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RABIES TREATMENT WITH VACCINE OF THE FUENZALIDA-PALACIOS TYPE[1]

Fernando P. Fábrega[2] and Carlos A. Sepúlveda[3]

The serologic responses of human volunteers vaccinated against rabies with Fuenzalida-Palacios vaccine indicate that good levels of immunization can be obtained with an eight-dose schedule of vaccinations. Overall, the results of this trial were better or comparable to those obtained by other investigators with longer vaccination schedules.

Introduction

Since July 1885, when Louis Pasteur first vaccinated a human subject against rabies, various different types of vaccines have been developed in an effort to find a biological product that will elicit a potent and assured immune response while causing minimal side-effects, discomfort, and expense. The most important of these vaccines use antigen obtained from nerve tissues, bird embryos, and (in recent years) cell cultures. Repeated doses of antigen must be administered in every case; and in some cases, owing to the gravity of the disease, an initial combination of antigen and hyperimmune antirabies serum must be given to ensure protection of the subject.

Because it is not possible to administer a viral challenge to humans for the purpose of determining vaccine potency (and hence the dosages needed for protection), vaccine potency and appropriate dosage schedules have in practice been determined by administering vaccine to human subjects and studying their serologic responses. Extensive studies of this nature have been conducted in the past by several research groups *(1-8)*.

The rabies vaccination schedule used until recently in Chile was based on schedules designed for vaccines such as the Fermi, Pasteur, and Semple types that are prepared from the nerve tissues of adult animals *(9, 10)*. These vaccines are quite different from vaccine of the Fuenzalida-Palacios type. The latter, developed in suckling mouse brains, achieves a virus concentration 100 times greater than the aforementioned types *(11)* and so is better able to confer immunity. It therefore appeared that the number of doses called for by Chile's vaccination schedule was excessive.

Moreover, the schedule officially recommended varied, depending on the type of animal biting and the bite site or point of infection *(12)*. These variations encouraged differing interpretations of the schedule by the medical or paramedical personnel prescribing treatment. Such variations also promoted a casual attitude on the part of some patients who, for reasons of discomfort or failure to comprehend the seriousness of their situation, did not complete the full treatment schedule on time, and who may therefore have failed to acquire an adequate immune response. In this regard, it should be noted that the average number of doses received per person treated in Chile in 1968 was 6.05 *(13)*.

The purpose of the study reported here was to determine the serologic response of human subjects to a reduced schedule of rabies vaccinations, with a view to contributing toward

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[2]Chief, Vaccines Subdepartment, Chilean Bacteriological Institute, P.O. Box 48, Santiago, Chile.

[3]Chief, Rabies Section, Chilean Bacteriological Institute.

formulation of a new uniform rabies vaccination schedule for Chile.[4] Such a schedule should be directed at the following basic goals:

- Obtaining at least 0.5 international units of antirabies seroneutralizing antibodies per ml of serum in order to ensure protection of the patient *(14, 15)*.
- Maintaining a high level of rabies antibodies by (1) administering booster doses *(16, 17)* and (2) lengthening the time interval between the primary doses and the boosters *(18, 19)*.
- Reducing the number of doses in order to reduce the chances of postvaccination complications *(20-22)*—and also to lessen the costs, patient time, and discomfort involved.
- Ensuring that booster doses are administered over the average (one to four month) rabies incubation period in man *(23-28)*.
- Ensuring application of the indicated antirabies treatment regardless of the bite site or animal involved.
- Obtaining a theoretically better and faster immunoglobulin G response than that obtainable with the traditional schedule, taking advantage of the booster effect *(29, 30)* and a lesser antigenic stimulus *(29)*.
- Incorporating the conclusions of the Rabies Vaccine Symposium held in Germany in 1977, which recommended administering nine doses (on days 0, 1, 2, 3, 4, 9, 13, 20, and 90) when using suckling mouse-brain vaccine *(14)*.

Materials and Methods

The subjects tested were 31 volunteers from the University of Chile's School of Livestock and Veterinary Medicine, none of whom had previously undergone rabies vaccination. Each subject was immunized with 2 ml doses of Fuenzalida-Palacios rabies vaccine for human use that were administered subcutaneously in the abdominal region on days 0, 1, 2, 3, 4, and 5. Two booster vaccinations with equivalent 2 ml doses were given on days 21 and 90.

Concurrently, three blood samples were obtained. The first was taken on day 0, at the time of the first vaccination; the second was taken on day 28, seven days after the first booster; and the third was taken on day 97, seven days after the second booster. Neutralization tests were then performed on these sera using Atanasiu's techniques *(31)*.

Titers indicating virus titration and seroneutralization levels were obtained by the Reed and Muench method *(32)*. Geometric means were calculated by the Paul and White method *(33)*. All of the titers obtained were expressed as international units of seroneutralizing antibody (using calculations based on the titer of the national reference serum) and also as logarithmic reciprocals (see Table 1). The statistical methods used corresponded to those recommended by Rodríguez *(34)*.

Results

Postvaccination reactions (erythema and pruritis) were observed in the local area of inoculation, especially after administration of the sixth vaccine dose on day 5; but these symptoms disappeared in 48 to 72 hours.

Table 1 shows the seroneutralizing rabies antibody titers obtained in the 31 subjects. These data indicate that antirabies seroneutralizing antibodies were absent in the first blood samples (taken on day 0) but were present in all of the sera taken on day 28. Values obtained for these second serum samples ranged from 2.0 to 287.9 international units, the geometric mean being 18.0 international units. (The logarithmic reciprocals of the titers involved ranged from 2.200 to 4.350, the geometric mean being 3.147.) Similarly, the results show that all of the third serum samples (taken on day 97) tested positively, the international unit values ranging from 0.9 to 164.5 (with a geometric mean of 27.5) and the logarithmic reciprocals ranging from 1.875 to 4.107 (with a geometric mean of 3.330). Student's "t" test did not reveal any significant differences between the results obtained with the second and third serum samples.

[4]In October 1979 the Ministry of Public Health of Chile modified its official antirabies treatment guidelines in accordance with recommendations derived from the work reported here.

Table 1. Titers of antirabies seroneutralizing antibodies found in sera from 31 previously unvaccinated subjects who received six initial and two booster doses of Fuenzalida-Palacios vaccine. These results are expressed in terms of international units of antirabies seroneutralizing antibody (IU) and also as logarithmic reciprocals (LR) of the actual titers.

Patient designation	Serum samples					
	No. 1 (day 0)		No 2 (day 28)		No. 3 (day 97)	
	IU	LR	IU	LR	IU	LR
A_3	0	0	18.1	3.150	44.5	3.540
A_6	0	0	10.2	2.903	34.6	3.430
B_8	0	0	16.9	3.120	164.5	4.107
C_3	0	0	15.1	3.070	164.5	4.107
C_4	0	0	11.9	2.970	44.5	3.540
C_6	0	0	8.8	2.840	5.1	2.602
C_8	0	0	5.3	2.620	36.2	3.450
D_1	0	0	2.5	2.301	1.6	2.100
D_2	0	0	2.0	2.200	41.1	3.505
D_4	0	0	27.4	3.330	58.7	3.660
E_6	0	0	20.5	3.204	28.7	3.350
E_7	0	0	128.5	4.000	48.8	3.580
F_2	0	0	10.2	2.903	5.1	2.602
G_1	0	0	3.3	2.420	22.3	3.240
G_3	0	0	52.3	3.610	41.1	3.505
G_5	0	0	20.5	3.204	31.5	3.390
H_2	0	0	16.1	3.100	16.9	3.120
H_4	0	0	57.4	3.650	32.3	3.400
I_8	0	0	6.9	2.730	41.1	3.505
J_3	0	0	15.8	3.090	41.1	3.505
J_4	0	0	287.9	4.350	164.5	4.107
J_5	0	0	6.4	2.700	6.7	2.720
L_1	0	0	131.5	4.010	47.7	3.570
L_3	0	0	28.7	3.350	95.3	3.870
L_7	0	0	6.4	2.700	3.4	2.430
M_7	0	0	17.7	3.140	22.3	3.240
N_2	0	0	41.1	3.505	28.1	3.340
N_3	0	0	57.9	3.654	25.0	3.290
N_4	0	0	17.7	3.140	36.2	3.450
N_4	0	0	4.0	2.500	0.9	1.875
R_6	0	0	164.5	4.107	164.5	4.107
Geometric mean	0	0	18.0	3.147	27.5	3.330

Discussion and Conclusions

It was to be expected that no antibodies would be found in the first blood samples, since none of the 31 individuals tested had ever before been vaccinated against rabies. Likewise, the 100 per cent positive serologic response obtained after administration of the first and second boosters was not surprising. However, comparison of these responses to those obtained with other kinds of vaccines indicated generally better antirabies antibody production. The responses also seemed better than those obtained using a different inoculation schedule for vaccine of the Fuenzalida-Palacios type, presumably because of the booster effect *(7, 16, 17, 35, 36)* or the longer interval allowed to elapse between the primary doses and the boosters *(18, 19)*.

Assessing the results on a case-by-case basis, it was found that in all cases both the first and second boosters had elicited defensive responses considered sufficient to protect against rabies (i.e., over 0.5 international units of antirabies seroneutralizing antibody per ml of serum). Despite the satisfactory response to the first booster, however, application of the second booster is considered necessary to guard against reduction of antibody levels over time *(8, 17, 36-39)* and to ensure protection of the subject for the average rabies incubation period of one to four months *(23-28)*. In this regard, it is worth noting that the second booster elicited a response 9.5 international units greater, on the average, than the first booster; and while this difference could not be proved statistically meaningful, it could very well have biological significance.

The reduction noted in certain individuals' rabies antibody levels after the second booster (as compared to the levels recorded after the first) could be attributable to a high rate of antibody production following the first booster and a resulting fast neutralization of the second booster antigen by these antibodies *(40)*.

In general, the results obtained after the first booster compare favorably with findings Fuenzalida and coworkers *(6)* obtained using 14 doses of Fuenzalida-Palacios vaccine. Indeed, there is a highly significant difference ($p<0.001$) between the two sets of results. There is also a statistically significant difference with respect to the results Godoy *(7)* obtained with three to five doses of the same vaccine plus a booster on day 25. The fourteen-dose schedule of Fuenzalida-Palacios vaccine used by Salido-Rengell *(41)* produced results statistically comparable to those reported here. However, the present procedure has the advantage of reducing the number of vaccine doses, thus lessening the risk of postvaccination complications *(20, 22)*.

All in all, the results reported here are superior to those obtained with most other types of rabies vaccines. For example, there is a highly significant difference ($p<0.001$) between these results and those that Atanasiu and coworkers *(4)* obtained by administering 14 doses of Semple vaccine plus two boosters. Likewise, Campillo and coworkers *(5)* obtained lower antibody titers with 14 or more doses of Semple vaccine, the logarithmic reciprocals of those titers ranging from 1.85 to 3.61. This was considerably below the range of 2.2 to 4.35 obtained in the present study. The reciprocal logarithms of the antibody titers obtained by Fox and coworkers *(42)* with Flury HEP vaccine were also relatively low, ranging from 1.51 to 2.11; and the same is true of the results obtained by Cohen and coworkers *(35)* with four doses plus one booster of duck embryo vaccine.

Comparison of our results with those obtained by Cox and Schneider *(16)*, Kuwert and coworkers *(17)*, Cabasso and coworkers *(19)*, and Hafkin and coworkers *(43)* using human diploid cell vaccines shows these latter vaccines elicited good rabies antibody responses while causing only mild local reactions *(43-45)*. Overall, the results obtained by these authors are similar to the results of the present study; however, the Fuenzalida-Palacios vaccine's lower cost and demonstrated safeness throughout the years it has been used in Chile are factors favoring its selection for our country.

It should be noted that the new vaccine schedule we tested was found to produce titers similar to or higher than those said to be obtained with reduced schedules or more doses of Fuenzalida-Palacios vaccine. These data suggest that the new proposed schedule is the safest of those compared—either because the antibody titers achieved with both boosters are higher or because of the smaller number of required doses.

Although no determination of specific immunoglobulins was made in our study, the findings of Rubin and coworkers *(29)* and Cho and coworkers *(30)* would lead one to expect a relatively great and fast immunoglobulin G response, this being the type that provides the greatest protection for the individual because it diffuses into the tissues *(29)*. How-

ever, there is clearly a need to perform the tests required to confirm this point.

Finally, attention should be drawn to the minimal postvaccination reactions that occurred (e. g., erythema and pruritis in the inoculation area that disappeared in 48 to 72 hours). This finding agrees with results reported by Lery *(46)* and others, who have indicated that using the brains of newborn animals like suckling mice to prepare rabies vaccine greatly reduces the chances that adverse reactions will occur *(11, 15)*.

SUMMARY

The study reported here sought to assess the serologic responses of human volunteers vaccinated against rabies with Fuenzalida-Palacios vaccine, with a view to contributing toward formulation of a new uniform rabies vaccination schedule for Chile. The subjects tested were 31 volunteers from the University of Chile's School of Livestock and Veterinary Medicine, none of whom had a prior history of rabies vaccination.

Eight 2 ml doses of Fuenzalida-Palacios vaccine for human use were administered subcutaneously in each subject's abdominal region on days 0, 1, 2, 3, 4, 5, 21, and 90 of the experiment. Blood samples for testing the antibody responses to these inoculations were taken on days 0, 28, and 97. The levels of seroneutralizing rabies antibodies present were determined in the manner described by Reed and Muench *(32)*.

The results showed that seroneutralizing rabies antibodies were absent in all the subjects' sera on day 0 but were present in all the sera on days 28 and 97 in sufficient amounts to provide protection. The serum samples taken on day 28 were found to contain between 2.0 and 287.9 international units of seroneutralizing rabies antibodies per ml, the geometric mean being 18.0 units. Similarly, samples taken on day 97 were found to contain between 0.9 and 164.5 international units, the geometric mean being 27.5. A minimum of 0.5 units per ml is the least deemed necessary to ensure protection.

These results seemed considerably superior to those obtained by other investigators using Semple, Flury HEP, and duck embryo vaccines. Likewise, they appeared equal or better than results obtained with other vaccination schedules employing Fuenzalida-Palacios vaccine. Workers using human diploid cell vaccines have obtained results comparable to those reported here. However, the Fuenzalida-Palacios vaccine's lower cost and demonstrated safety during the years of its use in Chile tend to favor its selection as the best product to be employed in that country for human vaccination against rabies.

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DISCONTINUATION OF SMALLPOX VACCINATION

In keeping with its declaration of the achievement of global smallpox eradication, the Thirty-third World Health Assembly (May 1980) recommended that smallpox vaccination be discontinued in every country, except for investigators at special risk. As of November 1981, obligatory smallpox vaccination of the general public has been abandoned in 144 countries, including China and India. Countries in which vaccination is still continuing include: Burma, Chad, Egypt, Kuwait, and Tunisia. Those whose present policy is unknown are: Albania, Algeria, Democratic People's Republic of Korea, Gabon, Republic of Korea, Romania, and San Marino.

While this policy pertains to internal vaccination, the practice of requiring a smallpox vaccination certificate from international travelers has been discontinued by all countries except Chad, which had not notified WHO of its current situation.

Source: World Health Organization, *Weekly Epidemiological Record* 56(45):353-354, 1981.