

REDUCED SCHEDULE OF HUMAN ANTI-RABIES IMMUNIZATION WITH FUENZALIDA & PALACIOS VACCINE. ADDITIONAL DATA

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SUMMARY

It was reevaluated a reduced schedule for anti-rabies post-exposure immunization with newborn mice nervous tissue vaccine (Fuenzalida & Palacios) in a group of 30 non exposed volunteers.

The vaccine was administered by intramuscular injections on days zero, 2, 4, 16 and 27, in the deltoid area.

Antibody levels were determined by a simplified serum neutralization microtest on days zero, 16 and 37.

On days 16 and 37 the antibody levels of the whole group was ≥ 0.5 IU/ml and ≥ 1.0 IU/ml, respectively.

The cell mediated immunity was precociously detected (on day 4) by the delayed type hypersensitivity skin test.

Our results show that this reduced schedule elicited an early and effective humoral and cellular immune response. However it is necessary other studies with larger groups of vaccinees in order to obtain definitive conclusion.

KEY WORDS: Human Anti-rabies Immunization; Reduced Schedule; Humoral and cellular immune responses.

INTRODUCTION

In Brazil as well as in most Latin American countries the most commonly used anti-rabies vaccine for human vaccination, during the last 25 years, has been prepared with nervous tissue of newborn mice (FUENZALIDA & PALACIOS)². This vaccine is usually administered following vaccination schedules that include a basic series of a relatively large number of daily doses plus two or three boosters.

In an attempt to reduce the risk of post vaccination accidents the original basic series of 14 doses was reduced to 10 and finally to the currently used schedule of 7 doses plus 2 additional boosters. When hyperimmune serum is administered the schedule is enlarged to 10 daily doses plus 3 boosters injections. However,

post-vaccination accidents still occur at dangerous levels.

Studies aimed at creating lower risk vaccination schedules for administering Fuenzalida & Palacios vaccine must be developed because an alternative vaccine, obtained from cell culture, is still far from being routinely used in Brazil.

Although encouraging results were obtained in a study following a 5 dose vaccination schedule¹³, more substantial data are needed based on a larger number of non-exposed volunteers before any schedule changes may be implemented.

Our aim was to reevaluate the same vaccination schedule in an additional larger group of non-exposed volunteers.

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MATERIAL AND METHODS

Human volunteers

Thirty healthy male volunteers aged 18-20 years without previous exposure to rabies virus antigens were studied.

Vaccine and vaccination schedule

We used the Fuenzalida & Palacios vaccine prepared by 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 PVI. It is inactivated by U.V. radiation, presents a final concentration of 2% nervous tissue, 0.5% phenol 1:10.000 thimerosal and antigenic value ≥ 0.6 .

The vaccination schedule consisted of 5 doses of the Fuenzalida & Palacios vaccine administered intramuscularly (deltoid area), on days zero, 2, 4, 16 and 27.

Evaluation of the immune response

- 1) The humoral immune response was evaluated by a simplified serum-neutralization microtest described elsewhere (in press) and based on the principle of the rapid fluorescent focus inhibition test (RFFIT)⁸ and fluorescent inhibition microtest (FIMT)¹² developed for detection of rabies virus neutralizing antibodies. The test was carried out on samples collected on days zero, 16 and 37 after the beginning of vaccination.
- 2) The cell-mediated response was evaluated as described by SANTOS et al⁶. Briefly, skin tests were performed by injecting intradermally in each arm, 0.1ml of the anti-rabies vaccine and control antigen (normal newborn mice nervous tissue prepared in the same manner as the vaccine), respectively. The tests were carried out on day 4, read on day 6 and considered positive when induration diameter was ≥ 5 mm.

Standard Serum

The standard serum used (containing 1577 IU/ml) was that distributed by the National Institute of Quality Control in Health (INCQS - Brazil), which was kindly provided by Dr. E.I.G. Valentin from Butantan Institute.

RESULTS

Skin Tests

The average diameter obtained with the antirabies vaccine was 12.4mm with individual diameter varying from 8mm to 20mm. The control antigen readings were either negative with no nodules or showed induration diameters smaller than 5mm.

Antibody Levels

As shown in table 1 all samples collected on day zero showed antibody levels lower than 0.25 IU/ml.

In contrast, all samples collected on day 16 were positive with antibody levels ≥ 0.75 IU/ml with only one exception showing titer between 0.50 and 0.75 IU/ml. On day 37 all the samples were positive with titers never below 1.0 IU/ml; 10 individuals presented titers ≥ 4.0 IU/ml. The median values for days 16 and 37 were similar, between 3.0 and 4.0 IU/ml.

DISCUSSION

In the current study, we demonstrated that all 30 volunteers submitted to the reduced immunization schedule of 5 doses showed early and satisfactory humoral and cellular immune responses.

On day 16, the antibody levels were all ≥ 0.5 IU/ml with 20 out of 30 studied individuals reaching levels ≥ 4.0 IU/ml. Our results are in accordance with WHO recommendations which require for new immunization schedules serum conversion with minimum titers of 0.5 IU/ml on day 21 after the beginning of immunization⁷.

Interestingly, no increase in the antibody levels median range was observed on samples from day 16 to 37, in spite of two additional vaccine doses administered during this period (see table 1). However, volunteers with the lowest antibody levels on day 16 showed increased levels on day 37.

These findings are in agreement with our previous data using the same reduced immunization schedule¹³ as well as those obtained by HERZOG et al. with reduced schedule too, using cell culture vaccines³.

Despite cellular mediated immunity (CMI) had been shown to play an important role in

TABLE I
Neutralizing antibody levels according to the sera collection dates.

Antibody level Ranges (IU/ml)	Collection days		
	0	16	37
< 0.24	30		
0.50 ─ 0.75		1	
0.75 ─ 1.00		3	
1.00 ─ 1.50		3	3
1.50 ─ 2.00		3	5
2.00 ─ 3.00		4	6
3.00 ─ 4.00		6	6
4.00 ─ 6.00		5	6
6.00 ─ 8.00		2	2
8.00 ─ 12.00		1	1
12.00 ─ 16.00		2	1
Media	< 0.25	3.00 ─ 4.00	3.00 ─ 4.00

induction of immunity to rabies^{10,11}, only a few experimental studies focused CMI in human vaccinees^{1,3,4,5,6,13}. Peripheral blood leukocyte proliferation^{1,3,4,13} and interleukin-2 production⁵ after *in vitro* specific stimulation have been the most commonly used tests. Nevertheless these techniques require supplies available only in sophisticated laboratories.

We used the delayed type hypersensitivity skin test to evaluate CMI. As previously shown it constitutes a simple, fast, reliable, sensible and low cost technique to detection CMI^{6,13}.

In this study the whole group presented positive results on day 4 when tested with anti-rabies vaccine, and negative ones with control antigen, showing a satisfactorily precocious CMI.

This study supports the idea that anti-rabies immunization may be performed with a smaller number of vaccine doses, administered on non-consecutive days with less trauma and lower post-vaccination risk for the patients.

RESUMO

Esquema reduzido de imunização anti-rábica humana com vacina Fuenzalida & Palacios. Dados adicionais.

Foi reestudado em um grupo adicional de 30 voluntários não expostos ao contágio um esquema reduzido para imunização pós-exposição com vacina de sistema nervoso de camundongos recém-nascidos (Fuenzalida & Palacios).

A vacina foi administrada nos dias zero, 2, 4, 16 e 27.

A resposta imune humoral foi avaliada por soroneutralização em cultura celular nos dias zero, 16 e 37.

Já no dia 16 os títulos de anticorpos foram ≥ 0.5 UI/ml e no dia 37 ≥ 1.00 UI/ml.

A resposta imune celular foi avaliada no dia 4 pelo teste cutâneo de hipersensibilidade tardia. Foram obtidos resultados positivos em todos os 30 voluntários estudados.

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