Assessment of the Plan for Pre-Exposition Vaccination with *Fuenzalida-Palacios* Anti-Rabies Vaccine

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ABSTRACT

Studies ere carried out to monitor the titres of anti-rabies antibodies taken from individuals exposed to the risk of infection. Fifty-seven individuals aged between 20 and 40, were vaccinated with a minimum titre of 1.3 IU Fuenzalida-Palacios type anti-rabies vaccine. The vaccination plan consisted of the application of three doses at two day intervals and a booster applied 28 days after the final dose. The accompainment of the antibody titres was achieved by means of the seroneutralization test over a total period of 18 months. Through the monitoring carried out, it was found to be necessary to apply a booster vaccination to part of the group. Some of these individuals were revaccinated with Fuenzalida-Palacios type vaccine with a minimum titre of 1.3 IU. And some with cellular vaccine with a minimum titre of 2.5 IU. All the individuals responded with titres greater than 0.5 IU, maintaining these levels until the end of the observation period, demonstrating a ready anamnestic response to both immunogens.

Key Words: Immunization, rabies vaccine, antibodies, neutralization, Fuenzalida-Palacios

INTRODUCTION

Rabies is a zoonosis which over the years has been the subject of much attention throughout the world, not only from health authorities but principally from the most varied research centres.

The prevention of human rabies is based, in essence, on the control of infection in animals, emphasizing in particular domestic carnivores and chiropteras as a principal breeding ground of the virus, in the Americas (Larghi, 1989; King et Alii, 1990).

In Brazil, according to the Ministry of Health's National Rabies Profilaxy Programme, the number of cases of canine rabies is particularly significant. Between 1980-1997, 22,032 cases were notified and in 1997 alone 1,454 cases of canine rabies were recorded.

In relation to human rabies, during the same period 1,230 deaths were recorded as being due to rabies infection. Dogs were responsible for 984 of these cases (80%) followed by bats which accounted for 77 cases (6.26%).

Considering the risk represented by the rabies virus to people who are in continuous contact with it, whether by means of contaminated material or by infected animals, there exists a risk group of considerable proportions. Therefore, it is essential that such people be periodically immunized and maintained under constant immunological assessment, pending the definition of the titres of neutralizing antibodies (Roumiantzeff, 1988; WHO, 1992).

In the case of rabies, measurement of specific immune response is performed by means of a seroneutralization test on mice (Roumiantzeff, 1988). It is unquestionable that the neutralizing antibodies are responsible for human protection against rabies infection. On the other hand, only individuals with titres equal to or greater than 0.5 IU are protected against infection by the rabies virus. Revaccination is recommended after a given period of time, determined in accordance with medical requirements, or when exposition to risk of infection is above this level. (Roumiantzeff *et alii*, 1988; WHO, 1992).

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It is important, however, to bear in mind that the immune response to rabies is not manifested, exclusively, at the expense of neutralizing antibodies. Thus, other types of antibodies take part in the process (Germano *et alii*, 1981), as do other immune mechanisms (Guillon *et alii*, 1980; Andral, 1982; Marcovistz *et alii*, 1987; Veiga et Alii, 1987; Zanetti *et alii*, 1989; Jayakumar *et al*, 1990).

The anti-rabies vaccines used in humans are not lacking in side effects, particularly those prepared in nervous tissue (Vodopija, 1988; Bahri *et alii*, 1996). However, with the advent of the Fuenzalida-Palacios (F&P) type vaccine, "suckling mouse brain" (Fuenzalida *et al*, 1965), the probability of the occurrence of post vaccinal accidents has reduced considerably; 1 : 263,377 according to the Brazilian National Health Foundation (May 97 - Bulletin). The reduction in the number of accidents is due to several technological improvements introduced in the production of this vaccine (Larghi, 1989).

In industrial countries, technological advances have made possible the adoption of other types of more sophisticated anti-rabies vaccines with virtually no risks as far as vaccinal accidents are concerned (Sureau, 1988; Chutivongse *et al.*, 1989; Wilde *et alii*, 1995; Fries *et alii*, 1996). Of special interest is the vaccine prepared from non tumourogenic VERO lines cells, derived from the kidney of the green African monkey *Cercopithecus aethiops* (Montagnon *et alii*, 1985; Fournier *et alii*, 1965). This production line vaccine routinely used in anti-rabies treatment and in prevention with risk groups provided very good results in relation to immune response (Kitala *et alii*, 1990).

MATERIAL AND METHODS

Serum Samples: Fifty-seven serum samples were taken from individuals between 20 and 40 years of age, who did not have an immune response to rabies and who underwent preventive anti-rabies treatment in accordance with World Health Organization standards for people involved in the production of biological products prepared from the rabies virus, using the method known as 3+1 (Zanetti *et alii*, 1995). After collect the sera were kept at -20ºC until use. When the seroneutralization test was done, the sera were inactivated at 56ºC for 30 minutes.

Vaccines: The following anti-rabies vaccines were used: Fuenzalida-Palacios, prepared in lactating mice brains, "suckling mouse brain" based on the CVS strain produced by the Paraná Institute of Technology – TECPAR, with a minimum titre of 1.3 IU and anti-rabies vaccine prepared on VERO cells by the Pasteur-Mérieux Institute of Lyon, France, having a minimum titre of 2.5 IU.

Animals: Albino Swiss mice of the CF1 strain with a minimum age of 21 days, weighing between 12 and 14 grams and originating from the TECPAR laboratory were used for the serological tests.

Standard Serum: The reference serum used was that of the TECPAR serology laboratory, having a titre of 5.2 IU and titrated by means of gama globulin produced by the Statens Seruminstitut – Center for Prevention and Control of Infectious Diseases and Congenital Disorders – an international reference laboratory for the WHO regarding biological standards for anti-rabies vaccines – in Denmark.

Virus: The CVC, strain VT 25 virus, with a titre equal to 10^7/0.03 ml. received from The National Quality Control and Health Institute (INCQS) in Rio de Janeiro was used in the serological tests.

Soroneutralization Test: The test was performed in TECPAR's Serology Laboratory in accordance with the technique described by Johnson (Johnson, 1974).

Experimental Delineation: Fifty-seven individuals, all TECPAR staff, who had not had any previous contact with the rabies virus were vaccinated for the first time against rabies using Fuenzalida-Palacios vaccine exclusively, with the intention of addressing the areas where the virus is handled. The vaccinal model consisted of the application of 3 doses of the vaccine at 2 day intervals, with a booster 28 days after the last dose. The first titration was performed 30 days after the booster; and the follow up of the titres took place at 90 day intervals after the first vaccination over a period of 18 months.

Those individuals who did not present protective titres (>0.5 IU/ml) after the first vaccination underwent a booster vaccination. In this way, three groups were obtained: the first comprised individuals who maintained a protective titre during the 18 month observation period; the second, formed by those who did not maintain a protective titre and who were given a Fuenzalida-Palacios booster vaccination; and the third, equal to the second group, but who were given a PV/VERO anti-rabies booster vaccination.

RESULTS

The following results were obtained based on the anti-rabies antibody titre readings, with the use of the classic seroneutralization test as standardised by the WHO:

- Of the total of 57 individuals vaccinated for the first time, 50 (87.7%) presented protective levels of anti-rabies antibody titres 30 days after the application of the preventive treatment.
- Of the 50 individuals with protective titres, 18 (32%) maintained the protective anti-rabies antibody titres up until the end of the observation period, 18 months after vaccination.
- Of the 7 individuals (12%) who did not present a protective titre against rabies 30 days after the first vaccination, plus a further 12 individuals who no longer presented a protective titre after 6 months, 3 and 6 of them respectively underwent an F&P anti-rabies booster vaccination and formed the first group, whilst 4 and 6 of them respectively received a PV/VERO booster vaccination and formed the second group. Thirty (30) days after the respective vaccine boosters these individuals showed titres greater than 0.5 IU and maintained them until the end of the observation period.
- Of the remaining 20 individuals who presented antibody titres of less than 0.5 IU 12 months after the first vaccination, 10 were given an F&P booster vaccination and the other 10 were given a PV/VERO booster vaccination and all of them formed the third group. After the boosters with the corresponding anti-rabies vaccines, all of them responded with titres greater than 0.5 IU and maintained this level until the end of the observation period.

The results referring to the individuals who received a booster vaccination after 6 and 12 months are shown in table 1 and figure 1.

Table 1 - Sampling of the individuals who received booster vaccinations after 6 and 12 months.

	Number of Individuals	Percent %
Individuals who received Booster vaccinations (no titre)	07 (3 + 4)	18
Individuals who received Booster vaccinations after 6 months	12 (6 + 6)	31
Individuals who received Booster vaccinations after 12 months	20 (10 + 10)	51
TOTAL	39	100

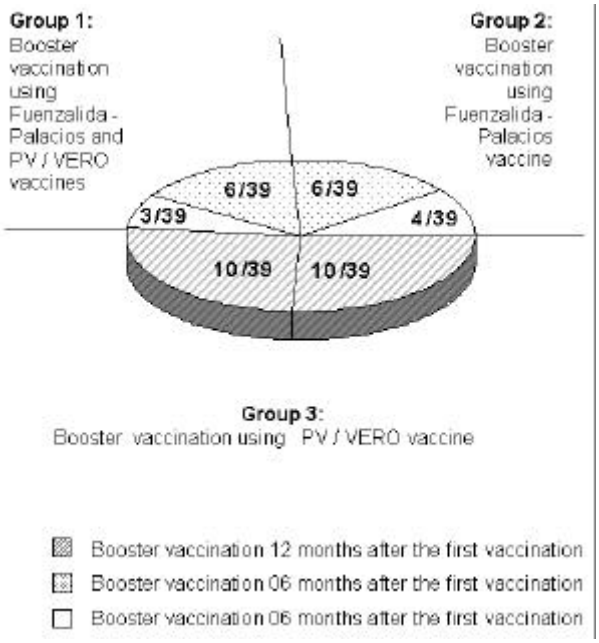

Figure 1 - Sampling of the individuals who received booster vaccinations after 6 and 12 months.

DISCUSSION

The study as a whole revealed that both vaccines presented similar results when used as boosters, thereby proving their effectiveness and demonstrating the rapid anamnestic response to both of the immunogens.

It is important to note that possible differences as to the size of the titres were not considered for the purposes of the study and that an option was made solely for the induction of anti-rabies antibodies at protective levels and their maintenance during the observation period; nor was the degree of inocuity

of each of these immunogens considered. However, it should be pointed out that for the doses used, for preventive purposes, according to TECPAR's Medical Service reports, no type of manifestation was observed which would suggest a post vaccinal allergic reaction in the individuals who underwent treatment.

Previous studies with both immunogens have already proven their qualities (Chuntivonse *et al.*, 1991; Zanetti *et alii*, 1989, 1995), with reservations only in relation to the F&P vaccine due to its being prepared in the brains of lactating mice and there therefore existing the possibility, albeit minimum, of it containing traces of myelin capable of producing an allergic response in vaccinated individuals (Suarez Hernandez *et alii*., 1984; Bahri *et alii*, 1996).

However, the PV/VERO vaccine is also not totally without side effects, although everything indicates that their proportion is minimum when compared to other immunogens, including the F&P vaccine (Chutivongse *et al.*, 1989; Bahri *et alii*, 1996).

In the case of developing countries, the F& P anti-rabies vaccine continues to be an immunogen which guarantees the development of immunity in humans submitted to it, at low cost, particularly when compared to more recent technology (Harry *et alii*, 1989). However, vaccine produced in cellular culture continues to be a safe alternative to be applied in cases where the individual has previously shown an allergic reaction to the F&P vaccine (Brazilian Ministry of Health, 1995; Paraná Health Department, 1996). No matter what, even considering the high cost of cellular cultivated vaccines (Roumantzieff *et alii*, 1985; Suntharasami, 1988; Sureau, 1988) in comparison with the F&P vaccine, it is recommended that both state-owned and private industries be encouraged to develop projects with the intention of making this immunogen available to the health services, principally for individuals with a history of sensitivity or immuno-depression (Brazilian Ministry of Health, 1995; Paraná Health Department, 1996) since its side effects are considered to be virtually nil when compared with immunogens prepared in other biological systems (Chutivongse *et al*., 1989; Bahri *et alii*, 1996).

RESUMO

Na raiva a mensuração da resposta imune específica é realizada através da prova de soro neutralização em camundongos. É inquestionável que os anticorpos neutralizantes são os responsáveis pela proteção dos indivíduos contra infecção rábica. Por outro lado, de acordo com a Organização Mundial da Saúde, somente os portadores de anticorpos neutralizantes com título igual ou superior a 0,5 UI estão satisfatoriamente protegidos. Este estudo mostra a monitorização de anticorpos anti-rábicos de indivíduos expostos ao risco de infecção rábica, realizada pelo Departamento Médico do Instituto de Tecnologia do Paraná. 57 indivíduos com idade entre 20 e 40 anos, foram vacinados com vacina tipo Fuenzalida-Palacios com título mínimo de 1,3 UI. O esquema vacinal constou na aplicação de três doses da vacina com intervalos de dois dias e um reforço vacinal 28 dias após a última dose. A monitorização dos títulos de anticorpos foi realizada através do teste de soro neutralização durante um período de 18 meses. Esta mostrou a nescessidade da aplicação de um reforço vacinal em parte do grupo. Parte destes indivíduos foram revacinados com vacina tipo Fuenzalida-Palacios com título mínimo de 1,3 UI e parte com vacina de cultivo celular com título mínimo de 2,5 UI. Todos os indivíduos responderam com título maior que 0,5 UI, mantendo estes níveis até o final do período de observação, demonstrando a pronta resposta anamnéstica de ambos os imunógenos.

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Received: June 15, 1999;
Revised: July 21, 1999;
Accepted: August 30, 1999.