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REDUCED SCHEDULE OF HUMAN ANTIRABIES IMMUNIZATION WITH FUENZALIDA & PALACIOS VACCINE

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SUMMARY

The present study evaluates the humoral and cellular immune responses in 35 volunteers submited to short antirabies vaccination schedules with the Fuenzalida & Palacios vaccine based on the administration of doses on non consecutive days.

The volunteers were divided into two groups. The first group received a total number of five doses given on days 0, 4, 7, 20 and 35. The other group received four doses, the first one being a double dose given on day 0 and than three other single doses on days 7, 20 and 35.

The evaluation of humoral immune response was carried out by serum neutralization (SN) and indirect immunofluorescense (IIF) tests, while the cellular immune response was evaluated by lymphoblastic transformation assay (LTA) and skin test (ST).

According to our results these reduced schedules elicited early and effective humoral and cellular immune responses to rabies antigen suggesting that new reduced schedules should be extensively studied in order to give the proper bases to the proposition of changes in the current long-term schedule.

KEY WORDS: Antirabies vaccination; Reduced schedule; Cellular immunity.

INTRODUCTION

The human antirabies vaccination schedule using nervous tissue vaccine tipically consist of a relative high number of sequential daily doses.

In Brazil as well as in the most Latin-American countries the Fuenzalida & Palacios vaccine[3] prepared with nervous tissue from newborn mice has been the most used over the last 25 years for human vaccination.

In our country, the original basic series of 14 doses was reduced to 10 and than to 7 doses. The latter is now employed with 3 additional booster doses.

Presence and titer of neutralizing antibodies have been the main elements used to justify these progressive reductions. The shorter schedules proved to be efficient and highly protective after

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human exposition to the virus, reducing costs and disconfort to the patients and mainly the risks of postvaccination accidents[1].

A reduction in the total number of doses as well as a more rational administration, i.e., in non consecutive days would probably result in a schedule as effective as the actual one. All other immunization procedures are based on schedules composed of few doses on non consecutive days, including those suggested for antirabies vaccine of cellular culture origin[6].

Recently protection against rabies has been shown to be much more related with cellular than humoral immune responses, at least in experimental conditions[7, 8, 10].

The above considerations justify, in our opinion, the study of new reduced schedules with non daily administration of Fuenzalida & Palacios vaccine that probably will remain in use in Brazil for many years. The evaluation of these schedules, however, must include the detection of cell mediated immune response (CMI) in addition to the titration of antibodies.

The objective of this paper is to present the first results obtained with two new vaccination schedules in groups of non-exposed volunteers.

MATERIAL AND METHODS

1 — Volunteers: 35 volunteers with ages ranging from 21 to 50 years, without previous contact with rabies antigen were divided into 2 groups A and B with 20 and 15 individuals respectively.

2 — Vaccination schedules: group A was submitted to a total number of five doses of Fuenzalida & Palacios vaccine administered by intramuscular route on days 0, 4, 7, 20 and 35. Group B was submited to a four doses schedule, but with a initial double dose (2 ml) on day 0 and single doses on days 7, 20 and 35.

3 — Immune response evaluation: the humoral immune response was evaluated by serum neutralization (SN) and indirect immunofluorescence (IIF) tests, while CMI was evaluated by skin tests (ST) and lymphoblastic transformation assay (LTA).

4 — Antirabies vaccine (for vaccination and skin tests): Fuenzalida & Palacios vaccine[3] was prepared at Instituto Butantan, São Paulo. Briefly, this vaccine consists of newborn mice brain (myelin free) previously inoculated by intra-cerebral route with fixed rabies virus, strain PV1. It is inactivated by U.V. radiation and presents a final concentration of 2% nervous tissue, 0.5% phenol and 1:10000 thimerosal. A control antigen for skin tests was prepared from normal newborn mice brain, according to the technique used to prepare antirabies vaccine.

5 — Antigen for LTA: rabies antigen (RAg) was prepared essentialy as the vaccine except that it contained 10% nervous tissue and no preservatives. Control rabies antigen (CAg) was the same as RAg but prepared from non inoculated newborn mice.

6 — Serum neutralization test: This test was performed essentially as described by ATANASIU[2], with sera diluted up to 1:625. All tests were performed against 25-40 LD50 and titer values ≤ 5 were considered negative.

7 — Indirect immunofluorescense: The IFI was carried out as described by GOLDWASSER & KISSLING[4], using slide imprints of mice nervous tissue containing street rabies virus with only one passage in laboratory.

8 — Lymphoblastic transformation assay: It was performed as described by HALL & GORDON[5] and adapted for the rabies system by VEIGA et al[9]. Each lymphocyte sample was cultured in the absence of antigens or in the presence of RAg, CAg or phytohaemagglutinin (PHA). All tests were carried out in triplicate and the result presented as lymphoblastic transformation index (LTI) was obtained as follows.

$$LTI = \frac{\text{Average CPM obtained with RAg, CAg or PHA}}{\text{Average CPM obtained without antigen}}$$

LTI $\geq$ 2.0 were considered positive for either RAg or CAg

LTI $\geq$ 2.0 were considered normal values for PHA

9 — Skin test: The skin test was performed inoculating i.d. 0.1 ml of antirabies vaccine and

the control antigen in right and left forearm respectively. Readings were done 48 to 72 hours later. The test was considered positive if induration was ≥ 5 mm.

RESULTS

The ST and LTA using the proper control antigens presented always negative results. All LTI obtained in the presence of PHA could be included within the limits of normality.

Table 1 shows the results of cellular and humoral immune responses of group A, composed of 20 volunteers submitted to the five-dose schedule.

It should be noted that in this group CMI detected by ST was present in 19 out of 20 volunteers as early as day 7. CMI, detected by LTA was also present since day 7 in all of the tested volunteers and LTI values kept raising up to day 35 when the last test was performed.

Antibodies could be detected in 5 out of the 20 volunteers on day 7 and from day 20 up to day 75, antibodies were detected by SN and IFI in all of the tested volunteers. In general, maximal antibody titers were obtained on day 45. The only volunteer which was ST negative on day 7 was not tested in LTA, but developed CMI later, as mesured by ST on day 75 since he is the one of the 14 tested at that time. The same volunteer developed a good humoral response, with a titer of 51 on day 20 reaching a titer > 625 on day 45 (data not shown).

TABLE 1
Cellular and humoral immune responses follow-up of the five-doses schedule group.

Immune Response	Assay		Days 0	7	20	35	45	75
Cellular	LTI	nº posit.	0	5	4	3	ND	ND
		nº tested	5	5	4	3		
		range	0,5 - 1,7	2,3 - 13	4,2 - 23	4,6 - 36		
	ST	nº posit.	0	19	ND	ND	ND	14
		nº tested	20	20				14
Humoral	SN	nº posit.	0	5	20	19	14	18
		nº tested	20	18	20	19	14	18
		titer range	> 5	5 - 19	51 - > 625	71 - > 625	255 - > 625	47 - > 625
	IIF	nº posit.	0	5	20	20	14	20
		nº tested	20	20	20	20	14	20

Table 2 shows the results of cellular and humoral immune responses of group B composed of 15 volunteers submitted to the four-doses schedule.

CMI detected by ST was presented in 8 out of the 13 tested volunteers as early as day 7. Antibodies could be detected by both techniques from 20th day on, and also showed maximal titers on day 45.

DISCUSSION

The Fuenzalida & Palacios vaccine was employed with good results in human experimental antirabies vaccination using two different reduced schedules with administration of doses in non consecutive days.

Both schedules elicited early cellular and humoral immune responses that were also satisfatory in terms of intensity and persistance.

In the last few years experimental evidences obtained suggested that CMI plays an essential role in protection against rabies[7, 8, 10] while antibodies may not participate as much as it was considered in the past.

TABLE 2
Cellular and humoral immune responses follow up of the four-doses schedule group.

Immune Response	Assay		Days					
			0	7	20	35	45	75
Cellular	ST	nº posit.	0	8	ND	ND	ND	10
		nº tested	15	13				10
Humoral	SN	nº posit.	0	0	10	10	10	10
		nº tested	15	15	10	10	10	10
		titer range	< 5	< 5	33 - 279	51 - > 625	63 - > 625	56 - > 625
	IIF	nº posit.	0	0	15	15	15	15
		nº tested	15	15	15	15	15	15

WIKTOR[10] demonstrated that resistance to challenge with street rabies virus depended on use of antigens that stimulated CMI and antibody production in mice. Antigenic stimulation that were good inducers of antibody and even interferon production but not elicited good CMI were not protective. In addition he showed that immunization with a single vaccine dose elicited a short term CMI which declined to undetectable levels in a few days.

On the other hand, immunization with several vaccine doses administered on non consecutive days elicited persistant CMI for at least four weeks, when the experiment was concluded.

These factors let us to includ the evaluation of CMI in the study of new reduced schedules for human antirabies vaccination.

The best result was obtained with the five-doses schedule administered to group A. In this group, cellular and humoral immune responses could be detected respectively in 19 and 5 out of the 20 volunteers as early as day 7. The levels of CMI measured by LTA in some volunteers increased up to day 35 after the beginning of the immunization, the last time LTI was determined. The persistance of detectable CMI was satisfactory as demonstrated by positive results in ST performed in 14 volunteers at the end of the follow-up on day 75.

Neutralizing antibodies were detectable in all volunteers on day 20 and SN test demonstrated increasingly higher titer up to day 45 when maximal values were usually obtained. Humoral immune response was still present in all volunteers tested by either technique (SN or IIF), at the end of the follow-up.

This reduced five-doses schedule represents a reduction of 50% in the number of doses when compared to the current schedule which consists of 7 doses plus three boosters, reducing patient disconfort and risks of postvaccinal accidents. Although these results are based on a small group of volunteers, the antigenic stimulation seems to be very effective and persistent.

The other schedule based on four-doses vaccine proved to be less efficient in stimulating an early response but was as effective as the five-dose one after the 20th day.

We do think it is very important to evaluate CMI in antirabies vaccination. The ST previously proposed to monitorize CMI constitutes a simple, fast, reliable, sensible and low cost technique. In our opinion the ST constitute an extremally valuable assay in the detection of CMI.

One must have in mind that changes in human vaccination schedule in diseases such as rabies need to be tested under experimental conditions in a much longer number of persons, before they can be adopted. Our preliminary results, however, have demonstrated that changes in the current vaccine schedule proposed almost empirically in the past must be revised in the lights of new knowledge of immunological aspects involved in protection against this disease.

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RESUMO

Esquema reduzido de vacinação anti-rábica humana.

O objetivo do presente trabalho foi estudar um novo esquema de vacinação anti-rábica humana, com um menor número de doses, administradas em dias não consecutivos (5 doses nos dias 0, 4, 7, 20 e 35).

A avaliação da resposta imune humoral foi feita pela prova de soroneutralização e pela reação de imunofluorescência indireta, enquanto que a resposta imune celular foi avaliada pela transformação linfoblástica em cultura de sangue total e pelo teste cutâneo de leitura tardia.

Foram estudados um total de 35 voluntários, submetidos ao esquema reduzido de vacinação, e os resultados encontrados permitem afirmar que, embora o número de casos seja relativamente pequeno, este novo esquema de vacinação mostrou-se capaz de induzir a produção de imunoglobulinas anti-rábicas, bem como de elicitar a resposta imune celular ao antígeno rábico.

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