was concentrated 1:15 by filtration through a porous (50 kDa) cellulose membrane (Pellicon XL Ultrafiltration Cassete, Millipore®) and then inactivated with β-propiolactone at a 1:8,000 mL dilution of viral suspension. The pH was stabilised at 8.2 using 400 µL of glicocola. Thimerosal (400 µL) was used to preserve the sample. An elevated pH was used to balance the acidic environment of the gastric tract. The final vaccine paste was produced by homogenising 3.0 mL of liquid vaccine in 3.0 g of lanovaseline, a neutral paste that served as a vehicle for vaccine administration. The vaccine paste had an infective potential of approximately $10^{7.5}$ DL_{50}/0.03 ml antigens per dose. Group A did not receive either neutral, or vaccine paste. Groups B, C1, C2, D, E1, and E2 received either vehicle (B and D), or the vaccine paste (C1, C2, E1, and E2). The vaccine paste was placed on the back of mice using the typical mouse cleaning behaviour of coat licking to orally deliver the vaccine. Twenty-four hours after paste application, all mice showed signs of paste ingestion, as demonstrated by a considerable reduction in the amount of paste on the coat. Wood shavings were only introduced into the cages 48 h after paste application.
Sera from all mice were tested for rabies virus neutralising antibody by a specific ELISA developed by the TECPAR. Sera were collected from group A at the beginning of the experiment, from B and C after 21 days, and from D and E after 42 days of the initial vaccination. Negative and positive sera obtained from mice (TECPAR) hyperimmunised with a rabies vaccine (via the intraperitoneal route) were used as controls. Statistical analysis was performed using the Mann-Whitney test.

RESULTS AND DISCUSSION

Lanovaseline was considered non-toxic to mice. This was confirmed through the constancy of their corporal mass, as determined by visual inspection. All mice survived the paste ingestion and only two mice presented signs of alopecia and epithelial flaking at their back, cervical region, scapulas and between the shoulders. The negative and positive sera standards used at the same dilution (1:25) were 0.127 and 1.989, respectively. Table 1 summarises the results from this experiment.

Table 1 - Sera obtained from 66 mice, which had received the oral anti-rabies vaccine PV strain (TECPAR), were diluted 1:25 and the optic density, measured by ELISA. Mean, median, and standard error of each experimental group are reported.

Animal Group	Mean	Median	Standard Error of Mean
A (n = 7)	0.21 [a]	0.2	0.024
B (n = 9)	0.17 [a]	0.2	0.013
C (n = 20)	0.15 [a, c]	0.2	0.008
D (n = 10)	0.13 [b, c]	0.1	0.012
E (n = 20)	0.43 [b]	0.3	0.08

Group A: Mice received neither vehicle nor vaccine paste and were sacrificed at day 0. Group B: Mice received 1 dose of vehicle and were sacrificed at day 21. Group C: Mice received 1 dose of vaccine and were sacrificed 21 days later. Group D: Mice received 2 doses of vehicle and were sacrificed at day 42. Group E: Mice received 2 doses of vaccine and were sacrificed 42 days after initial dose. Statistical analysis was performed using the Mann-Whitney test ($P < 0.05$ [a] e [b]; $P < 0.001$ [b] e [c]).

These results indicated that mice treated with two vaccine doses demonstrated a highly significant increase in the optical density, which was detected after 42 days of the initial administration.
Comparative analyses of groups A and E showed a 97% probability that a higher optical density would be detected in the doubly vaccinated group (E) compared to the negative control group (A). Another factor that might alter the efficacy of the vaccine was the method of delivery. This study showed for the first time that an immune response could be elicited against a PV strain virus using vaccination via oral route in a paste form without creating lesions in the oral mucosa, or forcing oral ingestion of the vaccine. Although no viral challenge was performed, results demonstrated that a paste form of PV vaccine was capable of inducing an antibody immune response, similar to the effect observed in intraduodenal (Atanasiu et al. 1982) and intraperitoneal (Ashraf et al. 2005) vaccinations.

Countries in the northern hemisphere have shown much progress in the control and elimination of sylvatic rabies in wild carnivores using oral immunisation (Artois et al. 1992; Mebatsion et al. 2001; Vos 2003; Rupprecht et al. 2004; Cross et al. 2007). Usually, prophylactic vaccination has emerged with the use of lyophilised SAG2 and V-RG vaccines to immunise dogs, raccoons, skunks, bats, and foxes as reported by Rupprecht et al. (1986), Fekadu et al. (1996), Lambot et al. (2001), and Follmann et al. (2004). Although the use of similar methodology has been attempted in vampire bat (Almeida et al. 2005; Almeida et al. 2008) with partial success, no comercial product is available in the Brazilian, or South American markets with this intent. The present study complemented the previous initiatives to produce an effective and viable bat vaccine, modifying also the vaccine delivering paste. The mouse model presented here was another step in this direction.

The formulation used in the present study enabled the ingestion of vaccine virus by mice. This was described earlier by Atanasiu et al. (1982), who used a vaccine concentrate produced by the Pasteur Institute, and by Artois et al. (1992), who used SAG-1 and V-RG vaccines. The need of a second vaccination to increase the immune response is an issue that must be addressed.

Results show that an oral paste vaccine produced from the PV TECPAR virus could be administered via topical application on the back of mice with capacity of eliciting a strong immune response.

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