

STUDIES ON HUMAN ANTI-RABIES IMMUNIZATION IN BRAZIL. II - PRELIMINARY EVALUATION OF THE 2-1-1 SCHEDULE FOR HUMAN PRE-EXPOSURE ANTI-RABIES IMMUNIZATION, EMPLOYING SUCKLING MOUSE BRAIN VACCINE

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SUMMARY

This study reports preliminary results of virus neutralizing antibody (VNA) titers obtained on different days in the course of human anti-rabies immunization with the 2-1-1 schedule (one dose is given in the right arm and one dose in the left arm at day 0, and one dose is applied on days 7 and 21), recommended by WHO for post-exposure treatment with cell culture vaccines. A variant schedule (double dose on day zero and another on day 14) was also tested, both employing suckling mouse brain vaccine. A complete seroconversion rate was obtained after only 3 vaccine doses, and almost all patients (11 of 12) presented titers higher than 1.0 IU/ml. Both neutralizing response and seroconversion rates were lower in the group receiving only 3 doses, regardless of the sample collecting day. Although our results are lower than those found with cell culture vaccines, the geometry mean of VNA is fully satisfactory, overcoming the lower limit recommended by WHO of 0.5 IU/ml. The 2-1-1 schedule could be an alternative one for pre exposure immunization, shorter than the classical 3+1 regimen (one dose on days 0, 2, 4 and 30) with only three visits to the doctor, instead of four.

KEY WORDS: Rabies; Human pre-exposure vaccination; 2-1-1 schedule; Mouse brain vaccine.

INTRODUCTION

The high costs of human rabies vaccines produced in cell cultures has led to attempts to use reduced schedules, employing lower amounts of vaccine.

Thus several new schedules arose, including the 2-1-1 one, consisting of administration of two doses on day zero (one dose in each deltoid region) and one dose on days 7 and 21. It was initially purposed in 1986 in Yugoslavia for post-exposure treatment, with different kinds of cell culture vaccines (potency value > 2.5 IU/ml), when good results were obtained¹³. The schedule was later tested in other countries, always with satisfactory results^{3,12}. The 2-1-1

regimen induces an early antibody response and may be particularly effective when post-exposure treatment does not include administration of rabies immunoglobulin¹², as stated by WHO¹⁴.

WHO states that new reduced schedules for post-exposure treatment should be considered satisfactory when seroconversion is attained by virtually all vaccines on day 21¹¹. The 2-1-1 regimen using Pittman-Moore (PM) rabies vaccines cultivated in Vero cells and evaluated on day 21 (before the fourth dose) induced high levels of virus neutralizing antibodies (VNA) against the CVS fixed rabies virus strain⁷

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On the other hand, in pre-exposure treatments strict harmonization of immunization schedules is considered of minimum significance since an acceptable neutralizing titer (0.5 IU/ml) can be attained to demonstrate seroconversion¹¹

The human rabies vaccine currently used in Brazil is prepared with the nervous system of suckling mice⁶, mostly by Instituto Butantan of São Paulo and Instituto de Tecnologia de Paraná, with the PV (Pasteur Virus) and CVS (Challenge Virus Standart) strain, respectively. The potency value is of at least 0.6 IU/dose as measured by the NIH test¹⁰.

Although this vaccine has demonstrated some failures, probably due to adverse conditions¹⁵, it has presented good results in well-controlled experimental groups receiving reduced schedules⁴, or the 3 + 1 regimen {3 doses on alternate days and the last dose on day 30}², the most commonly used one for pre-exposure immunization in Brazil.

Nevertheless, the 2-1-1 schedule, besides being recommended because of the excellent immune responses elicited with rabies vaccines produced in cell cultures⁷, may be faster than the 3 + 1 schedule in pre-exposure immunization and may reduce the number of clinical visits from 4 to 3.

The aim of the present study was to report preliminary results of VNA titers obtained on different days in the course of immunization with the 2-1-1 schedule and a variant one (double dose on day zero and another on day 14), employing suckling mouse brain vaccine.

MATERIAL AND METHODS

Subjects and vaccine: a total of 23 subjects (ranging in age from 22 to 38 years), never vaccinated before, whose professional activities justified their immunization, were divided into 2 groups. Group A (12 subjects) received the 2-1-1 schedule and Group B (11 subjects) received the abbreviated one. The vaccine doses were always administered i.m. into the deltoid region.

The volunteers were vaccinated with the same batch suckling mouse brain vaccine prepared with the CVS strain, and provided by the Instituto de Tecnologia do Paraná.

Serum samples and VNA determination: the serum samples were collected on days zero, 14, 28 and 42. Virus neutralizing antibodies against the PV strain were tested by the simplified fluorescence inhibition microtest as described elsewhere⁵, VNA is expressed as international units per ml (IU/ml) using as standard an equine hyperimmune serum adjusted to a concentration of 200 IU/ml. Seroconversion of each group was estimated as percentage of patients who developed VNA titer higher than 0.5 IU/ml.

RESULTS

VNA was not detected in the serum samples collected on day zero.

TABLE 1

Seroconversion rate (in %), VNA titer ranges and VNA titer geometric means (in IU/ml), according to the blood sampling day of Group A (vaccinated with the 2-1-1 schedule).

	Blood Sampling days		
	14	28	42
Seroconversion rates	100% (12/12)	100% (11/11)	100% (12/12)
Ranges	0.79 - 12.0	0.56 - 16.0	0.56 - 16.0
Geometric Means	2.17	3.11	2.04

TABLE 2

Seroconversion rates (in %), VNA titer ranges and VNA titer geometric means (in IU/ml), according to the blood sampling day of Group B (vaccinated with the 2-1 schedule).

	Blood Sampling days		
	14	28	42
Seroconversion rates	54.1% (6/11)	90.9% (10/11)	80% (8/10)
Ranges	0.10 - 2.25	0.20 - 2.25	0.15 - 2.25
Geometric Means	0.74	0.92	0.91

As shown in Table 1, group A had a complete seroconversion rate after only 3 vaccine doses. The whole group developed a neutralizing response on day 14 (10 out of 12 presented titers higher than 1.0 IU/ml). Only a slight rise in the geometric mean of VNA was observed on day 28 after the last vaccine injection, and with only one exception, all patients presented titers higher than 1.0 IU/ml. In the last blood sampling performed on day 42, we observed the same seroconversion rate and VNA titers obtained on day 14. Similar results had been previously reported using cellular culture vaccine⁷. One person presented very high neutralizing titers (12.0 IU/ml on day 14 and 16.0 IU/ml on days 28 and 42), suggesting that he belongs to the group of high responders to rabies vaccination⁸ or had been previously immunized.

Both neutralizing response and seroconversion rates were lower in group B (Table 2). The 2-1-1 schedule presented superior results regardless of the sample collecting day.

DISCUSSION

The results presented here, although involving a small group of vaccinees, are promising, especially those obtained with the 2-1-1 regimen. It should be

pointed out, however, that they were obtained under suitable conditions of transportation and storage of the vaccine, and always administered into the deltoid region, as recommended by WHO¹⁴.

Although our results are lower than those found with a vaccine produced with the PM strain in Vero cell⁷, the geometric mean of VNA is fully satisfactory, above the lower limit recommended by WHO¹⁴, and all but one subject showed VNA titers higher than 1.0 IU/ml.

We have already presented similar results using 3 doses of suckling mouse brain vaccine (days zero, 2 and 4) evaluating the VNA response on day 16⁴. Two aspects, however, impair comparison: 1) although all vaccine batches must contain a potency value of at least 0.6 IU/ml, as measured by the NIH test¹⁰, frequently some batches present 2 or 3 IU/dose¹. 2) in the previous study the immunization was performed with a PV suckling mouse brain vaccine, while in the present one the vaccinal strain was CVS. The evaluation of VNA titers was, however, always performed against the PV strain. This situation could importantly modify the relative VNA titers, as described before⁹. The VNA titers obtained on day 28 with the alternative schedule (2 doses on day zero and another on day 14) were lower than those achieved on day 14 with the 2-1-1 regimen, showing that a wider interval between the two initial doses and the third one importantly reduced the VNA response.

Our results point out that the study with the 2-1-1 regimen may be extended, comparing it to the 3 + 1 schedule under standardized conditions. Moreover, the titers achieved on day 14 open the possibility of eliminating the fourth dose. There is also the perspective of comparing the results of day 14 of the 2-1-1 regimen with the basic series of 7 doses on consecutive days employed for post-exposure vaccination in Brazil. Favorable results could reduce the number of injections from 7 to 3, with only 2 visits to the doctor, according to our currently used procedures for human anti-rabies immunization.

Each of these possibilities is being presently investigated with vaccinees immunized with either the CVS or PV strain, in well controlled groups.

RESUMO

Estudos sobre imunização anti-rábica humana no Brasil. II - Avaliação preliminar do esquema 2-1-1 para imunização anti-rábica humana pré-exposição, empregando vacina de tecido nervoso de camundongos recém-nascidos.

Este estudo apresenta resultados preliminares de títulos de anticorpos neutralizantes (AcN) obtidos em diferentes dias durante imunização anti-rábica humana empregando o esquema 2-1-1 (uma dose administrada em cada deltóide no dia 0, e uma dose nos dias 7 e 21), recomendado pela OMS para tratamento pós-exposição

com vacinas de cultivo celular. Um esquema variante (dose dupla no dia zero e outra no dia 14) também foi testado; em ambos esquemas utilizamos a vacina produzida em cérebro de camundongos recém-nascidos. Obteve-se taxa de soroconversão total após 3 doses de vacina, e quase todos os pacientes (11 de 12) apresentaram títulos maiores que 1,0 UI/ml. Tanto os títulos de anticorpos neutralizantes como a taxa de soroconversão foram menores no grupo que recebeu apenas 3 doses, independentemente do dia da coleta de soro. Embora nossos resultados tenham sido inferiores àqueles encontrados com vacinas de cultivo celular, a média geométrica de AcN foi inteiramente satisfatória, sobrepondo o limite mínimo de 0,5 UI/ml recomendado pela OMS. O esquema 2-1-1 pode ser alternativa para tratamento pré-exposição: mais curto que o esquema clássico 3 + 1 (uma dose nos dias 0, 2, 4 e 30) e com apenas três idas ao médico, ao invés de quatro.

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